



Hungarian University of Agriculture and Life Sciences

The effect of plant-based clarifying agents on the quality  
of musts and wines from organic production

Theses of the doctoral thesis

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## 1. INTRODUCTION AND AIMS OF THE STUDY

At the end of the 1990s, food production practices had to be reviewed due to bovine spongiform encephalopathy (BSE). This also affected the wine industry, as some of the fining agents were of animal origin. Many fining agents were based on protein extracted from cattle, the use of which was suspended as a precautionary measure. For this reason, the protein most commonly used for wine clarification today is pork gelatine, which binds high molecular weight tannins from the wine (Maury et al., 2001; Sarni-Manchado et al., 1999; Smith et al., 2015a). Due to various epidemics affecting farm animals, there is a growing demand for wines produced without animal-derived fining agents. This market trend is further reinforced by the growing demand for vegan and "natural" or "unprocessed" wines. An important area of research in wine fining agents is the evaluation of the fining effect of proteins extracted from plant materials (e.g., potatoes, legumes). Based on the above, it can be said that in the future, we should give preference to plant-based alternatives to the animal-derived fining agents traditionally used in wine fining. For this reason, my research focused on examining the effects of pea protein-based fining agents on groups of compounds that are also important from a physiological point of view. My research is also exploratory in nature, as the literature on so-called vegan wines treated with pea protein-based fining agents is extremely limited, and the relevance of these fining agents needs to be investigated.

1. I sought to answer the question as to what extent and how pea protein-based fining agents alter the polyphenol composition of musts and wines. I also sought to answer the question whether there are significant differences between individual plant-based fining agents in terms of

polyphenol composition: total polyphenol content, total antioxidant capacity (TAC), leucoanthocyanin content, catechin concentration, yeast assimilable nitrogen content (YAN).

When examining the polyphenol composition, I specifically examined the effect of plant-based clarifying agents on the polyphenol composition compared to untreated control samples.

2. As part of the fine analytical tests, I also studied nitrogen-containing compounds and their possible changes. My tests focused on determining the assimilable nitrogen content and proline concentration. I sought to answer the question as to what extent and how pea protein-based clarifying agents affect the assimilable nitrogen content. During the experiments, I paid particular attention to studying the extent of change in the assimilable nitrogen content (YAN) compared to the control sample.
3. Among physiologically active compounds, biogenic amines found in wines play a decisive role in oenological analytical research. In my experiments, I examined the biogenic amine composition when using certain plant-based clarifying agents. I investigated whether there was a significant difference between the control sample and the samples treated with the agents. I also examined whether pea protein-based treatments cause differences in biogenic amine concentration and composition compared to each other. My investigations focused on the three most important biogenic amines with physiological effects: histamine, serotonin, and tyramine.

4. I examined the differences between vintages in terms of the extent to which and how the vintage influences the fine analytical composition of musts and wines under the influence of pea protein-based treatments. I sought to determine whether nitrogen-containing components were influenced by the vintage effect and, if so, how.
5. During the statistical evaluation, I examined whether the analytical parameters of the wines could indicate whether they originated from a dry, drought-stricken vintage (immediately available nitrogen, polyphenols, biogenic amines). Does the vintage influence the development of nitrogen content?
6. Based on the effect of pea protein-based must treatment agents on analytical values, is it possible to determine which treatment agent can be used to produce stable, high-quality wine?

## 2. MATERIALS AND METHODS

I examined the effect of pea protein-based treatments on musts made from organically grown Chardonnay and Pinot noir grapes and on wines fermented from them under standardized conditions in the 2020, 2021, 2022, and 2023 vintages. The must treatments and fermentations took place at the Anonym Winery in Etyek. The grapes were processed by destemming and crushing. I treated the musts in 5-liter glass demijohns with the following pea protein-based fining agents at the concentrations specified by the manufacturer.

