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Technical Article

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PLANT PROTEINS IN ENOLOGY: WHICH PRODUCTS SHOULD BE CHOSEN TO ACHIEVE THE DESIRED ENOLOGICAL OUTCOME?

Plant protein fining agents are being increasingly used in modern enology and are well-aligned with current industry needs. To achieve optimal results when using these fining agents, it is essential to understand their characteristics and how they perform in different applications.

References to protein use in winemaking appear throughout the history of enology. However, both the fining materials and the objectives of their use have evolved significantly over time in response to industry demands and market expectations. This article summarizes the main aspects of plant-based proteins and their enological performance.

The Use of Proteins in Enology

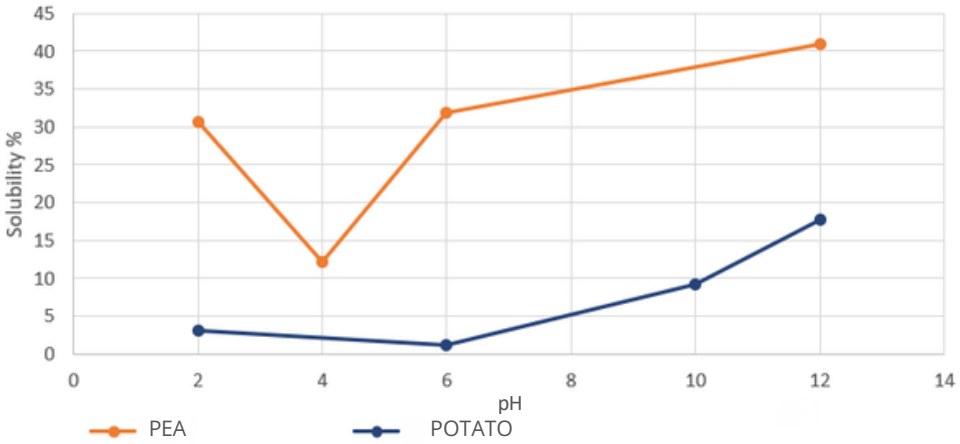
Approximately two thousand years ago, the Romans transformed wine from a luxury product enjoyed by a select few into a widely consumed beverage—for both nourishment and pleasure (Roma Caput Vini, Giovanni Negri, Elisabetta Petrini). As wine production expanded dramatically across the empire, the need for more sophisticated winemaking practices became evident. Large-scale production often resulted in questionable quality, prompting the development of techniques to improve stability, clarity, and flavor.

Alongside common practices like blending, the Romans used fining agents such as milk, eggs, and wild pea flour (Roman-Era Winemaking: Enological Practices – Annalisa Motta). These early treatments, based on materials readily available at the time, evolved gradually and can be considered the precursors of modern clarification practices.

Fast-forward to recent decades: while the core objective of improving wine quality remains unchanged, today's enological goals have become highly specific—for example, reducing astringency, improving color, or enhancing aromatic cleanliness. At the same time, fining agents have become increasingly purified and specialized (e.g., potassium caseinate from milk, albumin from eggs).

The emergence of bovine spongiform encephalopathy (mad cow disease, UK 1986), the subsequent ban on blood albumin, and growing concerns about milk and egg allergies created a strong need for safer, plant-based alternatives to traditional animal-derived fining agents.

During the past twenty years, extensive research has focused on proteins derived from legumes (soy, pea, lupine), cereals (wheat, corn, rice), and tubers such as potatoes. Among these sources, pea and potato proteins have gained the most widespread use due to their effectiveness, safety, and availability. Although current OIV monograph COEI-1-PROVEG (2016) permits the use of wheat, pea, and potato proteins, wheat (gluten) proteins will soon be excluded due to potential allergenicity. Despite evidence from ELISA (Enzyme-Linked Immunosorbent Assay) tests confirming the absence of gluten residues post-treatment, wineries prefer to avoid products associated with known allergens.



Graph 1 - Solubility as a function of pH.

