fermentation

MDPI

*Article*

# Influence of Plant Protein Fining Agents on the Phenolic Composition of Organic Grape Musts

**Áron Pál Szövényi [1], Annamária Sólyom-Leskó [1,*], Anna Szabó [2], Balázs Nagy [1], Zsuzsanna Varga [3] and Diána Ágnes Nyitrainé Sárdy [1]**

[1] Department of Oenology, Institute for Viticulture and Oenology, Buda Campus, Hungarian University of Agriculture and Life Sciences, Villányi Str. 29–43, H-1118 Budapest, Hungary; szovenyi.aron.pal@uni-mate.hu (Á.P.S.); nagy.balazs@uni-mate.hu (B.N.); nyitraine.sardy.diana.agnes@uni-mate.hu (D.Á.N.S.)

[2] Department of Vegetable and Mushroom Growing, Institute of Horticultural Sciences, Buda Campus, Hungarian University of Agriculture and Life Sciences, Villányi Str. 29–43, H-1118 Budapest, Hungary; szabo.anna@uni-mate.hu

[3] Department of Viticulture, Institute for Viticulture and Oenology, Buda Campus, Hungarian University of Agriculture and Life Sciences, Villányi Str. 29–43, H-1118 Budapest, Hungary; varga.zsuzsanna@uni-mate.hu

* Correspondence: solyom-lesko.annamaria@uni-mate.hu

**Abstract:** Protein-based clarification agents were tested to clarify Chardonnay grape musts during sedimentation. The experiments were conducted in the Etyek–Buda Wine Region in Northwest Hungary over four vintages between 2020 and 2023. The performance of the treatment agents was influenced by several factors, such as the composition of the grape must (the absolute concentration and the relative ratio of phenolic compounds) which varied with the vintage characteristics, the physiological and phenolic ripeness of the grapes, and the composition of the clarifying agents itself. Recent investigations show that fully ripe fruit juices can be clarified more effectively, and the effectiveness increases when different types of clarification agents are combined with the plant proteins, e.g., PVPP greatly facilitates the removal of phenolic compounds. The tested plant protein-based clarification agents did not influence the YAN source of the grape musts before fermentation. Our investigations proved an effective impact of these preparations even during the first steps of wine technology. Sensory properties and chemical stability are improved by decreasing the polyphenol content before fermentation, and, besides the good technological effects, wines treated with plant protein agents can be included in the vegan diet.

**Keywords:** plant protein fining agent; pea protein; PVPP; phenolic composition; yeast assimilable nitrogen; sedimentation; grape must clarification



**Citation:** Szövényi, Á.P.; Sólyom-Leskó, A.; Szabó, A.; Nagy, B.; Varga, Z.; Nyitrainé Sárdy, D.Á. Influence of Plant Protein Fining Agents on the Phenolic Composition of Organic Grape Musts. *Fermentation* **2024**, *10*, 642. https://doi.org/10.3390/fermentation10120642

Academic Editor: Alice Vilela

Received: 29 October 2024
Revised: 5 December 2024
Accepted: 12 December 2024
Published: 14 December 2024



## 1. Introduction

The phenolic compounds of grapes and wine have been one of the most important subjects of oenological research for decades due to their influence on technological and sensory characteristics on the one hand, and their physiological effects on the human body on the other. Phenolic components tend to oxidise, thus causing browning and a bitter or astringent taste. Polyphenols have strong antioxidant properties, offering positive effects on the human body. The generally accepted classification distinguishes non-flavonoid (simple) phenols, flavonoid phenols, and tannins [1]. Grape and wine flavonoids include catechins, leucoanthocyanins, and anthocyanin monomers, the latter of which are typically present in blue grapes. Leucoanthocyanins provide a significant part of the tannin content of must and wine, and they also play a decisive role in the development of organoleptic properties; their tannic, astringent taste depends on the degree of polymerisation and can be smoothed out with oenological treatments (clarification and fining). The organoleptic properties of wines of organic production are similar to those of conventional products, but their environmental benefits are indisputable. Often, if not always, this difference can also be observed in the chemical composition, even in terms of a higher polyphenol content [2]. Sparkling wines form a specific group of oenological products. Their production involves a second alcoholic fermentation of a sparkling wine base, namely the "cuvée", consisting of a dry wine, precisely dosed fermenting sugars, and an inoculation starter. The base wine should be light-bodied with a pronounced acidity and a decent polyphenolic content for a harmonious product. For best results, the pre-fermentation clarification and the final fining of the base wine should focus on smoothing the phenolic composition [3].

The Etyek–Buda Wine Region plays a key role in Hungarian sparkling wine production, where traditional and classic bottle fermentation methods are used. The base wines are produced from the varieties Chardonnay, Pinot blanc, Pinot gris, and Pinot noir [4], of which Chardonnay is the most widespread variety in the wine-growing area [5]. Several investigations have been published about the differing phenolic composition of the varieties, which point to the high concentration of simple phenolics and phenolic acids in Chardonnay musts [6–8]. Based on former experiments in producing Etyeki Pezsgő sparkling wines, (partial) removal of the phenolic composition results in better organoleptic properties of the products [9–11].

Juice clarification and wine fining agents are commonly used at various stages of the winemaking process. Their purpose is to remove certain undesirable (haze-forming or odour-masking) components: colloids or entire groups of compounds (e.g., tannins, phenolic compounds), which can affect not only the wine's aesthetic appearance (cloudiness) but also its taste and stability. The most commonly used agents are mineral-based (e.g., bentonite for binding proteins), protein-based (for precipitation of polyphenols), or polymer (e.g., polyvinylpolypyrrolidone (PVPP)) materials [12].

