

PEA PROTEINS AS AN ALTERNATIVE TO PROTEINS OF ANIMAL ORIGIN FOR WINE CLARIFICATION - A MINIREVIEW

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Abstract

Pea (Pisum sativum) is a leguminose crop cultivated worldwide for its high protein content. Pea protein is already used as a nutraceutical or food ingredient due to its low allergenic effects. In recent years it started to be applied in wine clarification as an alternative to the use of proteins of animal origin (casein, ovalbumin, gelatin), which are not suitable for vegetarians and can also cause allergic reactions in some people. As an adjuvant for wine fining, the pea protein removes some of the undesirable oxidisable polyphenols in wines and some other compounds, with good effect on the colour and taste. This paper discusses the types of pea extracts and their advantages and limitations as replacements of animal proteins in winemaking. Mechanisms of molecular interactions with the wine compounds and effects are presented in comparison with those produced by fining with traditionally used agents.

Key words: pea proteins, pea extracts, wine, fining agents.

INTRODUCTION

Pea (*Pisum sativum*) is a leguminous plant belonging to the *Fabaceae* family, cultivated worldwide as commercial crop, forage, rotational or cover crop (Pavek, 2012), due to its high protein content, reaching 20-25% (Shanthakumar et al., 2022).

Other compounds found in pea are fibres, starch, trace elements and some phytochemical substances which may also be important for antimicrobial, anti-inflammatory, antioxidant and even anticancer properties (Rungruangmaitree and Jiraungkoorskul, 2017).

Pea is relatively easy to grow, does not necessarily need fertilizers as it can fix atmospheric nitrogen (Wang et al., 2020), is drought tolerant because has a deep root system (Meena et al., 2018) and has a low carbon dioxide emission 0.98 CO₂ equivalents per kg of peas, significantly lower than 1.79 for beans or 99.48 for beef, 23.88 for cheese and 12.31 for pork (Ritchie et al., 2022).

As more and more people shift to vegetarian or vegan products for diet or health reasons, pea protein emerged as a very good alternative to replace ingredients of animal origin. Egg and dairy ingredients replacement with pea-based ingredients are very often researched (Hedayati et al., 2022; Wu, 2022).

Pea protein extracts became more popular as food supplements or for many applications in food industry, due to its affordability, availability, nutritional value and potential health benefits (Boye et al., 2010; Lam et al., 2016; Lu et al., 2020).

MATERIALS AND METHODS

Some of the most important scientific databases of references on life sciences were systematically searched using the terms “pea”, “pea protein”, “pea isolate”, “pea hydrolysate”, “vegetal protein” coupled with “wine”, “fining”, “fining agents”. The search was performed in ScienceDirect, Scopus, Elsevier and PubMed up to January 2025.

A separate literature search was performed using the terms “animal protein” or “vegetal protein” and “wine” in combination with “alternative”, “allergenicity” to document the reasons for replacing animal protein fining in wines.

Papers and some reviews specifically addressing the topic of pea protein as a fining agent for wines were favoured, but other papers related with the pea protein uses and reaction mechanisms were also included.

Pea protein research developed very much in the past years, but the research on fining wines with pea protein is still limited. For example,

on Science Direct Freedom Collection, a search with the term “pea protein” rendered 22 results for 2001, growing slowly to 111 in 2015 and then increasing abruptly and reaching 1554 in 2024 (Figure 1 a). In the same time, the search with “pea protein” coupled with “wine finding” rendered very few results (Figure 1 b), especially in the last years, showing that this new application in wine clarification is an emerging research topic.

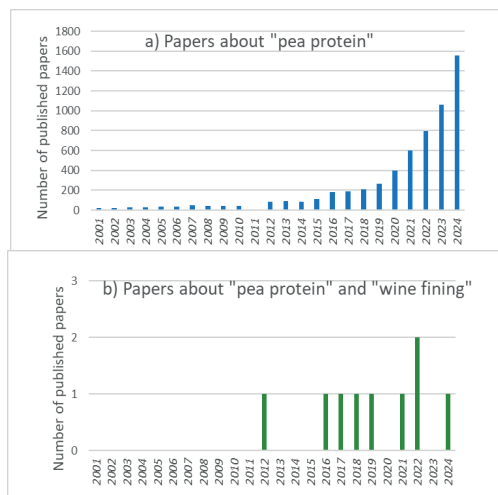


Figure 1. Evolution of published papers from 2001 to 2024 related to pea protein in general (a) and application of pea protein in wine fining (b)

RESULTS AND DISCUSSIONS

Pea protein as food

Plants have been used for centuries as part of human diet, providing energy and important nutritional compounds. Among plants, pulses include the nutritionally-dense edible seeds of legumes, such as beans, peas, chickpeas and lentils. Like any pulse, pea is composed mainly of starch embedded in proteins, fibres and lipids (Pelgrom et al., 2015).

Based on several studies included in a comprehensive review (Wu et al., 2023), the approximate composition of pea consists of 59.32–69.59% carbohydrates (more specifically 39.44% to 46.23% starch and 23.23% to 30.72% dietary fibres, of which 3.91-8.01% of soluble fibre and 19.32-23.1% of insoluble fibre). It also contains 20-25% proteins and 3.06-7.3% lipids.

However, when it comes to plant proteins, it is also known that they are not as well balanced as the animal proteins, regarding the content of essential and non-essential amino acids. Plant proteins may be deficient in several essential amino acids (Berrazaga et al., 2019) and pea is no exception, cysteine and methionine being the limiting amino acids in pea protein, as well as tryptophan (FAO, 2025). Lysin is, however, well represented in pea proteins (Shi et al., 2018), as well as arginine (Robinson and Domoney, 2021). Plant proteins are also less digestible than the animal ones (Kaur et al., 2022), due to the presence of fibres which inhibit the proteolytic enzymes (Doudou et al., 2003), the digestibility rate of cooked pea proteins being 73-94% (Khattab, S and Nyachoti, 2009).