Pea Proteins: Production Process and Chemical Characteristics

Pea proteins are extracted from the yellow pea (*Pisum sativum*) through a two-stage process that includes both dry and liquid operations. The first stage involves mechanical removal of the pea seed hull (tegument), which is primarily composed of insoluble fiber. The dehulled peas then undergo milling to produce a flour with uniformly sized particles containing proteins, starches, and partially soluble fibers. In the second stage, the pea flour is dispersed in water under controlled pH and temperature conditions. Through centrifugation, the soluble fraction—containing proteins—is separated from the insoluble components.

To obtain a protein concentrate, the dissolved proteins are then brought to their isoelectric point, causing them to precipitate. The precipitated proteins are subsequently collected and spray-dried (atomized), resulting in a fine, homogeneous powder with a protein content exceeding 85%. Temperature management throughout this process is critical, as it greatly influences the functional properties of the resulting protein concentrate.

Many studies on the chemical characterization of pea proteins show that they are primarily composed of globulins and albumins, which act as storage proteins and serve as a nitrogen source during seed germination. The globulin fraction—present in the highest concentration—is mainly represented by legumin, vicilin, and convicilin. Structurally, these proteins exhibit compact quaternary configurations. They form large multimeric assemblies such as hexamers and tetramers, with molecular masses ranging from approximately 380 kDa for the legumin hexamer to about 150 kDa for the vicilin trimer.

Fig. 1A - Pea Protein Rehydrated to 5% in Water at pH 3.5

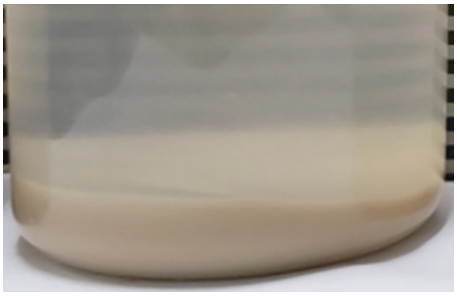


Fig. 1B - Potato Protein Rehydrated to 5% in Water at pH 3.5

Potato Proteins: Production Process and Chemical Characteristics

Potato (*Solanum tuberosum*) proteins are obtained through two primary industrial processes: starch extraction and potato chip production. In starch manufacturing, proteins are found in the residual potato juice once the starch has been removed, typically accounting for about 1.5% of the dry matter. In potato chip production, proteins are recovered from the wash water used to rinse sliced potatoes prior to frying, as well as from by-products generated during cutting, rasping, and trimming of the raw tubers.

As with other plant-based proteins, the steps involved in separating and purifying potato proteins—particularly those based on temperature control, isoelectric point manipulation, hydrophobic interactions, and filtration—play a decisive role in determining the functional and chemical properties of the final product.

Among the various proteins present in potato juice, the most uniform and well-characterized group is the *patatins*. These glycoproteins have an approximate molecular mass of 40 kDa and an isoelectric point between pH 4.5 and 5.2. At neutral pH and room temperature, patatin typically exists as a dimer stabilized by hydrophobic interactions. Its three-dimensional structure is highly sensitive to pH fluctuations, and exposure to temperatures above 60°C readily induces denaturation.