Protein-based clarifying agents form complexes with wine tannins, which can result in negatively charged hydrophobic colloids. These colloids can precipitate in the presence of metal cations. Other proteins may mainly associate with negatively charged particles in suspension or colloidal form. Certain proteins (such as casein) flocculate solely due to the low pH of the wine, but the presence of tannins is necessary for precipitation and clarification [12]. Proteins used in the food industry as raw materials or processing aids usually originate from animal sources; nevertheless, some plant products (such as cereals, potatoes, or pulses) may provide a useful source of proteins as well [13]. In the wine industry, commonly used animal proteins are gelatine, isinglass, casein, or egg albumin [14]. From the beginning of the 21st century, plant protein-based clarification agents have become increasingly widespread and can even be used in organic agriculture [15]. The use of vegetable proteins has become more emphasised since various (ethical, sustainability, health, or allergenic) concerns arise from time to time regarding the agents of swine, bovine, or poultry origin. On the other hand, attention is focused on new clarification agents because of allergenic properties or technological disadvantages (removal of favourable aromas and flavours) [16–18].

According to early tests, wines treated with clarifying agents—even egg albumin—do not pose a health risk, especially if bentonite fining is also used to remove protein residues and the clarification process is combined with efficient filtration [19]. However, it remains controversial whether treated wines can cause allergies, and this applies equally to plant-derived proteins such as wheat proteins [20]. With more precise detection methods, the presence of allergenic proteins in products can be excluded (or confirmed) more and more reliably [21]. The range of consumers who completely avoid or reduce the use of animal-derived foods and other products (cosmetics) due to environmental considerations is also becoming wider. The population with a vegetarian or vegan lifestyle is constantly growing [22]. There have, therefore, been many attempts to replace animal protein-based wine treatment agents with vegan alternatives. During the Vienna Assembly, in July 2004, the International Organisation of Vine and Wine (OIV) approved the use of some plant protein-based fining agents in grape juice and wine [23], and in December 2005, the use of wheat gluten and pea protein was permitted in the European Union [24].

Since 2006, wheat-based vegetable proteins have been available on the market for wine treatment processes. These materials were first used for wines made for consumers with special dietary requirements (vegans). Due to the risks of allergenic wine treatment substances and the obligation to indicate them on the label, the research was directed to pea and other vegetable-based proteins [25]. There has been little practical experience with plant-based treatments compared to gelatine, isinglass, and casein. For the removal of monomeric and dimeric flavanols, clarifying agents based on wheat protein and lentil protein have proven to be effective; nevertheless, wheat protein may have a gentler effect on the wine's aromatics [26].

In addition to wheat gluten, proteins extracted from lupins, peas, potatoes, and soy have become the subject of other investigations. Based on first experiences, plant-based proteins with a protein content of over 80% are effective in wine treatment processes, and most vegetable proteins were utilised in coarse clarification processes achieving a turbidity close to 10 NTU [27]. The total polyphenol content can be reduced with pea proteins. In addition, by binding oxidised phenols, the colour of rosé wines becomes more vivid. Since plant proteins have a limited capacity and cannot remove bitter-tasting polyphenols, combinations with other treatment agents (PVPP, bentonite, silicates) can provide a good solution for clarifying treatments [27]. Pea protein or patatin can be an excellent alternative to the highly effective PVPP [28]. The efficiency of patatin is similar to that of gelatine and exceeds that of egg albumin and casein. Proteins from legumes (soy, lentils, peas), wheat gluten, and corn protein have also been tested. These are effective in removing tannins, although the exact impact depends on the protein composition and dose [29] or even on the chemical structure (e.g., modifications with hydrolysis) of the plant protein molecules [30].

In current studies, various plant-based juice clarification agents were tested during the base wine preparation for Etyek sparkling wines. The main goal was to analyse the change in the phenolic composition crucial for sensory evaluation, and the effect of the agents on the yeast assimilable nitrogen content was also investigated.

## 2. Materials and Methods

This paper presents the results of grape must clarification before fermentation, with the addition of four commercial plant-protein-containing clarification agents. The experiments were conducted on Chardonnay grape musts of organic production [15] in the Etyek–Buda Wine Region (NW-Hungary) in four consecutive vintages from 2020 to 2023.

### *2.1. Grape Processing and Vinification*

The harvested grape material was processed at the Anonym Winery, Etyek/Hungary, which specialises in sparkling wine production. The grapes were pressed in a pneumatic wine press, applying a maximum pressure of 1.2 bar, achieving 65% extraction of the grape juice. The grape juice was divided into 5 portions and poured into 5 stainless steel sedimentation tanks. The juice portions were settled at 16 °C using one of four commercial clarifying agents in each, and one part was left settling without any treatment agents. After 12 h, the cleaned musts were racked from the sediment into fermentation tanks. At this point, samples were collected, and a chemical analysis of the grape juice batches was conducted.

The fermentation was conducted at 16 °C, using a commercial yeast product for organic wine production, purchased from Erbslöh Austria GmbH (Siegendorf, Austria). After fermentation, the wines were racked from the coarse lees, and samples were collected for analysis.

The effectiveness of a treatment agent largely depends on the mechanism of action of its components. The products used in the present investigation contained pea protein, PVPP, chitin-glucan, microcrystalline cellulose, and silicates (bentonite) as clarifying ingredients. Pea protein can easily bind phenolic components with a harsh, green, unripe taste which also oxidise rapidly and give wines a brown colour. The PVPP binds most bitter substances (catechins) and green notes; in addition, it reduces the risk of oxidation.

Cellulose and silicate remove phenolic compounds based on their adsorptive effect. Chitin-glucan, a polysaccharide, improves the must clarification efficiency and binds non-specific polyphenols [31–34]. Table 1 summarises the plant-protein treatment agents used in the experiments. Dosage was applied according to the manufacturer's recommendations.