Moreover, structural differences are also found, plant proteins having in their structures fewer α -helixes and more β -sheets than animal proteins, which increase with heating and adversely affects their digestibility (Carbonaro et al., 2012).

To improve the nutritional value and to better valorise peas, protein is often extracted for various applications.

Pea protein extraction

Plant proteins, including pea's, are classified in accordance to their solubility in four major classes: globulin, albumin, prolamin, and glutelin (Markgren and Johansson, 2020), most of them having storage functions for the plant, especially globulin. Globulin (legumin and vicilin) is the fraction soluble in solutions of salt, representing 55-65% of pea protein (Lu et al., 2020).

Pea protein is obtained by removing the outer shell of the peas and milling the rest into flour. The fibres and starch are subsequently removed from the flour. Pea protein concentrate is the least processed and contains also carbohydrates and lipids, while pea protein isolates and hydrolysed pea protein are higher in protein.

To extract the pea protein several methods can be applied to obtain enriched protein fractions or isolates. The most used methods are wet fractionation, which is based on the solubilization of the starch and protein in water at different conditions, and dry fractionation,

which is based on separation by density and particle size (Klupšaitė & Juodeikienė, 2015).

The wet fractionation method leads to higher protein concentration (up to 94%), but is very demanding in time and energy, while the dry fractionation reaches a concentration of up to 75%, but is more sustainable (Allotey et al., 2022) and preserves better the natural structure and function of the proteins (Pelgrom et al., 2015; Pelgrom et al., 2013).

More sophisticated extraction methods include the use of enzymes, ultrasounds, radio-frequency, microwaves, high pressure, pulsed electric fields, intense pulsed light and so on (Rajpurohit and Li, 2023).

These extracts have many uses and certain reviews are available to point out this diversity from food applications (Shanthakumar et al., 2022) to food supplements and pharmaceutical products, edible coatings for fruits and vegetables, emulsifiers, drug delivery and non-edible applications (Kumar et al., 2022).

These proteins can be used as such or can be modified by various treatments such as heat, pressure, extrusion, plasma, ultrasounds, chemical modifications, fermentation, enzymatic transformations and so on, in order to improve their functional properties (Shanthakumar et al., 2022).

Wine fining with proteins

Of all the applications of the pea protein isolates, the use in wine is only recent and is mainly based on their capacity to bind with the tannins, a property discussed in the subchapter which follows.

Pea protein is applied in wine as an adjuvant and the technological process for which is used is called fining. This is a practice based on using some substances, called fining agents, to clarify and improve the filterability of a liquid product, such as wine, juice, beer - thus preventing unwanted sensory effects and the forming of sediments after bottling.

Generally, the fining agents are selected to be able to remove undesirable particles, haze and, in some beverages, yeasts after fermentation.

In wine, fining is applied to modulate the organoleptic properties, including, but not

limiting to the visual ones. Thus, the expected effects of wine fining are as follows:

- Reduce turbidity for a better visual effect, but also as a means for reducing unwanted compounds. Turbidity is reduced through adsorption on the fining agent molecules, either in gravitational sedimentation or in flotation.
- Remove C6-aldehydes and C6-alcohols inducing herbaceous notes.
- Remove enzymes such as polyphenol-oxidases which lead to wine browning or pinking, and esterases, which promote the loss of aroma, and elemental sulphur, which could lead to reductive aroma.
- Remove phenolic compounds to reduce white wine oxidability and avoid browning and pinking in the presence of oxygen and oxidoreductase enzymes.
- Remove phenolic compounds to avoid bitterness, which is mainly imputable to flavan-3-ols, but may also be caused by some flavanols and derivatives of benzoic and hydroxycinnamic acids (Ferrer-Gallego et al., 2014; Ferrer-Gallego et al., 2016).
- Remove phenolic compounds to avoid the astringency determined by flavanols and their polymers with low molecular weight from the classes of procyanidins/prodelphinidins sub-class of condensed (or proanthocyanic) tannins (OIV Resolution Oenological Tannins OIV-OENO 675A-2022; Vignault, 2019). These compounds are present especially in red wines and they naturally decrease by polymerization and precipitation during wine maturation and aging (Quijada-Morín et al., 2014; Ramos-Pineda et al., 2017), but their removal by fining speeds up the process of taming an unbalanced astringency and is especially useful for wines, white or red, which are not matured or aged for long times or at all.

Protein based fining agents, pea protein isolates included, cannot induce all the effects underlined above, but they have important and various effects at the polyphenol compounds level, due to their ability to interact with several phenolic classes in selective ways in accordance to their composition (Río Segade et al., 2020).

Animal protein fining agents, their allergenic potential and vegetal alternatives for wine fining

Proteins of animal origin, such as gelatine, ovalbumin and caseins, are the most regularly used protein-based fining agents for wine, an overview of these agents being synthetically presented by Obreque-Slier et al. in support of their applicative research done in 2023 (Obreque-Slier et al., 2023). Gelatine of porcine origin is frequently used as it is rich in proline and selectively removes tannins with high molecular weight, thus removing up to 20% of the initial tannin (Maury et al., 2001).

Gelatine is most effective in reducing the bitter aftertaste, making wines softer or thinner; casein prevents oxidation in both white and red wines; and albumin is a very good fining agent for tannic red wines (AWRI, 2024; Braga, et al., 2007). Isinglass, protein originating in fish, is also used in white wines to intensify yellow colour (AWRI, 2024).

Despite their long-time application and demonstrated effectiveness, animal-based fining agents could present a risk for individuals with allergies or food intolerances (Peñas et al., 2015) and legislative decisions have been made for their regulation or labelling (Regulation (EU) No 1169/2011; Regulation (EU) 2019/33).

As fining agents are defined as processing aids (Resolution OIV-OENO 567A-2016) and are eliminated themselves through subsequent finings, decanting and filtration operations before wine is bottled, there is a reasonable concern that some small residues may still remain in the wine.