- CH 0 – control, untreated
- CH 1 – treated with LittoFresh Rosé at a dose of 100 g/hl
- CH 2 – treated with LittoFresh ChitoFlot at a dose of 200 ml/hl
- CH 3 – treated with LittoFresh Most at a dose of 100 g/hl
- CH 4 – treated with LittoFresh Origin at a dose of 50 g/hl
- PN 0 – control, untreated
- PN 1 – treated with LittoFresh Rosé at a dose of 100 g/hl
- PN 2 – treated with LittoFresh ChitoFlot at a dose of 200 ml/hl
- PN 3 – treated with 100 g/hl of LittoFresh Most
- PN 4 – treated with 50 g/hl of LittoFresh Origin

I set up pea protein-based treatments in each experimental year using the above sample names, in three parallel samples. To observe the effectiveness of the treatments, I also set up control, untreated must samples for both grape varieties.

I carried out sedimentation for 12 hours, then fermented the musts under standardized conditions. After fermentation, I racked the sample wines from the coarse lees and applied a basic dosage of 50 mg/l to the new wines.

## **2.1. Determination of polyphenols**

- 2.1.1. Total polyphenol determination using Folin-Ciocalteu reagent, calibrated to gallic acid (OIV-MA-AS2-10)
- 2.1.2. Catechin content, at 500 nm, by spectrophotometry (Rebelein, H., 1965)
- 2.1.3. Leucoanthocyanin content (Flanzy, M. et al., 1969), based on a modified method
- 2.1.4. Anthocyanin content at 550 nm using a spectrophotometer (Flanzy, M. et al., 1969), based on a modified method

## **2.2. Determination of nitrogen-containing compounds**

- 2.2.1. YAN, determination of immediately available nitrogen content (Kállay M. & Nyitrainé Sárdy D., 2006)
- 2.2.2. Determination of proline concentration using a spectrophotometric method (Kállay M. & Leskó A., 2007)

## **2.3. Determination of biogenic amines using HPLC**

Among the biogenic amines, I examined the amounts of histamine, serotonin, and tyramine, which have important physiological effects.

### **3. RESULTS AND DISCUSSION**

I evaluated the results separately for musts and wines aged under standardized conditions. I examined whether pea protein-based treatments had a significant effect in different vintages and whether the vintage had a significant effect on the parameter under investigation. I also examined whether the treatment x vintage interaction was significant.

#### **3.1. Evaluation of the results of the musts**

##### **3.1.1. Evaluation of polyphenol results**

The total polyphenol values of the 2022 vintage Chardonnay musts stand out among the values of the vintages examined. Based on the results, it can be stated that must treatment has a significant effect on total polyphenol content. It can also be stated that the vintage has a strong influence on the development of total polyphenol values, while the effect of must treatments based on pea protein of different compositions on total polyphenol content does not differ significantly, but the interaction is significant, i.e., the effect of must treatment depends on the vintage. In the case of Pinot noir musts, it can also be said that the total polyphenol values of the 2022 vintage are exceptionally high. Based on the data, it can be stated that the effect of must treatments and vintages is significant on the total polyphenol content, and that the effect of must treatment depends on the vintage. In the case of Chardonnay and Pinot noir musts, the vintage and must treatment have a significant effect on total antioxidant capacity, and the effect of must treatment depends on the vintage. It can be stated that the 2022 vintage showed significantly higher total antioxidant capacity values for both varieties than any other vintage studied. In the case of Chardonnay musts, must treatments and vintage have a

significant effect on catechin content, and the interaction between treatment and vintage is also significant. In the case of Pinot noir musts, must treatments have no significant effect on catechin content, but it can be stated that the vintage strongly influences the catechin content of musts, with the 2022 vintage showing exceptionally high values. In the case of Chardonnay and Pinot noir musts, both must treatments and vintage had a significant effect on leucoanthocyanin content. A significant interaction between vintages and must treatments can be observed. Among the vintages, the 2022 vintage shows significantly higher leucoanthocyanin values for both varieties than the other vintages. Based on the statistical evaluation, it can be stated that the vintage has a strong influence on the anthocyanin content of the musts, while must treatment alone does not have a significant effect on anthocyanin content. From a winemaking technology perspective, this finding is very important, as it can be stated that the colorant content of Pinot noir musts, which is already low, is not affected by pea protein-based fining agents of different compositions.

#### 3.1.2. Evaluation of the results for nitrogen-containing compounds

In the case of Chardonnay and Pinot noir musts, the vintage has a significant effect on the yeast assimilable nitrogen content (YAN), but must treatments have no significant effect on YAN. The vintage has a significant effect on the proline content of both varieties of must, but must treatments have no significant effect.