**Table 1.** Summary of the clarification agents.

| In Further Discussion Referred to As | Basic Description | Composition | Applied Dose |
|---|---|---|---|
| Rosé | Casein-free plant-based clarification agent for rosé musts/wines [31] | Pea proteins<br>PVPP | 100 g/100 L |
| ChiF | Plant-based clarification, and flotation agent for white, and rosé musts/wines [32] | Pea proteins<br>Chitin-glucan<br>L(+)-tartaric acid<br>Potassium-metabisulfite | 200 mL/100 L |
| Most | A vegan clarification agent for white/rosé grape musts<br>Preventing oxidation and bitterness by the removal of phenolic compounds [33] | Microcrystalline cellulose<br>Pea proteins<br>PVPP<br>Bentonite | 100 g/100 L |
| Orig | A plant-based clarification agent for white/rosé musts, and white/rosé/red wines with specific flocculation properties, for the absorption of browning phenolics [34] | Pea proteins | 50 g/100 L |

### *2.2. Chemical Analysis Methods*

Measurements included the determination of reducing sugar content, acidity, pH, total polyphenolics (TP; all kinds of phenolic components, which form a blue-coloured complex with the Folin–Ciocalteu reagent), catechins, leucoanthocyanins, and yeast assimilable nitrogen content (YAN; immediately absorbable inorganic (ammonium) and organic (mainly amino acids) nitrogenous components). YAN was measured to monitor the nutrient supply for the yeasts, as one requirement for clarification agents is that these do not affect the YAN levels of the musts.

Reducing sugar content was measured by the Rebelein method [35]. Acidity was determined by titrimetry [36] (pp. 433–435), and pH values were determined by combined electrodes [36] (pp. 491–493).

TP was determined according to the Folin–Ciocalteu method with some modifications, as results were calculated based on a calibration with gallic acid. Results are, therefore, expressed in mg gallic acid per litre [36] (pp. 119–120). Leucoanthocyanin content was determined after heating with hydrochloric acid by spectrophotometry at 550 nm, according to Flanzy's method [37]. The results are expressed in mg malvidine-3,5-diglucoside per litre. Catechin content was determined by spectrophotometry at 500 nm after a colour reaction with vanillin in a sulfuric acid–ethanol medium, according to Rebelein [38].

YAN levels were determined by spectrophotometry, with results expressed in mg valine per litre, using ninhydrin reagent (50 mL reagent containing 1.5 g ninhydrin, 1.25 mL glacial acetic acid, and 6.3 g Na-acetate $\times$ $3H_2O$ diluted in 2-methoxy ethanol): 1 mL reagent was added to 0.5 mL of a 10-times diluted grape juice (dilution with distilled water) in a screw test tube. The sealed test tube was placed in boiling water (100 °C) for 15 min. After cooling, 5 mL of a 1:1 dilution of water and isopropanol was added, and the absorbance was measured at 570 nm.

All measurements were conducted at the Hungarian University of Agriculture and Life Sciences, Department of Oenology, Budapest. Spectrophotometry was performed using a Dynamica Halo RB-10 (Precisa Gravimetrics AG, Dietikon, Switzerland) UV-VIS spectrophotometer.

## 3. Results and Discussion

### 3.1. Overview of the Vintages

The grape material in the four vintages had distinct characteristics reflecting the unique features of each vintage. In 2020, the winter and spring were mild with low precipitation. Starting in May, the drought was followed by a balanced vegetation period. The summer weather was free from extremes, without prolonged heat waves. The harvest began at the beginning of September, at the usual harvest time. The musts were generally extremely concentrated, with high acidity, high extract content, high sugar content, and normal polyphenol content. The grapes for the experiment were harvested in technological ripeness for a sparkling wine cuvée.

Due to the dry and cold spring, flowering was also delayed in the vintage 2021. The harvest date was also postponed, with the harvests starting in the first decade of September. A slow ripening process could be observed: the sugar content increased rapidly, but the acidity decreased slowly. By mid-September, all varieties were suddenly ripe. For our experimental material, an ordinary sugar content could be achieved with an outstanding acidity, and the nitrogen source seemed to be available in sufficient quantities for the fermentation.

The year 2022 was an extremely drought-prone vintage. At the end of August, the grapes were still characterised by a sluggish sugar accumulation. Some varieties had parameters typical of forced ripening: high acidity and moderate sugar content. In most varieties, the acidity was decomposed quickly by mid-September. The polyphenol content of the grape musts was outstanding, causing intense browning processes. These circumstances resulted in rapidly maturing wines.

The character of the 2023 vintage was the opposite of 2022. The vegetation period was particularly rich in precipitation, causing many plant protection issues: unprecedented downy mildew pressure, followed by powdery mildew. The Chardonnay plantation was defoliated in early August to enhance the effectiveness of plant protection treatments permitted in organic cultivation. The harvest was carried out in the first days of September, with slightly thinner musts and a lower sugar content than in previous years. Chardonnay contained somewhat higher phenolic content than usual due to defoliation; however, the nitrogen source was expected to be insufficient (Table 2).

**Table 2.** Basic composition of organic Chardonnay grape juices in four vintages.

| Vintage | Harvest Date | Reducing Sugars (g/L) | Titratable Acidity (g/L) | pH | YAN (mg/L) |
|---|---|---|---|---|---|
| 2020 | 3 September | 148 | 9.6 | 3.10 | 1036 |
| 2021 | 30 August | 193 | 11.8 | 3.05 | 2176 |
| 2022 | 29 August | 267 | 8.4 | 3.15 | 1587 |
| 2023 | 30 August | 152 | 8.4 | 3.14 | 644 |

The quantity of phenolic substances varied significantly between the different vintages. The data confirm that as the grape reaches full maturity, the concentration of phenolic components in the juice also increases, paralleling the phenolic composition and the physiological ripeness (Table 3). The leucoanthocyanin content of the 2020 grape juice was exceptionally high, although its sugar content remained relatively low.