A team of researchers determined in model wines that 24-58% of initial proteins remained after fining, the values varying in accordance with the quantification method used (Maury et al., 2019). But these values were obtained in model wines, not in actual wines, which have a more complex composition and in which many technological operations are applied (fining with bentonite to remove proteins, decanting and filtering). Peñas et al. (2015) showed in their review with data obtained from real wines that all studies used for the review indicated that most wines at bottling time were free from allergenic proteins as residues of allergenic fining agents, but they also showed that in

some cases relatively high quantities of especially egg white proteins, as well as some amounts of milk proteins, were still present. Casein is less likely to be found in wines fined with this agent (Restani et al., 2012).

According to Article 51 of Regulation (EU) No. 579/2012 allergenic products which were used for wine treatments, including milk-based and eggs-based products, have to be declared on the label (Commission Implementing Regulation (EU) No 579/2012) if they are detected in the final wine by using the OIV ELISA method OIV-MA-AS315-23 for the quantification of potentially allergenic residues of fining agent proteins (Resolution OIV-Oeno 427-2010 modified by OIV-COMEX 502-2012; Weber et al., 2007). Other methods for simultaneous quantitative detection of protein residues by High-Resolution Mass Spectrometry (Monaci et al., 2013) UPLC-MS/MS (various caseins, α -lactalbumin, β -lactoglobulin, lysozyme, ovalbumin and ovotransferrin) have also been proposed (Yang et al., 2021).

In the light of these drawbacks, alternatives from vegetal sources are actively sought and new products started to be commercially proposed, to be used in various winemaking stages, in doses between 10 and 30 g/hl.

A few licence-protected plant-based products are available for wine fining or wine clarification through flotation, but they are not usually composed only of proteins, although some contain mostly proteins, such as:

- pea proteins: Plantis L (Enartis, Trecate, Italy), Proveget Premium and Proveget 100 (Agrovin, Ciudad Real, Spain), GreenFine® Mix/Rosé, Nature, Must, X-Press, Must-L, Intense etc. (Lamothe Abiet Canéjan, France);
- Fitoproteina P (Enologica Vason, Verona, Italy), Protein Clair Liquid and Special (LaFood, Fasano, Italy);
- potato proteins/patatin: Plantis® PQ (Enartis, Trecate, Italy), Proveget Fine (Agrovin, Ciudad Real, Spain), Vegefine™, Vegeflot™, Oenofine™ Pink, Nature and RedY etc. (Laffort, Floirac, France), Fitoproteina XP (Enologica Vason, Verona, Italy), Protein Clair VP, VP Special, PP (LaFood, Fasano, Italy).

Products containing combinations of several proteins were classified according to the main protein in their composition. The list provided

is not exhaustive and is only showing the present state, as products may be discontinued in the future and replaced by other new ones.

However, plant proteins may also generate immune responses in humans, as it is the case of gluten, which has negative effects on people prone to IgE-mediated allergic reactions (Simonato et al., 2001) or suffering from celiac disease (Cohen et al., 2019). For this reason, in spite of some studies showing their technological efficacy, glutens or other wheat proteins were removed by the OIV in 2024 from the list of fining agents approved for wine (Antoce, 2025). Pea protein is approved since 2004 (resolution OIV-OENO 28/2004) as part of the same resolution in which wheat protein was also approved, but later on removed (Resolution OIV-OENO 723-2024).

In compensation, patatin from potato was included since 2013 (resolution OENO 495 - 2013), along with the pea protein, as both have a very low allergenic potential, with very few cases reported. From pea, for example, the protein 7S globulin Pis s1 was found to be potentially allergic for some children (Popp et al., 2020), but not for adults.

Potato and pea proteins rarely induce allergies by themselves, but, because of common IgE epitopes, a cross-reaction cannot be excluded in the case of people sensitized to latex (Schmidt et al., 2002) or legumes (Robinson et al., 2022), such as peanut, soy or lupine.

Proteins from maize, rice, other legumes such as lentil, soybean or faba bean are not commercially available, even though some research shows that some of them have good prospects, with an efficiency comparable with that of the gelatine (Marangon et al., 2019).

Grape seed protein extract (GSPE) with a minimum 40% protein is also under evaluation (Gazzola et al., 2017.). Yeast protein extract is also considered a protein of vegetal origin and is present in various composed fining agents (Gaspar et al., 2019).

Pea protein for wine fining – properties, traits and reaction mechanisms

For the use of pea protein in food there is no quantitative limit, and this is also recognized by its inclusion the FDA's GRAS (Generally Recognized as Safe) database (GRAS Notice No. GRN 000182, 2025).

As a processing aid in wine there are, however, limits set. The OIV recommends that the maximum usage dose to be used for fining be less than 50 g/hl and should be established based on laboratory trials (OIV-International Code of Oenological Practices, 2025). Same dose is also allowed in the USA since 2022, when permission was given for continuing the use of pea protein in wine and grape juice (Alcohol and Tobacco Tax and Trade Bureau, 2025).

The effectiveness of the pea protein depends on the type, but also on the composition of the wine which is fined with it, especially on the polyphenol classes (Segrade et al., 2019).

A drawback of wine fining is the loss of some colour pigments. Proteins interact differently with anthocyanins or with colourless phenolics, plant proteins being more protective of the red wine colour (Gordillo et al., 2021). However, some degree of colour loss is inherently reported when using plant proteins, too. A pea-based protein used in high dose in Primitivo and Montepulciano red wines fining decreased the anthocyanin levels by 7.7% and 3.5%, respectively (Segade et al., 2019). The same study also showed that the colour intensity is affected differently depending on the pea protein type, so that some proteins may not lower visibly the colour intensity, while others can remove also flavonols, beside anthocyanins, contributing further to the loss of colour. The initial quantity and types of anthocyanins and their stability is also a factor in resistance to colour loss due to fining, which makes Syrah wines more resistant to visible colour loss, while Nebiolo wine colour was strongly affected.