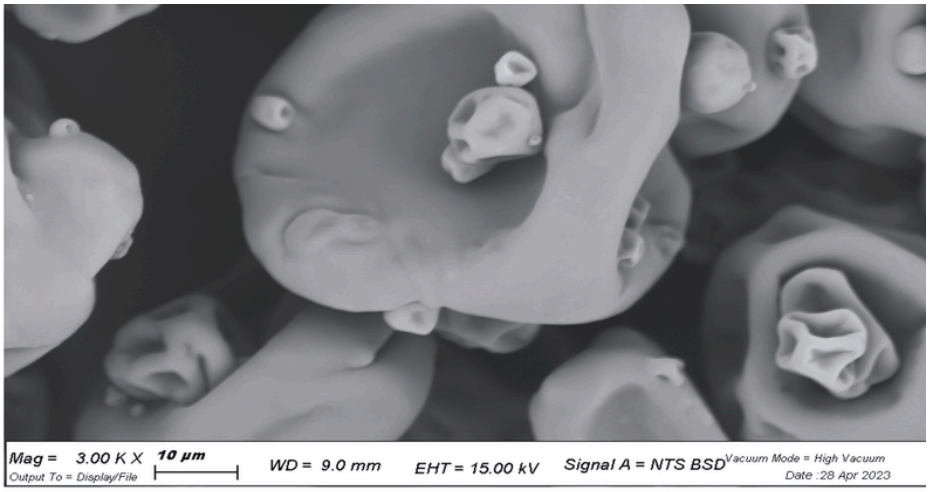


Fig. 2A - Pea Protein

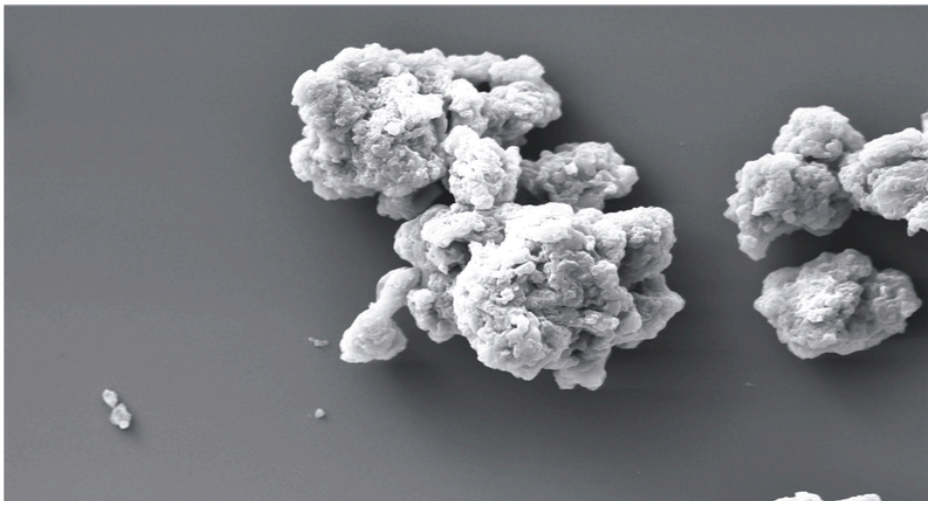


Fig. 2B - Potato Protein

Solubility: A Key Difference Between Plant and Animal Proteins

Unlike animal proteins, which are typically fully soluble, plant proteins exhibit only partial solubility. As a result, a substantial —and sometimes very large—portion of plant protein remains in suspension. This suspended fraction contributes functional effects related to its surface properties.

Graph 1 illustrates, as an example, the solubility profiles of the two proteins across a range of pH values.

Fig. 1A and **1B** show the suspensions formed by the two plant proteins at pH 3.5. **Fig. 2A** and **2B** provide electron microscope images (3000× magnification) highlighting the microstructural details of these protein suspensions.

The soluble fraction of each protein was analyzed using SDS-PAGE electrophoresis to determine the molecular weight distribution. The results, shown in **Fig. 3**, reveal that potato protein is dominated by a major band at approximately 40 kDa (patatin), whereas pea protein displays numerous fragments spanning a broader range of 10–100 kDa.

What Is Their Enological Behavior and Performance?