**Table 3.** Polyphenolic composition of organic Chardonnay grape juices in four vintages.

| Vintage | Total Polyphenolics (mg/L) | Total Catechins (mg/L) | Leucoanthocyanins (mg/L) |
|---|---|---|---|
| 2020 | 209 | 289 | 1015 |
| 2021 | 252 | 478 | 682 |
| 2022 | 842 | 983 | 1156 |
| 2023 | 492 | 386 | 563 |

### *3.2. Effect of the Clarification Treatments*

It is challenging to compare the effectiveness of the treatment agents because the initial grape composition varies greatly depending on the vintage. An obvious solution is comparing the ratio of the removed amounts of the phenolic compounds (Table 4) and the YAN (discussed later, see Table 5).

**Table 4.** The ratio of removed phenolic components in Chardonnay grape juices during clarification before fermentation with plant-based fining agents in four consecutive vintages. Data were calculated as the difference between non- and after-treatment concentrations, expressed as percentages (%).

| Rosé | | | | ChiF | | | | Most | | | | Orig | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2020 | 2021 | 2022 | 2023 | 2020 | 2021 | 2022 | 2023 | 2020 | 2021 | 2022 | 2023 | 2020 | 2021 | 2022 | 2023 |
| Removed total polyphenolics (%): | | | | | | | | | | | | | | | |
| 31.6 | 4.0 | 36.3 | 16.5 | 3.3 | 0 | 13.2 | 14.0 | 28.2 | 0.8 | 27.3 | 6.1 | 54.5 | 2.0 | 22.0 | 7.7 |
| Removed catechins (%): | | | | | | | | | | | | | | | |
| 35.6 | 0 | 54.0 | 15.3 | 43.6 | 1.5 | 5.3 | 14.0 | 45.0 | 2.3 | 47.5 | 10.4 | 54.7 | 3.3 | 35.5 | 8.5 |
| Removed leucoanthocyanins (%): | | | | | | | | | | | | | | | |
| 29.7 | 0 | 29.5 | 9.1 | 36.7 | 0 | 0 | 14.6 | 59.5 | 0.4 | 46.7 | 10.5 | 57.2 | 1.2 | 13.8 | 6.4 |

**Table 5.** Clarification effect of plant protein-based agents on the YAN levels in organic Chardonnay grape musts in four consecutive vintages. YAN levels are expressed in mg valin per litre. Removed YAN values were calculated as the difference between non- and after-treatment grape musts, expressed as percentages.

| Vintage | Initial YAN (mg/L) | YAN Removed Before Fermentation | | YAN in Wines (mg/L) |
|---|---|---|---|---|
| | | by Agent | (%) | |
| 2020 | 1036 | Rosé | 0 | 639 |
| | | ChiF | 0 | 612 |
| | | Most | 9.4 | 708 |
| | | Orig | 0 | 554 |
| 2021 | 2176 | Rosé | 7.5 | 963 |
| | | ChiF | 0 | 941 |
| | | Most | 2.3 | 1012 |
| | | Orig | 4.1 | 1065 |
| 2022 | 1587 | Rosé | 0.5 | 1190 |
| | | ChiF | 0 | 1246 |
| | | Most | 4.6 | 1283 |
| | | Orig | 3.7 | 1182 |
| 2023 | 644 | Rosé | 9.6 | 307 |
| | | ChiF | 4.2 | 286 |
| | | Most | 7.1 | 263 |
| | | Orig | 2.5 | 304 |

The vintage characteristics should be considered when analysing the effectiveness of treatment materials.

#### 3.2.1. Change of the Phenolic Composition

When phenolic substances are present in higher concentrations, their removed proportion is also greater during the clarification process (Figure 1).

Depending on the composition of the treatment agents, some specificity in the reduction of the tannin compounds can be observed. Although no statistically significant differences can be observed due to the large standard deviation, important trends emerge from a technological point of view. In the 2021 vintage, pre-fermentation clarification treatments with all tested agents have had an insufficient effect. In this vintage, we observed the highest acid content (11.8 g/L), with a pH value of around 3.0. Possibly, this outstanding acid content hindered the effectiveness of grape juice cleaning. Nevertheless, during fermentation, a satisfactory decrease in the phenolic content could be observed in the experimental wines.