#### 3.1.3. Evaluation of biogenic amine results

The vintage has a significant effect on the histamine content of Chardonnay musts, but must treatments have no significant effect. Both the vintage and must treatments have a significant effect on the

histamine content of Pinot noir musts. The vintage and must treatments have no significant effect on the serotonin content of Chardonnay musts. In contrast, the vintage and must treatment have a significant effect on the serotonin content of Pinot noir musts. The vintage and must treatments have a significant effect on the tyramine content of Chardonnay musts. The vintage has a significant effect on the tyramine content of Pinot noir musts, but must treatments have no significant effect.

### **3.2. Evaluation of wine results**

#### 3.2.1. Evaluation of polyphenol results

In the case of vegan Chardonnay wine samples, both the vintage and the treatments have a significant effect on the total polyphenol content. In the case of vegan Pinot noir wine samples, the vintage has a significant effect, but the treatments have no significant effect on the total polyphenol content. For vegan Chardonnay and Pinot noir wine samples, both treatments and vintage have a significant effect on total antioxidant concentration (TAC). For vegan Chardonnay and Pinot noir wine samples, both treatments and vintage have a significant effect on catechin content. The leucoanthocyanin content of vegan Chardonnay and Pinot noir wine samples is significantly influenced by both must treatments and vintage. The anthocyanin content of vegan Pinot noir wine samples is significantly influenced by both vintage and treatments.

#### 3.2.2. Evaluation of results for nitrogen-containing compounds

The vintage has a significant effect on YAN content of vegan Chardonnay and Pinot noir wine samples, but the effect of must treatments is not significant. The vintage has a significant effect on

the proline content of vegan Chardonnay and Pinot noir wine samples, but must treatments have no significant effect.

### 3.2.3. Evaluation of biogenic amine results

The vintage has a significant effect on the histamine content of vegan Chardonnay wine samples, but the treatments have no significant effect. The vintage and treatments have a significant effect on the histamine content of vegan Pinot noir wine samples. The vintage and must treatments have a significant effect on the serotonin content of vegan Chardonnay and Pinot noir wine samples. The vintage and must treatments have a significant effect on the tyramine content of vegan Chardonnay wine samples. However, the vintage has a significant effect on the tyramine content of vegan Pinot noir wine samples, but the must treatments do not have a significant effect.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

The use of pea protein-based fining agents, which is the subject of my research, has not yet been incorporated into "everyday" winemaking technology. However, it can be said that vegan wines produced using pea protein-based fining agents can reach a growing consumer base. Therefore, the focus of my research, which is to examine the effects of pea protein-based fining agents on the fine analytical parameters of musts and wines, can help in the practical application of these fining agents. Between 2020 and 2023, I carried out must treatments on Chardonnay and Pinot noir varieties with pea protein-based fining agents to examine their effect on polyphenols, nitrogen-containing compounds, and biogenic amines. Based on my research, I make the following conclusions and recommendations:

- The pea protein-based fining agents used during must treatment showed varying results in terms of polyphenol content in the musts. Of the phenolic components examined, the pea protein-based fining agents were effective in reducing the total polyphenol content in the musts. Lower total polyphenol content is the basis of modern winemaking, as wines produced in this way are less sensitive to oxidation and require less sulfurization. The pea protein-based fining agents examined were able to significantly reduce the amount of catechins across vintages. We know that catechins are prone to oxidation, and modern winemaking technology minimizes the amount of catechins because they cause a bitter taste in wines.

- When using fining agents, it is important to treat the must and wine gently, which in practice means that fining should not have a negative effect on other components. The selectivity of pea protein-based fining agents on nitrogen-containing compounds, tested during must treatment, is demonstrated by the fact that the treatments have no effect on the yeast assimilable nitrogen content. Their use preserves the assimilable nitrogen content of the musts and has no negative effect on the alcoholic fermentation of the musts.
- Pea protein-based fining agents had different effects on the biogenic amine content of the Chardonnay and Pinot noir varieties used in my research. Of the biogenic amines examined, the histamine and serotonin content decreased significantly in Pinot noir musts, while the tyramine and cadaverine content decreased in Chardonnay musts. In other cases, must treatments did not cause significant changes in biogenic amine content.
- The total polyphenol content of vegan wine samples treated with pea protein-based fining agents shows small differences. In the case of vegan Chardonnay wine samples, I demonstrated a significant effect of the treatments. As a result of the treatments, I detected a lower total polyphenol content in all vintages examined compared to the control sample. My data show that the total polyphenol content of vegan Pinot noir wine samples also decreased compared to the control sample, but the decrease is not significant.
- The catechin content of the vegan wine samples was lower than that of the control sample in both test varieties in all of the vintages examined.