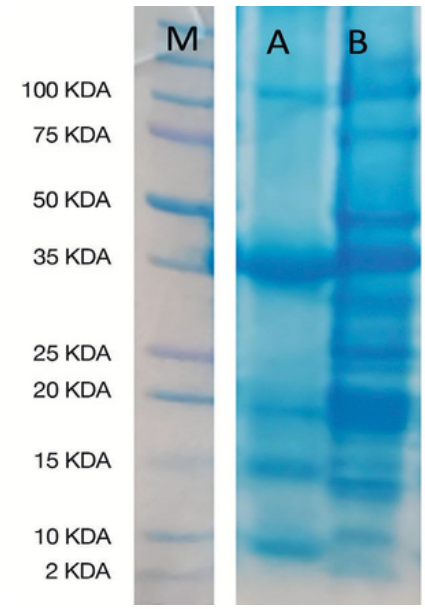
The impact of the two protein categories on wine was assessed by monitoring several key parameters: clarification efficacy, oxidation control, reduction of astringency, influence on colloidal stability, and overall sensory effects. In enology, recommended dosage ranges for pea and potato proteins typically fall between 5 and 30 g/hL, depending on the stage of application and the desired outcome. For the experimental trials, a standardized dosage of 10 g/hL was applied in all tests except flotation, for which a dosage of 5 g/hL was used.

Clarification Efficacy

The molecular weight distribution of plant proteins plays a significant role in determining their clarifying performance, as illustrated in **Graph 2**. Controlled enzymatic hydrolysis can enhance this clarifying ability by producing smaller protein fragments that interact more effectively with wine colloids.

For comparison, a gelatin with a low degree of hydrolysis (100 Bloom) reduced turbidity to 5 NTU in Trebbiano wine and 2 NTU in Sagrantino wine. **Fig. 4** below shows the clarifying effects of potato proteins with different degrees of hydrolysis in two wines. To highlight turbidity differences among the treated samples, the wines were intentionally photographed against a background of horizontal black lines, making variations in clarity more visually evident.

Fig. 3 - Electrophoretic profile of the soluble fraction of potato (A) and pea (B) protein representative of the average characteristics of products on the market. (M) Molecular weight markers.



Graph 2 - Turbidity values at 48 hours after product addition in white wine (Trebbiano, pH 3.22) and red wine (Sagrantino, pH 3.45).

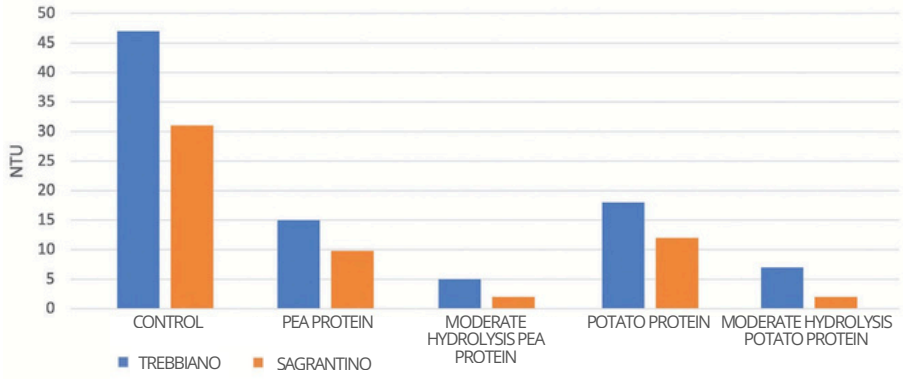
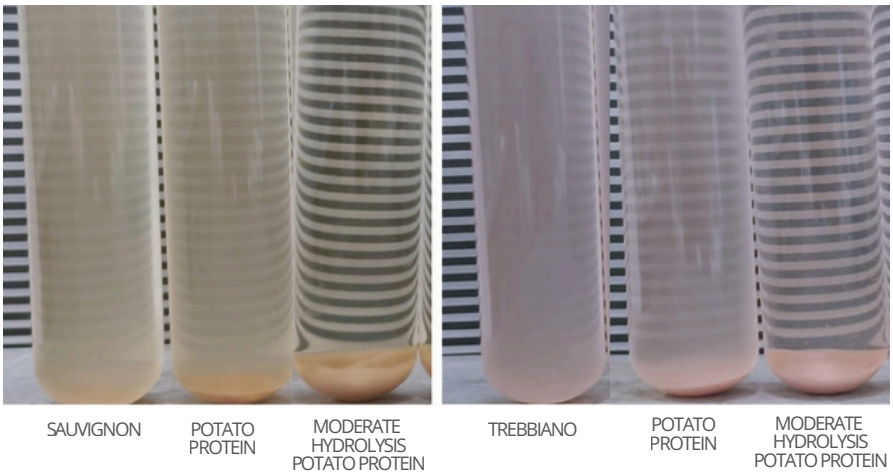


Fig. 4 - Clarification tests. Turbidity after 48 hours following product addition in Sauvignon Blanc wine (pH 3.3) and Trebbiano wine (pH 3.22).