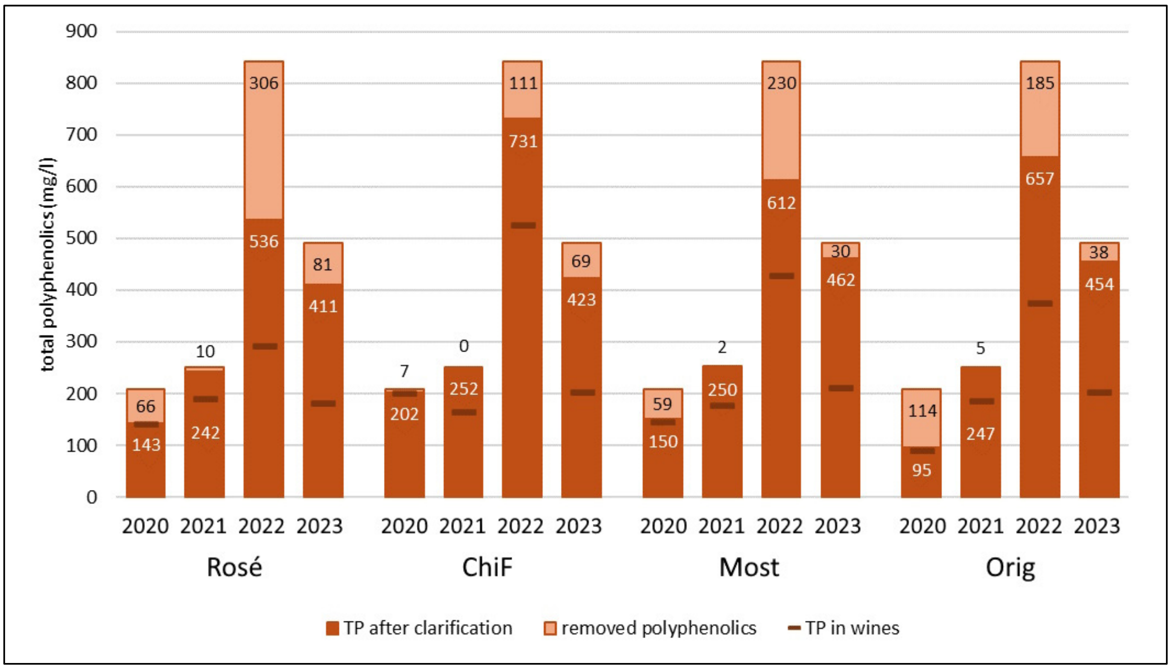


**Figure 1.** Total polyphenolic content of clarified grape juices and wines in four vintages, clarified with different plant protein agents before fermentation. (Deep-coloured sections with white digits show TP in the clarified grape juices; light-coloured sections with black digits indicate the removed phenolic content (calculated data); full-height columns show the initial TP of grape juices; dark notch markers indicate TP in the wines after fermentation. Data are expressed in mg gallic acid per litre).

"Rosé" (pea protein and PVPP) and "Most" (pea protein, PVPP, bentonite, and cellulose) had a similar effect in reducing the TP. They were highly effective in 2022, when the grape material had reached both physiologic and phenolic ripeness with a high polyphenol content. When less ripe grapes were harvested for sparkling wine in 2020, both cleaning agents proved similarly effective in removing around 30% of the phenolic content before the fermentation.

In the "ChiF" samples (a combined flotation agent containing pea protein, chitin-glucan, tartaric acid, and potassium hydrogen sulphite), the treatment's overall effectiveness tended to be lower. Its effect on TP was outstanding only in the well-ripened 2023 vintage, performing similarly to the "Rosé". Results were similar in 2022, with other clarification agents being more effective that year.

The efficacy of the "Orig" (pure pea protein) in the 2020 grape juice was outstanding by removing more than half of the TP. This is the highest TP value in the whole series of experiments. In addition, the treatment gave an appreciable result in the 2022 vintage, but its effect in the 2021 and 2023 vintages was negligible in terms of the TP.

In some cases, the treatment agents were particularly effective in reducing catechin (Figure 2). Removing the catechin content of the 2022 grape juice was the easiest—similarly to TP—and a similar performance was achieved in 2020 as well. The most striking result is the low efficiency of "ChiF" in 2022. The catechin content of the 2021 vintage grape juice was generally difficult to remove with any of the tested agents.

In the 2023 vintage, approximately 10–15% of catechins were removed during juice clarification, with no statistically significant differences.

The removal of leucoanthocyanins more or less followed the pattern observed for catechins (Figure 3). This suggests that the clarification agents are almost equally specific for the two types of flavonoids, namely catechins and leucoanthocyanins.

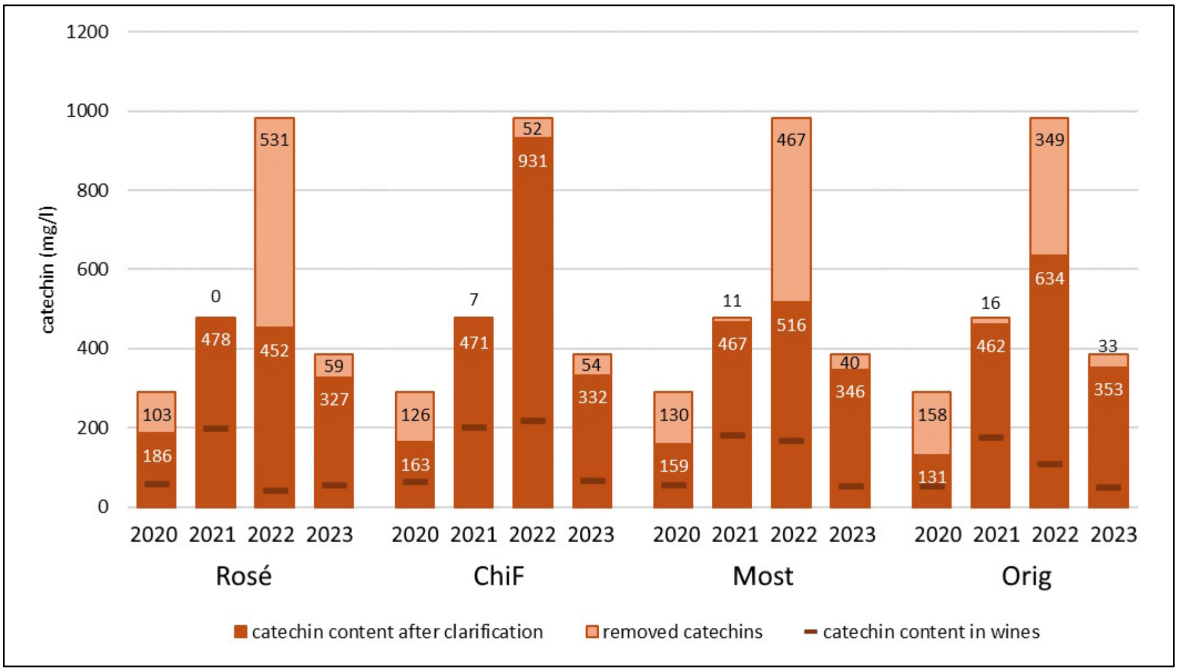


**Figure 2.** Catechin content of clarified grape juices and wines in four vintages, clarified with different plant protein agents before fermentation. (Deep-coloured sections with white digits show the catechin content of the clarified grape juices; light-coloured sections with black digits indicate the removed catechins (calculated data); full-height columns show the initial catechin content of the grape juices; dark notch markers indicate the catechin content of the wines after fermentation. Data are expressed in mg catechin per litre).