Similarly with egg protein, pea protein is able to decrease the Syrah wine colour intensity by 5%, increase lightness by 5%, without significantly affecting the hue measured by CIELab method, indicating that it reduces better the content of copigments rather than other colour components (Gordillo et al., 2021). Other studies showed that while gelatine can remove monomeric anthocyanins and anthocyanin-flavanol copolymers, the pea protein does not do this to a significant degree. Of all monomeric anthocyanins, pea protein influenced most the cyanidin-3-glucoside, but not the other glycosides (Granato et al., 2018)

This can explain also why greater colour loss is observed in varieties richer in cyanidin-3-glucoside, such as Nebiollo and Primitivo (Segrade et al., 2019). Pea protein is selective in removing anthocyanidins. In the young wines pea protein removed about 6% of malvidin derivatives, such as Mv-3-glc (from 51.95 ± 0.20 to 49.18 ± 0.54), Mv-3-acetylglc (from 14.22 ± 0.31 to 13.07 ± 0.14), Mv-3-p-coumglc (from 8.05 ± 0.08 to 7.35 ± 0.18), as well as Pt-3-glc (from 11.16 ± 0.18 to 10.18 ± 0.24), which is similar to the effect of egg albumin on these compounds. But, unlike egg albumin, pea protein also removed as well Dp-3-glc (from 9.88 ± 0.08 to 9.06 ± 0.20) and Cy-3-glc (from 1.32 ± 0.02 to 1.23 ± 0.01) (Gordillo et al., 2021).

The mechanisms involved in these protein-polyphenol binding are determined by the formation of hydrogen bonds and hydrophobic interactions. If the protein concentration is small, the polyphenols cover their surface, lowering their hydrophilic character, which leads to flocculation and precipitation. Conversely, if the protein concentration is high, the protein is covered by phenolic compounds which also lead to precipitation (Ribéreau-Gayon et al., 2021). As compared to plant proteins, gelatine is forming more hydrogen bonds (Zoecklein et al., 1999). Plant proteins have their affinity for combining with polyphenols explained by their high proline content. The proline residues force the protein into a more irregular structure which provides higher accessibility of binding sites which interact with phenolic compounds (Kieserling et al., 2024).

The study made by Granato and their team in 2018 shows that the natural pea protein is not binding very well with proanthocyanidins (tannins) in a red wine, irrespective if the wine is young or aged. This behaviour of the pea protein also correlates with the observations that the hue of the wine colour is not affected, which can be a very good effect sometimes, but which also means that is less effective in removing browning in white wines (Cosme et al., 2012). However, the affinity can be increased if the pea protein is enzymatically hydrolysed to reduce its size, while keeping the hydrophobic binding sites (Granato et al., 2018). Pea protein was similarly effective in

removing monomeric and dimeric flavonols, as much as the other commercial fining agents tested, but not as effective as lentil proteins (Granato et al., 2014).

By binding and precipitating polyphenols proteins reduce not only colour, but also the astringency and bitterness induced by certain classes of polyphenols. The perceptions of astringency and bitterness are produced by molecules in the class of flavanols, a group of polyphenols including various compounds, from the monomeric flavan-3-ols to oligomeric flavanols, and to polymeric procyanidins also called condensed tannins.

As pea protein has a limited affinity for tannins, its efficiency is lower than that of the gelatine or potato protein when it comes to removing astringency, but the reduction of astringency is demonstrated to be similar to the one produced by polyvinylpolypyrrolidone (PVPP), a synthetic molecule very efficient for wine fining (Kang et al., 2018). Some studies show that, for removing astringency, potato protein could be a better choice (Gambuti et al., 2012; Gambuti et al., 2016).

For the removing of bitterness, pea protein can be a very good alternative. Segade et al. (2019) showed that pea protein is very effective in reducing polymeric and oligomeric flavanols by 7.1% and 11.1%, respectively, being better for this effect than other fining agents tested. These oligomeric flavanols are highly correlated with the bitterness of the wine (Griffin et al., 2020).

The degree of flavanol polymerization is correlated to the intensity of bitterness and astringency, thus the longer the molecular chain, the less bitterness and the highest the perceived intensity of astringency (Sun et al., 2013).

The effect of pea protein on flavanols depends also on the dose, as well as on the phenolic matrix of the treated wine. In Montepulciano wines Segade et al. (2019) observed a reduction of 7.0% of oligomeric flavanols for a low dose of pea protein used, and a 10.8% reduction in case of a high dose, but no significant reduction of polymeric flavanols, irrespective of the dose. In Syrah, one of the pea proteins tested had a significant effect only on polymeric flavanols, irrespective of the dose, while in Nebiollo another pea protein removed equally

oligomeric and polymeric flavanols (Segade et al., 2019). The flavanols composition may also play a role in their interaction with the pea protein, galloylation percentage being cited as a factor of increased binding with proteins (de Freitas, 2012).

The use of pea protein as a fining agent in wine can also have an impact on the flavour, either by removing some of the volatile compounds, but also by contributing some. Pulses and their proteins are known to have a specific flavour, which is not always appreciated by the consumers, and there is a concern that certain flavours can be transferred in the wine during the process of fining. Such compounds identified in the flavour of pulses are the 2-penten-1-ol and 2-octenal (Bi et al., 2020), hexanal and 3-*cis*-hexenal, aldehydes which have a beany, grassy and green-leaf aroma and result from the oxidation of lipids catalysed by the enzyme lipoxygenase (LOX) (Bi et al., 2022; Roland, et al., 2017), as well as 3,5-octadien-2-one, nonanol (Trikusuma et al., 2020). In addition, the presence of some pyrazines in higher content, such 2-methoxy-3-isopropyl-(5 or 6)-methyl pyrazine (Zhang et al., 2020; Trikusuma et al., 2020) lead to pea off-flavours of earthy type. Many other substances are cited in the literature as participating to the formation of the typical off-flavour of pea (Karolkowski, et al., 2021; Murray et al., 1976; Murray et al., 1970), but all these substances are in small quantities and were not reported so far to determine an off flavour in the wines treated with pea protein or hydrolysates, although this possibility cannot be excluded. As well, there may also be some non-volatile compounds that could be transferred to wine, for example caffeic acid or saponins, which are known to be present in the pea flour or extracts and confer a bitter taste (Curl et al., 1985).