- The anthocyanin content of vegan Pinot noir wine samples is significantly lower than that of the control sample in all treatments. In vintages with higher heat sums, especially in the case of rosés, the anthocyanin-reducing effect of fining agents can have a positive impact on the wine profile.
- Among the nitrogen-containing compounds measured in vegan wine samples, the treatments did not cause a significant reduction in immediately available nitrogen content. The gentle clarification properties of the pea protein-based clarifying agents tested during must treatment were also evident in the wine samples.
- The biogenic amine content of vegan wine samples showed similar variation to that of musts. In the case of vegan Chardonnay wine samples, the treatments had a significant effect on serotonin and tyramine content. In the case of vegan Pinot noir wine samples, the treatments had a significant effect on histamine and serotonin content.

Due to their selective polyphenol-reducing effect, pea protein-based fining agents can be used effectively in the technology of fruity white and rosé wines. Their use is recommended in must, as their gentle fining effect does not reduce the assimilable nitrogen content of the must. Due to their effect on biogenic amines, they can be effective supplements to traditionally used bentonite fining agents. The use of pea protein-based fining agents in must certainly has an effect on the fine analytical components of the finished wine, but these are only slightly affected.

## 5. NEW SCIENTIFIC RESULTS

1. I was the first to examine the effect of pea protein-based fining agents of different compositions on the fine analytical composition of wines in Hungary. In my scientific work, I examined how pea protein-based fining agents of different compositions change the polyphenol composition of musts and wines. I examined whether there is a significant difference between pea protein-based fining agents with different compositions in terms of total polyphenol content, leucoanthocyanin content, catechin concentration, and TAC value. *I was the first to establish that pea protein-based fining agents with different compositions reduce:*

*the polyphenol composition of musts: total polyphenol content by 55%, maximum leucoanthocyanin content by 60%, maximum catechin concentration by 55%, maximum TAC value by 55%.*

*the polyphenol composition of wines: total polyphenol content by a maximum of 51%, leucoanthocyanin content by a maximum of 84%, catechin concentration by a maximum of 84%, TAC value by a maximum of 51%.*

2. First, I examined the assimilable nitrogen content and proline concentration of musts and wines from the perspective of how and to what extent pea protein-based fining agents of different compositions affect the assimilable nitrogen content and proline concentration. *I found that the yeast assimilable nitrogen content of musts did not change significantly as a result of the treatments, and I also found that pea protein-based fining agents of different compositions do not affect the*

*assimilable nitrogen content of wines. I found that there was no significant difference in the proline concentration of the musts as a result of the treatments. Furthermore, I found that there was no significant difference in the proline concentration of wines treated with pea protein-based fining agents of different compositions. Overall, it can be concluded that the amount of assimilable nitrogen and the proline concentration are not affected by treatments with pea protein-based fining agents of different compositions.*

3. Among the physiologically active compounds, I also examined the changes in the biogenic amine composition of musts and wines during the use of pea protein-based fining agents of different compositions. *I found that the use of pea protein-based fining agents of different compositions resulted in significant differences in the biogenic amine composition of musts, and that the treatments also had a significant effect on the biogenic amine composition of wines. Pea protein-based fining agents with different compositions significantly reduced the histamine concentration, serotonin content, and tyramine content of the musts. The histamine content, serotonin concentration, and tyramine content of the wines were significantly reduced as a result of the treatments. Based on my investigations, it can be concluded that pea protein-based fining agents of different compositions reduce the concentration of histamine, serotonin, and tyramine in musts and wines.*
4. I examined the differences between vintages in terms of the extent to which and how vintage influences the fine analytical composition of musts and wines under the influence of pea

protein-based treatments of varying composition. *I found that treatments based on pea protein of different compositions interact with the influence of the vintage, which significantly affects the fine analytical composition of musts and wines. Based on my results, a clear and significant difference between vintages could be detected in the fine analytical composition of wines, in terms of assimilable nitrogen content and proline concentration. A clear significant difference was found in the assimilable nitrogen content and proline concentration in musts treated with pea protein-based fining agents of different compositions. Furthermore, based on my results, a significant difference was detected between vintages in the assimilable nitrogen content and proline concentration of wines treated with pea protein of different compositions.*