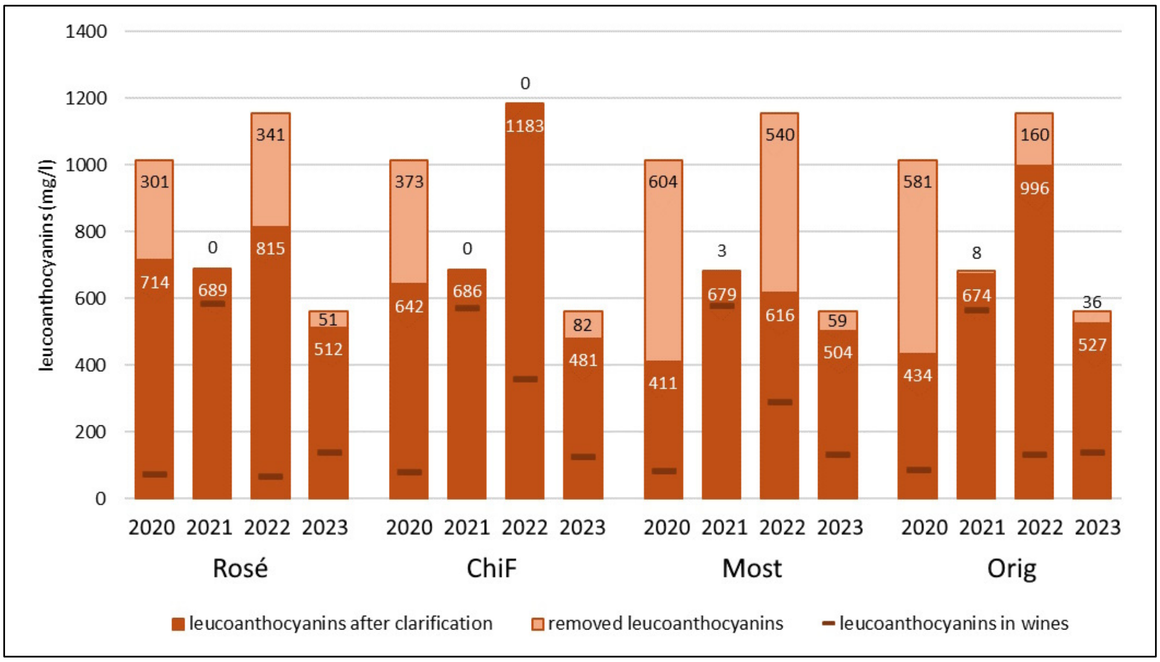


**Figure 3.** Leucoanthocyanin content of clarified grape juices and wines in four vintages, clarified with different plant protein agents before fermentation. (Deep-coloured sections with white digits show the leucoanthocyanin content of the clarified grape juices; light-coloured sections with black digits indicate the removed leucoanthocyanins (calculated data); full-height columns show the initial leucoanthocyanin content of the grape juices; dark notch markers indicate the leucoanthocyanin content of the wines after fermentation. Data are expressed in mg malvidine-3,5-diglucoside per litre).

The lowest overall efficiency was shown by the "ChiF" treatment. Apart from the difficult-to-clarify 2021 vintage, it did not remove leucoanthocyanins from the 2022 grape juice with phenolic ripeness. We have to mention that the leucoanthocyanin content was the highest in these two years. "ChiF" performed a measurable reduction of the leucoanthocyanins only in 2020, but the other agents also turned out to be effective in that year. The agents with complex ingredients, "Rosé" and "Most", performed similarly, perhaps with a slightly greater clarifying efficacy in the case of the latter. Achievements with the "Orig" (pure pea protein) were similar, although, in the well-ripened 2022 vintage, it lagged behind them in efficiency.

#### 3.2.2. Change in the YAN-Levels

Grape juice treatments based on plant protein did not significantly affect the nitrogen source (Table 5). Considering the entire duration of the study (four vintages), neither of the four treatment agents caused a higher than 10% decrease in nitrogen content. The tests were carried out before inoculation with a commercial starter and the related nutrient dosing, so the results only refer to the effect on the natural YAN levels of the grape juices.

## 4. Conclusions

The effect and performance of the plant protein-based grape juice treatment agents depend on several factors. Based on present investigations, influencing factors can be, without claiming to be complete, (1) the characteristics of the vintage, which fundamentally influence the composition and behaviour of the grapes (e.g., spontaneous settlement, cleanability); (2) the analytical composition of the must, including the absolute concentration and the relative ratio of phenolic compounds; (3) the physiological and phenolic ripeness of the grapes; and (4) the composition of grape juice clarifying agents, and possible combinations. Some of these factors are related to, or derived from, each other or can be traced back to the same physical, chemical, and biological causes.