Conversely, pea protein added in must or wine may selectively remove volatile compounds, by reversible or non-reversible mechanisms (Bi et al., 2022), with various effects. Low hydrophobicity of certain molecules correlates with low retention by pea protein. While fining agents such as PVPP especially reduce the volatile ethyl esters, pea protein was found to especially reduce the amount of terpenes (Antoce and Cojocaru, 2024), a fact that may

affect the aromatic profile of some muscat type-aroma. A study performed on a terpenic variety of grapevine called Tamâioasa românească shows that, when it is applied in must, before fermentation, the effects on volatile profile of pea protein treatments in dose of 20 g/hL are not statistically significant compared to the PVPP, thus making it a good alternative to PVPP in winemaking (Antoce and Cojocaru, 2024). Further research is going to be necessary to prove that the application of pea protein for wine fining does not impart unwanted flavours and/or that does not remove key aroma compounds, but the results so far are encouraging. In a study comparing PVPP with pea protein and K-caseinate fining agents it was showed that, from the viewpoint of the sensory effect, there were no significant differences (Cosme, 2012).

Other properties of pea protein and hydrolysates may also be of interest for the application in wine fining, such as their solubility or foaming ability. As it is the case with all proteins, the solubility depends on the pH of the media, being the least soluble at the isoelectric points, which for pulse proteins are between pH 4 and 6 (Ma et al., 2022), values which are, in general, above those found in wines. Granato (2014) experimented with the use of insoluble protein isolates from pea for fining white wines, as insolubility is an important trait of fining agents which must be separated from the wine after the treatment, by decanting or filtration. Foaming properties were mostly studied in connection with the preparation of other foods (Ma et al., 2022), but for the compounds based on pea protein destined to be used in the technique of wine flotation, foaming could also be relevant.

Other issues which have not yet been addressed in the scientific literature are the influence of fining must or wine with pea protein on the microorganisms (some of which are useful in alcoholic or malolactic fermentation, or undesirable such as spoilage lactic or acetic bacteria or *Brettanomyces* yeasts). Also, it may be of interest to determine if soluble peptides could be formed through protein hydrolysis, which might remain in wine after fining.

While the over-fining phenomenon, when too much fining agent remaining in the final product also creates turbidity, is not reported to

be associated with the use of pea protein, it should also be of interest to check the compactness of the formed deposit, so that not too much wine is caught in the lees.

ACKNOWLEDGEMENTS

This work was supported by the grant of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, project number 849, acronym Fine4Pietroasa, within IPC 2023.

REFERENCES

- Allotey, D.K., Kwofie, E.M., Adewale, P., Lam, E., Ngadi, M. (2022). A meta-analysis of pulse-protein extraction technologies: Impact on recovery and purity. *Journal of Food Engineering*, 327, 111048.
- Antoce, O.A. (2025). Overview of oenological practices recently approved or under consideration for approval by the OIV, *Acta Hort.*, 1418, ISHS 2025, V EHC – Proc. IS on Viticulture and Winemaking between Tradition and Innovation.
- Antoce, O.A., Cojocar, G.A. (2024). Effects of Pre-Fermentative Treatments with Non-Synthetic Ternary Component Fining Agents Based on Pea Protein on the Volatile Profiles of Aromatic Wines of Tămăioasă Românească. *Beverages*, 10, 81.
- Berrazaga, I., Micard, V., Gueugneau, M., Walrand, S. (2019). The Role of the Anabolic Properties of Plant-versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review. *Nutrients* 11(8), 1825.
- Bi, S., Lao, F., Pan, X., Shen, Q., Liu, Y., Wu, J. (2022). Flavor formation and regulation of peas (*Pisum sativum* L.) seed milk via enzyme activity inhibition and off-flavor compounds control release, *Food Chemistry*, 380, 132203.
- Boye, J., Zare, F., Pletch, A. (2010). Pulse proteins: processing, characterization, functional properties and applications in food and feed, *Food Res. Int.*, 43, 414-431.
- Braga, A., Cosme, F., Ricardo-da-Silva, J.M., Laureano, O. (2007). Gelatine, Casein and Potassium Caseinate as Distinct Wine Fining Agents: Different Effects on Colour, Phenolic Compounds and Sensory Characteristics. *OENO One*, 41, 203.
- Carbonaro, M., Maselli, P., Nucara, A. (2012). Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: a Fourier transform infrared (FT-IR) spectroscopic study. *Amino Acids*, 43(2), 911-21.
- Cohen, I.S., Day, A., Shaoul, R. (2019). Gluten in Celiac Disease-More or Less? *Rambam Maimonides Med J*, 10(1), e0007
- Cosme, F., Capão, I., Filipe-Ribeiro, L., Bennett, R.N., Mendes-Faia, A. (2012). Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physicochemical and sensory characteristics. *LWT Food Sci. Technol.*, 46, 382–387.
- Curl, C.L., Price, K.R., Fenwick G.R. (1985). The quantitative estimation of saponin in pea (*Pisum sativum* L.) and soya (*Glycine max*), *Food Chemistry*, 18(4), 241-250.
- de Freitas, V., Mateus, N. (2012). Protein/Polyphenol Interactions: Past and Present Contributions. Mechanisms of Astringency Perception. *Current Organic Chemistry*, 16(6), 724–746.
- Duodu, K. G., Taylor, J. R. N., Belton, P. S., Hamaker, B. R. (2003). Factors affecting sorghum protein digestibility, *J. Cereal Sci.*, 38, 117–131.
- Ferrer-Gallego, R., Bras, N. F., Garcia-Estevéz, I., Mateus, N., Rivas-Gonzalo, J. C., de Freitas, V., Escribano-Bailon, M. T. (2016). Effect of flavonols on wine astringency and their interaction with human saliva. *Food Chemistry*, 209, 358–364.
- Ferrer-Gallego, R., Hernandez-Hierro, J. M., Rivas-Gonzalo, J. C., Escribano-Bailon, M. T. (2014). Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: Synergistic effect and modulation by aromas. *Food Research International*, 62, 1100–1107.
- Gambut, A., Rinaldi, A., Moio, L. (2012). Use of patatin, a protein extracted from potato, as alternative to animal proteins in fining of red wine. *Eur. Food Res. Technol.*, 235, 753–765.
- Gambut, A., Rinaldi, A., Romano, R., Manzo, N., Moio, L. (2016). Performance of a protein extracted from potatoes for fining of white musts, *Food Chem.*, 190, 237–243.
- Gaspar, L. M., Machado, A., Coutinho, R., Sousa, S., Santos, R., Xavier, A., Figueiredo, M., Teixeira M. de F., Centeno, F., Simões, J. (2019). Development of Potential Yeast Protein Extracts for Red Wine Clarification and Stabilization, *Frontiers in Microbiology*, 10, 02310.
- Gazzola, D., Vincenzi, S., Marangon, M., Pasini, G., Curioni, A. (2017). Grape seed extract: the first protein-based fining agent endogenous to grapes *Aust. J. Grape Wine Res.*, 23(2), 215-225.
- Gordillo, B., Chamizo-González, F., González-Miret, M.L., Heredia, F.J. (2021). Impact of alternative protein fining agents on the phenolic composition and color of Syrah red wines from warm climate. *Food Chem.*, 342, 128297.
- Granato, T.M., Ferranti, P., Iametti, S. Bonomi, F. (2018). Affinity and selectivity of plant proteins for red wine components relevant to color and aroma traits, *Food Chemistry*, 256, 235-243.
- Granato, T.M., Nasi, A., Ferranti, P., Iametti, S., Bonomi, F. (2014). Fining white wine with plant proteins: effects of fining on proanthocyanidins and aroma components., *Eur. Food Res. Technol.*, 238, 265–274.
- Griffin, L. E., Diako C., Miller, L. E., Neilson A. P., Ross, C.F., Stewart, A.C. (2020). Preference for and sensitivity to flavanol mean degree of polymerization in model wines is correlated with body composition, *Appetite*, 144, 104442.
- Hedayati, S., Jafari, S.M., Babajafari, S., Niakousari, M., Mazloomi, S.M. (2022). Different food hydrocolloids