Graph 3 - Flotation tests performed on Muscat must with starting turbidity of 600 NTU. Turbidity values one hour after the process.

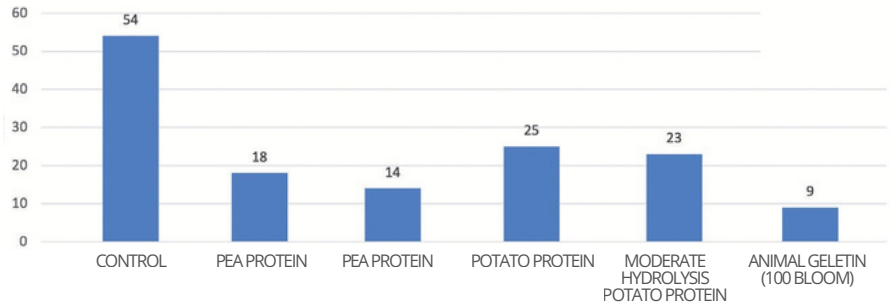
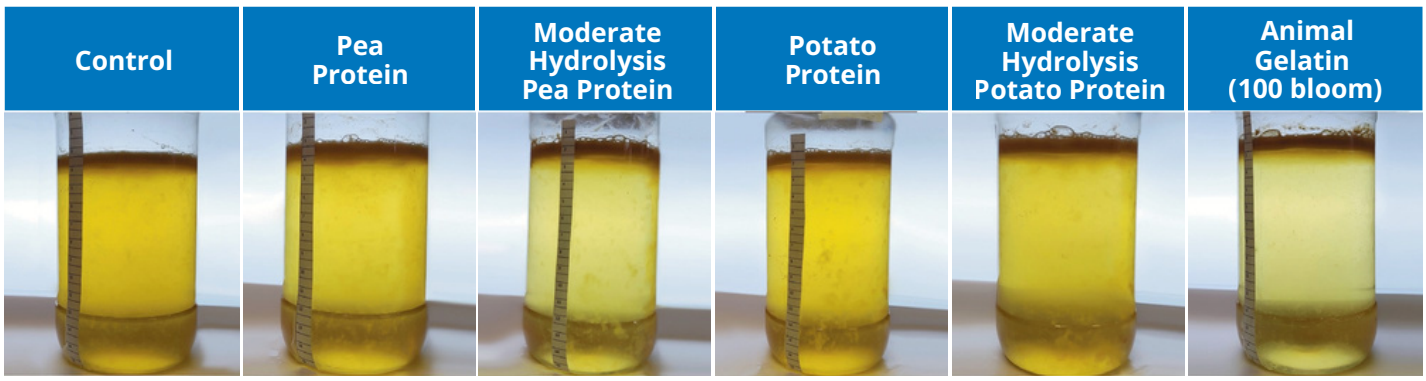


Fig. 5 - Flotation tests performed on Muscat must with starting turbidity equal to 600 NTU. Images acquired one hour after the process.



Plant proteins are also commonly used for must flotation. Note how pea protein with a medium degree of hydrolysis, in addition to clarifying, can improve the color of the treated must. **Graph 3** and **Fig. 5**.