The results of the present investigations can be summarised as follows:

- The clarification of juices from fully ripe grapes is easier, practically any must-treatment agent can be effective.
- Grape juices with a higher acidity and a low pH can become difficult to clarify, or the process may even fail. In this case, the role of incomplete ripeness of the fruit also arises, reducing efficiency.
- Although pure pea protein preparations can work sufficiently, the efficacy and effectiveness increase when other types of clarification agents are used in combination, e.g., PVPP greatly facilitates the removal of phenolic compounds.
- The pea protein preparation containing chitin-glucan, L(+)-tartaric acid, and potassium-metabisulfite in combination showed lower efficiency in gravity-settling grape juice clarification in recent experiments. According to the manufacturer's recommendations, this agent is more suitable and therefore recommended for flotation.
- The effect of plant protein-based clarification agents is insignificant on the YAN source.

The clarifying effect of plant protein-based preparations can be detected in many analytical parameters of grape juice, especially phenolic components. Proteins of plant origin may have an effective impact even at the first steps of wine technology. As a result of the treatments, the changes (decreasing) in the polyphenol content of the grape juice can improve both the sensory properties and the chemical stability of future wines, which can also be consumed by those following a vegan diet.

Due to the characteristics of the Chardonnay variety, as well as the difference between vintages, further experiments are considered. Many factors affect the phenolic and YAN composition and their behaviour in grape juice and wines. Plant-based fining agents are also likely to play a major role in the natural and organic wine segment, so more studies in this field may be justified. Since manufacturing such agents is more acceptable from various sustainability points of view than those of animal origin (e.g., gelatin, casein), they can be more easily integrated into the market segment that follows the philosophy and ethical issues involved.

While the studies need to be expanded to other—both autochthonous and international—white wine grape varieties, it will also be important to study the fining effect in wine products made from red grapes—rosé musts, blanc de noir sparkling wines, etc.—especially in terms of their interaction with anthocyanins and other pigments.

**Author Contributions:** Conceptualization, Á.P.S. and D.Á.N.S.; methodology, Á.P.S., A.S.-L. and D.Á.N.S.; investigation, Á.P.S. and D.Á.N.S.; resources, Á.P.S. and B.N.; writing—original draft preparation, Á.P.S., A.S.-L. and A.S.; writing—review and editing, A.S.-L. and Á.P.S.; visualization, A.S.-L. and A.S.; supervision, Z.V. and D.Á.N.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is available upon request to the corresponding author.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

1. Peri, C.; Pompei, C. An Assay of Different Phenolic Fractions in Wines. *Am. J. Enol. Vitic.* **1971**, *22*, 55–58. [CrossRef]
2. Cravero, M.C. Organic and Biodynamic Wines Quality and Characteristics: A review. *Food Chem.* **2019**, *295*, 334–340. [CrossRef]
3. Puig-Deu, M.; López-Tamames, E.; Buxaderas, S.; Torre-Boronat, M.C. Quality of Base and Sparkling Wines as Influenced by the Type of Fining Agent Added Pre-Fermentation. *Food Chem.* **1999**, *66*, 35–42. [CrossRef]
4. Etyeki Pezsgő PDO Product Specification. Etyeki Pezsgő Termékleírása. Available online: https://boraszat.kormany.hu/download/6/fa/d2000/ETYEKI%20PEZSG%C5%90%20term%C3%A9kle%C3%ADr%C3%A1sa.pdf (accessed on 4 December 2024).
5. Statistical Data, National Council of the Wine Communities, Hungary. Etyek-Budai-Borvidek. Available online: https://www.hnt.hu/wp-content/uploads/2024/10/Etyek-Budai-Borvidek.pdf (accessed on 4 December 2024).
6. Rocchetti, G.; Gatti, M.; Bavaresco, L.; Lucini, L. Untargeted Metabolomics to Investigate the Phenolic Composition of Chardonnay Wines from Different Origins. *J. Food Comp. Anal.* **2018**, *71*, 87–93. [CrossRef]
7. Somers, T.C.; Pocock, K.F. Phenolic Assessment of White Musts: Varietal Differences in Free-Run Juices and Pressings. *Vitis* **1991**, *30*, 189–201.
8. Merkytė, V.; Longo, E.; Windisch, G.; Boselli, E. Phenolic Compounds as Markers of Wine Quality and Authenticity. *Foods* **2020**, *9*, 1785. [CrossRef]
9. Szövényi, Á.; Steckl, S.; Varga, Z. Növényi Fehérje Alapú, Vegán Musttisztító Anyagok Alkalmazása Chardonnay és Pinot Noir Rozé Mustokon. *Borászati Füzetek* **2021**, *31*, 33–36.
10. Szövényi, Á.; Simon, D.; Sólyom-Leskó, A.; Nyitrainé Sárdy, D.Á. Erjedés Során Alkalmazott Bentonit Fehérjemegkötő Hatásának Vizsgálata Magas Fehérjetartalmú Fehér Mustok Esetén. *Borászati Füzetek* **2022**, *32*, 38–41.
11. Szövényi, Á. Borderítés a 2023-as Évjáratban, de Hogyan? *Agrofórum* **2023**, *34*, 20–23.
12. Ribéreau Gayon, P.; Glories, Y.; Dubourdieu, D.; Maujean, A. *Handbook of Enology, Volume 2: The Chemistry of Wine Stabilization and Treatments*, 3rd ed.; Wiley: Chichester, UK, 2021; pp. 389–395.
13. Wadhave, A.A.; Jadhav, A.I.; Arsul, V.A. Plant Proteins Applications: A Review. *World J. Pharm. Pharm. Sci.* **2014**, *3*, 702–712.
14. Mierczynska-Vasilev, A.; Smith, P.A. Knowledge and Challenges in Wine Clarification. *Aust. J. Grape Wine Res.* **2015**, *21*, 615–626. [CrossRef]
15. Consolidated TEXT: 32018R0848—EN—21.02.2023. Regulation (EU) 2018/848 of the European Parliament and of the Council. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02018R0848-20230221 (accessed on 14 July 2024).
16. Maury, C.; Sarni-Manchado, P.; Lefebvre, S.; Cheynier, V.; Moutounet, M. Influence of Fining with Plant Proteins on Proanthocyanidin Composition of Red Wines. *Am. J. Enol. Vitic.* **2003**, *54*, 105–111. [CrossRef]
17. Marangon, M.; Vincenzi, S.; Curioni, A. Wine Fining with Plant Proteins. *Molecules* **2019**, *24*, 2186. [CrossRef]
18. Kemp, B.; Marangon, M.; Curioni, A.; Waters, E.; Marchal, R. 8—New Directions in Stabilization, Clarification, and Fining. In *Managing Wine Quality*, 2nd ed.; Reynolds, A.G., Ed.; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing: Cambridge, UK, 2022; pp. 245–301. [CrossRef]