- and biopolymers as egg replacers: A review of their influences on the batter and cake quality, *Food Hydrocolloids*, 128, 107611.
- Kang, W., Niimi, J., Bastian, S.E.P. (2018). Reduction of red wine astringency perception using vegetable protein fining agents, *Am. J. Enol. Vitic.*, 69, 22–31.
- Karolkowski, A., Guichard, E., Briand, L., Salles, C. (2021) Volatile Compounds in Pulses: A Review. *Foods*, 10, 3140.
- Kaur, L., Mao, B., Abhilasha, A.S.B, Kaur, R., Chian, F.M., Singh, J. (2022). Alternative proteins vs animal proteins: The influence of structure and processing on their gastro-small intestinal digestion, *Trends in Food Science & Technology*, 122, 275–286.
- Khattab, R.Y., Arntfield, S.D., Nyachoti, C.M. (2009). Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation, *Lebensmittel Wissenschaft und Technologie - Food Sci. Technol.*, 42 (6), 1107–1112.
- Kieserling, H., Bruijn, W. J. C., Keppler, J., Yang, J., Sagu, S. T., Güterbock, D., Rawel, H., Schwarz, K., Vincken, J.-P., Schieber, A., Rohn, S. (2024). Protein–phenolic interactions and reactions: Discrepancies, challenges, and opportunities. *Comprehensive Reviews in Food Science and Food Safety*, 23, e70015.
- Klupšaitė, D., Juodeikienė, G. (2015). Legume: composition, protein extraction and functional properties. A review, *Chemine Technologija*, 1(66).
- Kumar, M., Tomar, M., Punia, S., Sangram Dhumal, J. D.-L., Changan, S., Senapathy, M., Berwal, M.K., Sampathrajan, V., Sayed, A.A.S., Chandran, D., Pandiselvam, R., Rais, N., Mahato, D. K., Udikeri, S. S., Satankar, V., Anitha, T., Radha, R., Singh, S., Amarowicz, R., Kennedy, J.F. (2022). Plant-based proteins and their multifaceted industrial applications, *LWT*, 154, 112620.
- Lam, A. C. Y., Can Karaca, A., Tyler, R. T., Nickerson, M. T. (2016). Pea protein isolates: Structure, extraction, and functionality. *Food Reviews International*, 34(2), 126–147.
- Lu, Z.X., He, J.F., Zhang, Y.C., Bing, D. J. (2020). Composition, physicochemical properties of pea protein and its application in functional foods, *Crit Rev Food Sci Nutr.*, 60(15), 2593–2605.
- Ma, K.K., Grossmann, L., Nolden, A.A., McClements, D.J., Kinchla, A.J. (2022). Functional and physical properties of commercial pulse proteins compared to soy derived protein, *Future Foods*, 6, 100155.
- Marangon, M., Vincenzi, S., Curioni, A. (2019). Wine Fining with Plant Proteins, *Molecules*, 24, 2186.
- Maury, C., Sarni-Manchado, P., Cheynier, V. (2019). Highlighting protein fining residues in a model red wine, *Food Chemistry*, 279, 272–278.
- Maury, C., Sarni-Manchado, P., Lefebvre, S., Cheynier, V., Moutounet, M. (2001). Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. *Am. J. Enol. Vitic.*, 52, 40–145.
- Meena, R.S., Das, A., Gulab., Y, Rattan, L. (2018). Legumes for soil health and sustainable management, first ed., Springer Nature Singapore.
- Monaci, L., Losito, I., De Angelis, E., Pilolli, R., and Visconti, A. (2013). Multi-Allergen quantification of fining-related egg and milk proteins in white wines by high-resolution mass spectrometry. *Rapid Commun. Mass Spectrom.* 27, 2009–2018.
- Murray, K.E., Shipton, J., Whitfield, F.B. (1970). 2-Methoxypyrazines and flavour of green peas (*Pisum sativum*), *Chem. Ind.*, 27, 897–898.
- Murray, K.E., Shipton, J., Whitfield, F.B., Last J.H. (1976). The volatiles of off-flavoured unblanched green peas (*Pisum sativum*), *J. Sci. Food Agric.*, 27, 1093–1107.
- Obreque-Slier, E., Cortés-Araya, K., Medel-Marabolí, M., & López-Solis, R. (2023). Different effectiveness of protein fining agents when tested for interaction and precipitation with tannic acid, a seed polyphenol extract and seven wines. *OENO One*, 57(2), 13–28.
- Pavek, P.L.S. (2012). Plant fact sheet for pea (*Pisum sativum* L.). *USDA-Natural Resources Conservation Service*, Pullman, WA.
- Pelgrom, P.J.M., Boom, R. M., Schutyser, M.A.I. (2015). Functional analysis of mildly refined fractions from yellow pea, *Food Hydrocolloids*, 44, 12–22.
- Pelgrom, P.J.M., Visser, A.M., Boom, R.M., Schutyser, M.A.I. (2013). Dry fractionation for production of functional pea protein concentrates, *Food Research International*, 53(1), 232–239.
- Peñas, E., di Lorenzo, C., Uberti, F., Restani, P. (2015). Allergenic proteins in enology: a review on technological applications and safety aspects. *Molecules*, 20(7), 13144–64.
- Popp, J., Trendelenburg, V., Niggemann, B., Randow, S., Völker, E., Vogel, L., Reuter, A., Spiric, J., Schiller, D., Beyer, K., Holzhauser, T. (2020). Pea (*Pisum sativum*) Allergy in Children: Pis s1 is an Immunodominant Major Pea Allergen and Presents IgE Binding Sites with Potential Diagnostic Value., *Clin. Exp. Allergy*, 50, 625–635.
- Quijada-Morín, N., Williams, P., Rivas-Gonzalo, J.C., Doco, T., Escribano-Bailón, M.T. (2014). Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency, *Food Chem.*, 154, 44–51.
- Rajpurohit, B., Li, Y. (2023), Overview on pulse proteins for future foods: ingredient development and novel applications, *J. Future Foods*, 3(4), 340–356.
- Ramos-Pineda, A. M., Garcia-Estevéz, I., Bras, N. F., Martín del Valle, E. M., Duenas, M., & Escribano Bailon, M. T. (2017). Molecular approach to the synergistic effect on astringency elicited by mixtures of flavanols, *J. Agric. Food Chem.* 65(31), 6425–6433.
- Rasheed, F., Markgren, J., Hedenqvist, M., Johansson, E. (2020). Modeling to understand plant protein structure-function relationships-implications for seed storage proteins. *Molecules*, 25(4), 873.
- Restani, P., Uberti, F., Danzi, R., Ballabio, C., Pavanello, F., Tarantino, C. (2012). Absence of allergenic residues in experimental and commercial wines fined with caseinates, *Food Chem.*, 134, 1438–1445.
- Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., Towey, J. (Translation Editor).