Oxidation Management

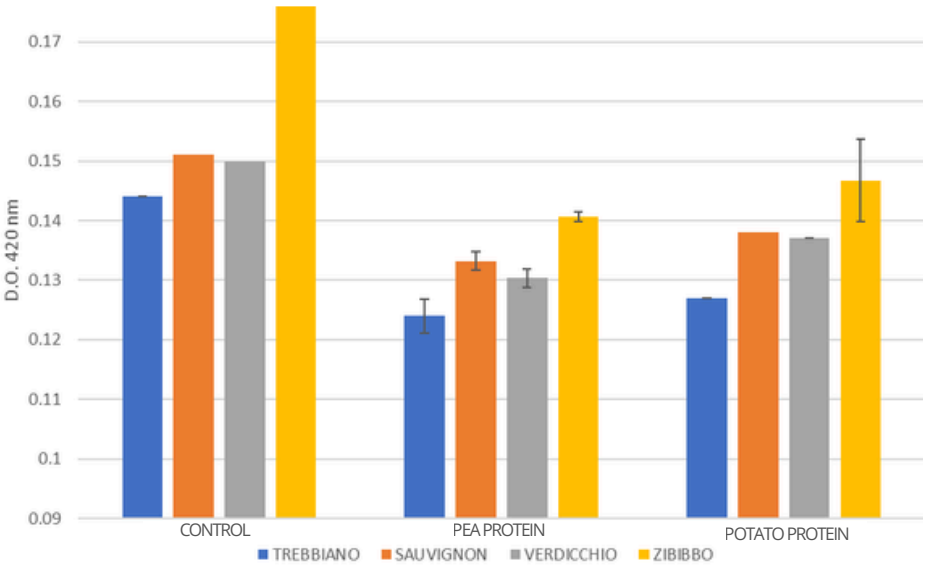
Pea proteins are particularly effective in reducing browning. This activity can be evaluated by measuring the decrease in optical density at 420 nm, as illustrated in **Graph 4**. In this case, molecular size does not appear to significantly influence performance. Pea proteins also demonstrate superior efficacy in lowering the concentration of iron—a metal that catalyzes oxidative reactions. **Graph 5** shows this effect, with the performance of PVI/PVP included as a reference.

Astringency Reduction

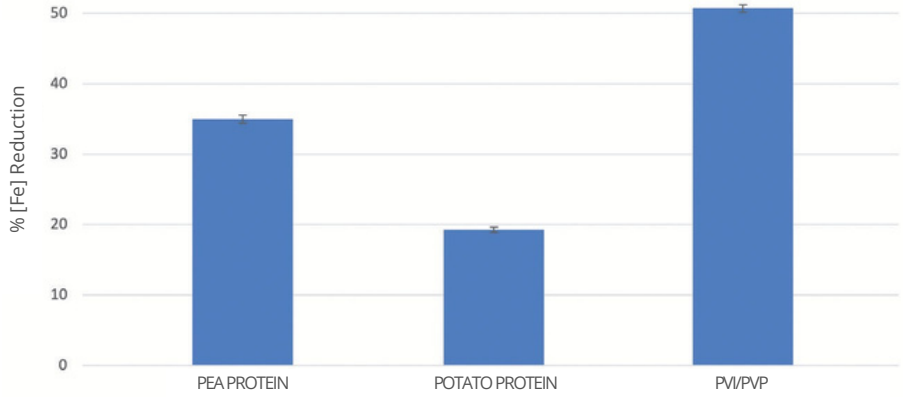
Potato proteins exhibit the strongest reactivity toward highly astringent tannins. Clarification trials conducted on different wines confirmed this characteristic, as shown in **Graph 6**. For comparison, the performance of a hydrolyzed gelatin is also reported.

Graph 7 demonstrates that an appropriate degree of hydrolysis applied to pea protein enhances its effectiveness in reducing astringency.

Graph 4 - Average optical density reading at 420 nm following clarification with plant proteins.