19. Kirschner, S.; Belloni, B.; Kugler, C.; Ring, J.; Brockow, K. Allergenicity of Wine Containing Processing Aids: A Double-Blind, Placebo-Controlled Food Challenge. *J. Investig. Allergol. Clin. Immunol.* **2009**, *19*, 210–217.
20. Penas, E.; Di Lorenzo, C.; Uberti, F.; Restani, P. Allergenic Proteins in Enology: A Review on Technological Applications and Safety Aspects. *Molecules* **2015**, *20*, 13144–13164. [CrossRef]
21. Bongiorno, D.; Avellone, G.; Napoli, A.; Mazzotti, F.; Piazzese, D.; Censi, V.; Indelicato, S. Determination of Trace Levels of Organic Fining Agents in Wines: Latest and Relevant Findings. *Front. Chem.* **2022**, *10*, 944021. [CrossRef] [PubMed]
22. Vegan Statistics | Veganism Around the World. Worldwide Growth of Veganism. Available online: https://www.vegansociety.com/news/media/statistics/worldwide (accessed on 14 July 2024).
23. Matieres Proteiques Vegetales. Resolution Oeno 28/2004. Codex–Protein Plant Origin. Available online: https://www.oiv.int/public/medias/653/oeno-28-2004-en.pdf (accessed on 14 July 2024).
24. Consolidated TEXT: 32019R0934—EN—08.02.2022. Commission Delegated Regulation (EU) 2019/934. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02019R0934-20220208 (accessed on 14 July 2024).
25. Sobe, M.; Bausinger, S. Vegane Mostvorklärung. *Dtsch. Weinmag.* **2020**, *15*, 34–35.
26. Granato, T.M.; Nasi, A.; Ferranti, P.; Iametti, S.; Bonomi, F. Fining White Wine with Plant Proteins: Effects of Fining on Proanthocyanidins and Aroma Components. *Eur. Food Res. Technol.* **2014**, *238*, 265–274. [CrossRef]
27. Meinl, J. Einsatz von Pflanzenproteinen. *Dtsch. Weinmag.* **2010**, *4*, 32–33.
28. Cojocaru, G.A.; Antoce, A.O. Use of Vegetable Proteins as Alternatives to PVPP and Caseinate for Removing Polyphenols from White Grape Musts. *Sci. Pap. Ser. B Hortic.* **2022**, *66*, 237–244.
29. Smith, P.A.; McRae, J.M.; Bindon, K.A. Impact of Winemaking Practices on Tannins. *Aust. J. Grape Wine Res.* **2015**, *21*, 601–614. [CrossRef]
30. Tschiersch, C.; Nikfardjam, M.P.; Schmidt, O.; Schwack, W. Degree of Hydrolysis of Some Vegetable Proteins Used as Fining Agents and its Influence on Polyphenol Removal from Red Wine. *Eur. Food Res. Technol.* **2010**, *231*, 65–74. [CrossRef]
31. LittoFresh Rosé—Erbslöh Geisenheim. Pflanzliches Kombinationsprodukt zur Rosébereitung. Available online: https://erbsloeh.com/deutsch/produkt/littofresh-rose/ (accessed on 14 July 2024).
32. LittoFresh ChitoFlot—Erbslöh Geisenheim. Veganes Kombiprodukt Besonders zur Flotation. Available online: https://erbsloeh.com/deutsch/produkt/littofresh-chito-flot/ (accessed on 14 July 2024).
33. LittoFresh Most—Erbslöh Geisenheim. Vegane Prävention von Oxidationsnoten und Bitterkeit. Available online: https://erbsloeh.com/deutsch/produkt/littofresh-most/ (accessed on 14 July 2024).
34. LittoFresh Origin—Erbslöh Geisenheim. Erbsenprotein zur Klärung, Stabilisation und Schönung. Available online: https://erbsloeh.com/deutsch/produkt/littofresh-origin/ (accessed on 14 July 2024).
35. *MSZ 9479:1980*; Borok Cukortartalmának Meghatározása Rebelein Módszerrel. Hungarian Standard: Budapest, Hungary, 1980.
36. Compendium Vol1_EN.pdf. Compendium of International Methods of Wine and Must Analysis. Available online: https://www.oiv.int/public/medias/7907/oiv-vol1-compendium-of-international-methods-of-analysis.pdf (accessed on 14 July 2024).
37. Flanzy, M.; Aubert, S.; Marinos, M. New Technique for Determination of Leucoanthocyanic Tannins. *Ann. Technol. Agric.* **1969**, *18*, 327–328.
38. Rebelein, H. Beitrag zur Bestimmung des Catechingehaltes in Wein. *Dtsch. Lebensm. Rundschau.* **1965**, *61*, 182–183.