- (2021). Handbook of Enology, Volume 2: The Chemistry of Wine Stabilization and Treatments; 3rd Edition, John Wiley & Sons, NJ, USA.
- Río Segade, S., Paisonni, M.A., Vilanova, M.; Gerbi, V., Rolle, L., Giacosa, S. (2020). Phenolic composition influences the effectiveness of fining agents in vegan-friendly red wine production, *Molecules*, 25, 120.
- Ritchie, H., Rosado, P., Roser, M. (2022). Environmental impacts of food production, OurWorldinData.org, <https://ourworldindata.org/environmental-impacts-of-food>, accessed February 17, 2025.
- Robinson, G.H., Domoney, C. (2021). Perspectives on the genetic improvement of health-and nutrition-related traits in pea, *Plant Physiol. Biochem.*, 158, 353–362.
- Robinson, K.A., St-Jacques, A.D., Bakestani, I.D., Benjamin, A.G., Beavington, M., Loewen, C. (2022). Pea and lentil 7S globulin crystal structures with comparative immunoglobulin epitope mapping, *Food Chemistry: Molecular Sciences*, 5, 100146.
- Roland, W.S.U., Pouvreau, L., Curran, J., van de Velde, F. and de Kok, P.M.T. (2017). Flavor Aspects of Pulse Ingredients. *CHEM*, 94, 58-65.
- Rungruangmaitree, R., Jiraungkoorskul, W. (2017). Pea, *Pisum sativum*, and its anticancer activity. *Pharmacogn Rev.*, 11(21), 39-42.
- Schmidt, M.H.H., Raulf-Heimsoth, M., Posch, A. (2002). Evaluation of patatin as a major cross-reactive allergen in latex-induced potato allergy. *Ann Allergy Asthma Immunol.*, 89(6), 613-8.
- Segade, R.S., Paisonni, M.A., Vilanova, M., Gerbi V., Rolle, L., Giacosa, S. (2019). Phenolic composition influences the effectiveness of fining agents in vegan-friendly red wine production, *Molecules*, 25, 120.
- Shanthakumar, P., Klepacka, J., Bains, A., Chawla, P., Dhull, S.B., Najda, A. (2022). The current situation of pea protein and its application in the food industry, *Molecules*, 27(16), 5354.
- Shi, L., Arntfield, S.D., Nickerson, M. (2018). Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses, *Food Res. Int.*, 107, 660–668.
- Simonato, B., de Lazzari, F., Pasini, G., Polato, F., Giannattasio, M., Gemignani, C., Peruffo, A.D., Santucci, B., Plebani, M., Curioni, A. (2001). IgE binding to soluble and insoluble wheat flour proteins in atopic and non-atopic patients suffering from gastrointestinal symptoms after wheat ingestion, *Clin Exp Allergy.*, 31(11), 1771-8.
- Sun, B., Sá, M. de, Leandro, C., Caldeira, I., Duarte, F. L., & Spranger, I. (2013). Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *Journal of Agricultural and Food Chemistry*, 61(4), 939–946.
- Trikusuma, M., Paravisini, L., Peterson, D.G. (2020). Identification of aroma compounds in pea protein UHT beverages, *Food Chemistry*, 312, 126082.
- Vignault, A. (2019). Oenological Tannins: characteristics, properties and functionalities. Impact on wine quality, *Agricultural sciences*. Thesis University of Bordeaux; University Rovira i Virgili (Tarragona, Spain).
- Wang, C., Reid, J.B., Foo, E. (2020). The role of CLV1, CLV2 and HPAT homologues in the nitrogen-regulation of root development, *Physiol Plant.*, 170(4), 607-621.
- Weber, P., Steinhart, H. A., and Paschke, A. (2007). Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA, *J. Agric. Food Chem.* 55, 3127–3133.
- Wu, D.T., Li, W.X., Wan, J.J., Hu, Y.C., Gan, R.Y., Zou, L.A. (2023). Comprehensive review of pea (*Pisum sativum*): chemical composition, processing, health benefits and food applications. *Foods*, 12, 2527.
- Wu, J. (2022). Emerging sources and applications of alternative proteins: Chapter One - An introduction, Editor: Wu, J., *Advances in Food and Nutrition Research*, Academic Press, 101, 1-15.
- Yang, Y., Yang, Y., Zhang, J., Yao, K., Liu, J., Shao, B. (2021). Combination of polyvinylpyrrolidone extraction and standard addition strategy for the accurate determination of multiple allergen residues in red wine by UPLC-MS/MS, *Journal of Chromatography B*, 1179, 122849.
- Zhang, C., Hua, Y., Li, X., Kong, X., Chen, Y. (2020). Key volatile off-flavor compounds in peas (*Pisum sativum* L.) and their relations with the endogenous precursors and enzymes using soybean (*Glycine max*) as a reference, *Food Chemistry*, 333, 127469.
- Zoecklein, B.W., Fugelsang, K.C., Gump, B.H., Nury, F.S. (1999). Wine analysis and production. Chapman & Hall, New York.
- ***Alcohol and Tobacco Tax and Trade Bureau (2025), Wine and Juice Treating Materials and Processes for Domestic Wine Production, www.ttb.gov/wine/treating-materials, accessed 18 February, 2025.
- ***AWRI. Fining Agents; AWRI: Urrbrae, SA, Australia (2024) https://www.awri.com.au/industry_support/winemaking_resources/frequently_asked_questions/fining_agents/, accessed 12 March, 2025.
- ***Commission Implementing Regulation (EU) No 579/2012 of 29 June 2012 amending Regulation (EC) No 607/2009 laying down certain detailed rules for the implementation of Council Regulation (EC) No 479/2008 as regards protected designations of origin and geographical indications, traditional terms, labelling and presentation of certain wine sector products.
- ***FAO (2025). Food and Agriculture Organization of the United Nations, Dry legumes and legume products, www.fao.org/3/ac854t/AC854T14.htm, accessed 12 March 2025.
- ***GRAS Notice No. GRN 000182 (2025). www.fda.gov/food/gras-notice-inventory/agency-response-letter-gras-notice-no-grn-000581, accessed March 2025.
- ***OIV-International Code of Oenological Practices (2025), www.oiv.int/standards/international-code-of-oenological-practices, accessed January, 15, 2025.
- ***OIV-Oeno 427-2010 modified by OIV-COMEX 502-2012, Criteria for the methods of quantification of potentially allergenic residues of fining agent proteins in wine, www.oiv.int/public/medias/