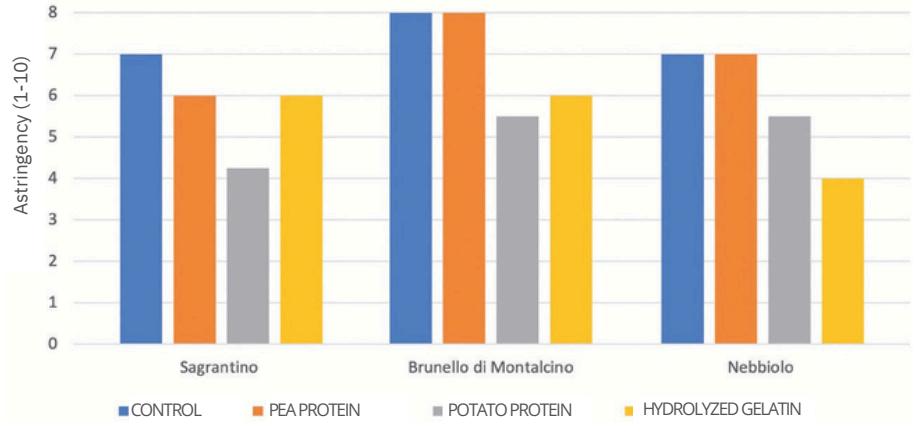
Graph 5 - Average percentage reduction in iron concentration achieved after clarification in various wines using the main plant-based proteins available on the market.



Reduction in Protein and Colloid Instability

Protein-unstable white wines clarified with plant proteins and subsequently subjected to heat testing exhibit a smaller increase in turbidity, as shown in **Graph 8**. This behavior suggests a potential stabilizing effect associated with the use of plant proteins. However, detailed HPLC analyses of the unstable protein fractions—specifically chitinases and thaumatin-like proteins—revealed no significant differences in their concentrations following treatment.

Graph 6 - Astringency values of wines clarified with plant proteins and hydrolyzed gelatin.



These findings indicate that additional mechanisms may be involved, likely linked to interactions among polyphenols, proteins, and other colloidal components. In this context, polyphenols may function as destabilizing agents within the protein–colloid matrix, contributing to the observed behavior. Further investigation will be necessary to fully elucidate these interactions.

Sensory Impact

It is well established that the use of high dosages of plant proteins can impart aromas to wine that are generally described as "vegetal notes."

Application trials conducted on numerous products currently available on the market have demonstrated that both the selection of the appropriate product and its use at the correct dosage are critical factors determining the success of the treatment.

During the clarification trials, a wide range of wine samples treated with pea protein, potato protein, and proteins of animal origin were analyzed by gas chromatography (GC), focusing on the main classes of aromatic compounds.

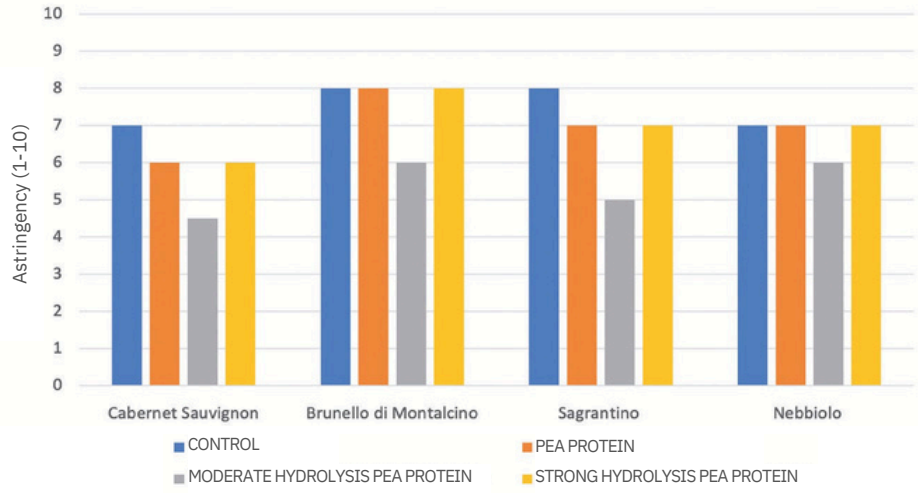
In summary, the results indicate that these treatments do not lead to significant losses of the aromatic compounds typically present in the wine. Moreover, it is important to highlight that, in sensory evaluations, the clarified samples generally exhibited a cleaner and more refined overall profile.

An example of these results is shown in **Graph 9**.