5. I examined whether it is possible to determine, based on the fine chemical composition of musts and wines, whether a must or wine has undergone pea protein-based clarification. *I found that there was no clear significant difference in the fine analytical composition of musts and wines as a result of the treatments, either within vintages or between vintages. Meaning, that based on the polyphenol composition, biogenic amine composition, assimilable nitrogen content, and proline concentration of musts and wines, it cannot be determined whether the must or wine was treated with a pea protein-based fining agent.*
6. I examined the effect of pea protein-based must treatment agents with different compositions on fine analytical values to determine which treatment agent can be used to produce

stable, high-quality wine. *Based on my results, I concluded that the amount of nitrogen-containing compounds was not significantly reduced by the various pea protein-based must treatment agents, meaning that they do not adversely affect alcoholic fermentation or the quality of the resulting wines. Based on my results, I concluded that, in terms of polyphenol composition, the best quality Chardonnay must can be achieved with CH4 and pure pea protein treatment. I concluded that, in terms of polyphenol composition, the best quality Pinot noir must can be achieved with PNI, pea protein and PVPP treatment. Based on my results, I concluded that the biogenic amine content of Chardonnay and Pinot noir musts was significantly reduced by must treatment agents containing other clarifying agents in addition to pea protein, which enables the production of high-quality wine.*

## 6. PUBLICATIONS

### Basic requirements

#### Scientific publications with impact factors:

1. Szövényi, Áron Pál; Sólyom-Leskó, Annamária; Nagy, Balázs; Varga, Zsuzsanna; Németh, Noémi Aletta; Nyitrai Sárdy, Diána Ágnes

Comparison of Gelatin and Plant Proteins in the Clarification of Grape Musts Using Flotation Techniques

FERMENTATION11: 10 Paper: 569, 14 p. (2025)

*Folyóirat szakterülete: Scopus - Biochemistry, Genetics and Molecular Biology (miscellaneous) SJR indikátor: Q2 Folyóirat*

2. Szövényi, Áron Pál; Sólyom-Leskó, Annamária; Szabó, Anna; Nagy, Balázs; Varga, Zsuzsanna; Nyitrai Sárdy, Diána Ágnes

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

FERMENTATION10: 12 Paper: 642, 11 p. (2024)

*Folyóirat szakterülete: Scopus - Biochemistry, Genetics and Molecular Biology (miscellaneous) SJR indikátor: Q2 Folyóirat*

#### Refereed scientific publications without impact factors:

Szövényi, Áron; Steckl, Szabina; Varga, Zsuzsanna

Növényi fehérje alapú, vegán musttisztító anyagok alkalmazása Chardonnay és Pinot noir rozémustokon

BORÁSZATI FÜZETEK31: 2pp. 33-36., 4 p. (2021)

*IV. Agrártudományok Osztálya IVAO A*

#### Further scientific publications:

1. Szövényi, Áron

Érzékszervi harmonizáció, polifenol csökkentés a gyümölcsösebb borokért

BOR ÉS PIAC: 11-12p. 11 (2017)

2. Szövényi, Áron

Littofresh Rosé, stabil rozé szín növényi fehérjével

BOR ÉS PIAC: 7-8p. 11 (2016)

Conference proceedings:

1. Szövényi, Áron Pál; Sólyom-Leskó, Annamária; Steckl, Szabina;  
Nyitrai, Sárdy Diána Ágnes

Növényifehérje-alapú kezelőszerek hatása a musttisztítás során

In: Varga, Zsuzsanna; Oláh, Róbert; Nyitrai, Sárdy, Diána (szerk.) A  
VI. Országos Szőlész –Borász Konferencia összefoglalói: A  
konferencián bemutatott tudományos poszterek összefoglalói

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