- 2154/oivcomex-502-2012-en.pdf, Accessed February 17, 2025.
- ***Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 Oct.2011 on the provision of food information to consumers, Article 21; Commission Delegated Regulation (EU) 2019/33 of 17 Oct. 2018 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards applications for protection of designations of origin, geographical indications and traditional terms in the wine sector, Article 41.
- ***Resolution OIV-OENO 28/2004, CODEX- protein plant origin, protein plant origin from wheat and peas, www.oiv.int/public/medias/653/oeno-28-2004-en.pdf, accessed March 12, 2025.
- ***Resolution OIV-OENO 495-2013 Monograph on protein plant origin - modification of the file, www.oiv.int/public/medias/1572/oiv-oeno-495-2013-en.pdf, accessed March 12, 2025.
- ***Resolution OIV-OENO 567A-2016 Distinction between additives and processing aids – Part 1, www.oiv.int/public/medias/4954/oiv-oeno-567a-2016-en.pdf, accessed March 12, 2025.
- ***Resolution OIV-OENO 675A-2022, Oenological Tannins - Specific monograph for oenological tannins containing procyanidins/prodelphinidins, www.oiv.int/node/3099#:~:text=Procyanidins%2Fprodelphinidins%20are%20a%20sub,included%20in%20this%20sub%2Dclass, accessed on February 17, 2025.
- ***Resolution OIV-OENO 723-2024 Update of monograph and Code on protein of plant origin (withdrawal of wheat), [/www.oiv.int/node/3810](http://www.oiv.int/node/3810), accessed March 12, 2025.