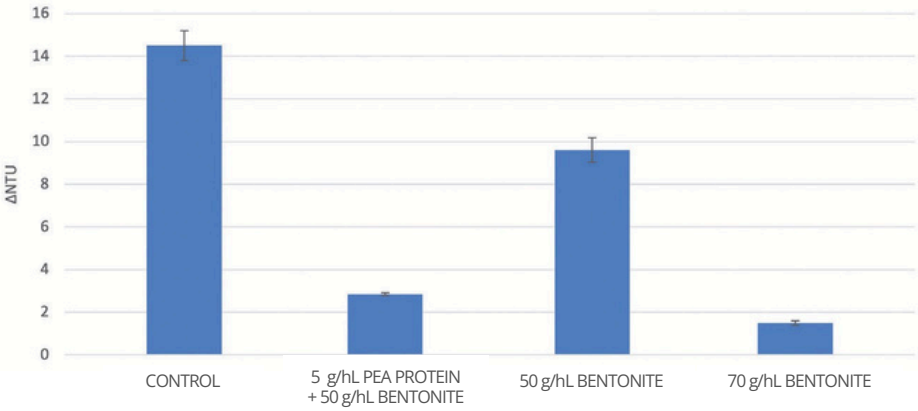
Conclusion

The aim of this study was to describe the characteristics and primary enological functions of pea and potato proteins, as well as to evaluate the performance of the most widely used plant-based clarifiers currently available to the wine industry. The results obtained highlighted the distinctive strengths of the two protein categories examined and demonstrated that, through targeted processes capable of modifying their reactivity, it is possible in certain cases to enhance their performance, making them better suited to specific technological needs within the sector.

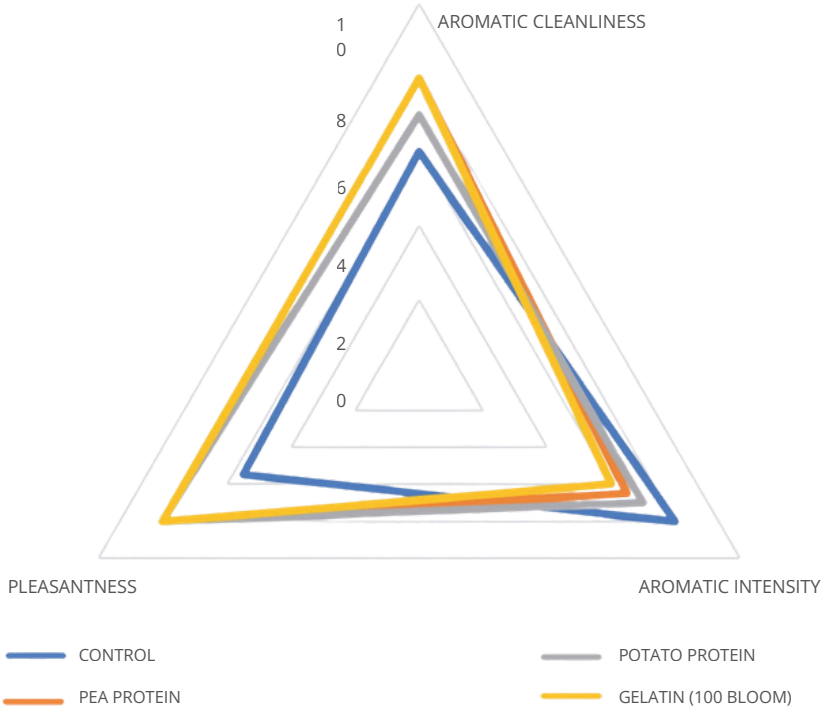
Graph 7 - Astringency values of wines clarified with pea proteins at different degrees of hydrolysis.



Graph 8 - ΔNTU values of heat tests (30 minutes at 80°C) carried out on Traminer wine.



Graph 9 - Sensory evaluation of Nebbiolo wine. Average scores obtained after treatment with the main plant-based proteins; gelatin included as a comparison.



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