# Stability of two allergens usually used as fining agents in the winemaking process



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## Introduction

DE LA VIÑA

A variety of fining products are used in wineries to eliminate suspended particles and unstable elements present in the wine, as excessive tannins, polyphenols that cause high astringency, proteins, metals, and thus obtain a clean, transparent and stable wine over time.

An example of these are Egg White Proteins (EWP) and Casein, a major milk protein. Due to the allergenic potential of both molecules and in order to comply with labeling regulations it is necessary to detect their presence in treated wines [1]. Values equal to or greater than the maximum limit of 0.25 mg/l established by the methods of the International Organization of Vine and Wine (OIV), considered official by the European Union, must be declared on the label [1-4]. The aim of this work is to document the stability of these proteins in a matrix as complex as wine. After reviewing the literature, no studies have been found on this subject.

## Material and Methods

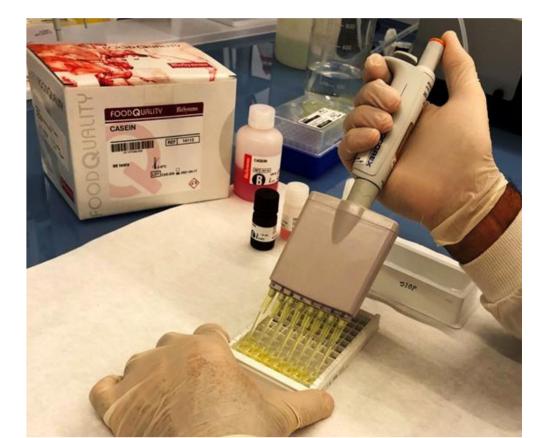
The stability of EWP and Casein was studied in eighteen wine samples analyzed at the Oenological Station of Haro (EEH) between 2019 and 2022.

The sandwich ELISA technique was used:

- RIDASCREEN FAST Egg Protein (Ref; R6402) from R-Biopharm for the quantification of EWP with a limit of quantification of 0.13 mg/kg.
- BioSystems CASEIN (Ref; 14113) for the quantification of Casein with a limit of quantification of 0.20 mg/l).
- EEH has these two accredited tests under the UNE-EN ISO/IEC 17025 Standard [5] with the limits of quantification stated above.
- EQUIPMENT: Plate reader: Tecan Sunrise at 450 nm.

### Results

Out of 1643 samples tested for EWP and 1822 for Casein, during this period at the EEH, only 1.2% and 0.7% exceeded the established limit for each protein respectively. Eighteen of these samples with different allergen concentrations were selected for the study. The assays showed the instability of these analytes in wine, a decrease in its quantification was observed during the duration of the assays (Figure 1). This instability was shown to be independent of allergen concentration and sample storage



### temperature.

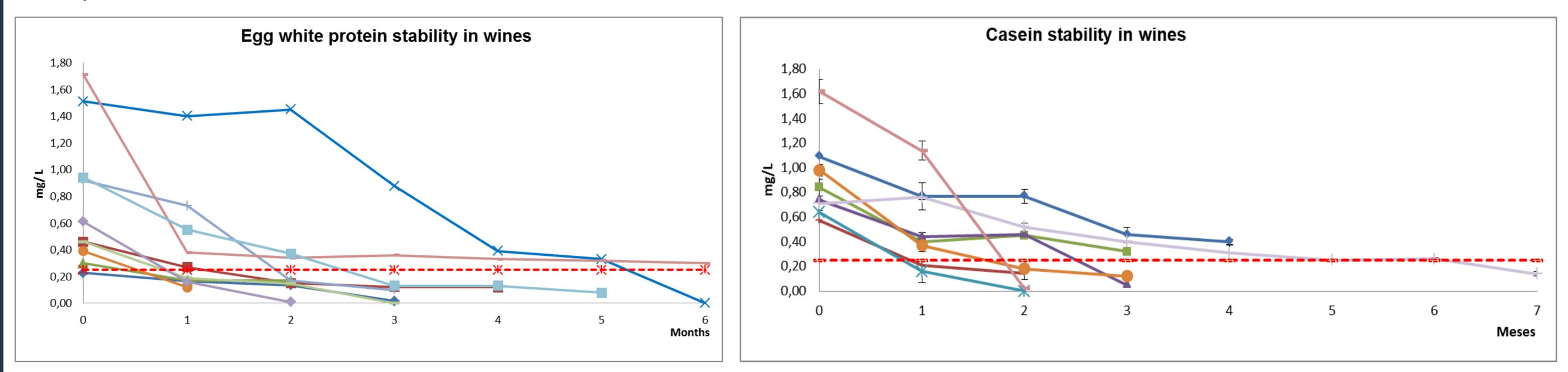


Figure 1. - Stability of Egg White Protein and Casein in Wine Samples.

### Conclusions

- EWP and Casein allergens are unstable during wine storage. This may be due either to degradation and/or to a conformational change of these molecules because of complexity of the matrix, which may affect its detection by the kit's antibody detectors. This conformational change does not necessarily imply an allergenicity loss of the proteins studied, which will have to be demonstrated in other assays.
- The present study confirms that the date of analysis of these allergens is decisive for their detection, suggesting that wineries should extend the time between clarification of their wines and the assay for the statement on the label.
- About 1% of the wines analyzed in the EEH showed concentrations that exceeded the maximum limit for mandatory declaration. However, it is important to note that these wines reached values below 0.25 mg/L of allergenic proteins in a period of approximately one to six months, depending on each wine sample.

### References

[1].-Regulation (EU) N<sup>o</sup> 1169/2011 of the European Parliament and of the Council of 25 October 2011.

[2].- Regulation (EU) Nº 1308/2013 of the European Parliament and of the Council of 17 December 2013.

[3].- OIV. Criteria for the Methods of Quantification of Potentially Allergenic Residues of Fining Agent Proteins in Wine. Resolution OIV/OENO 427/2010.

[4].- Mazzoni, C., Tirard, A. & Boubetra, A. Allergenic proteins in wine: an overview of results obtained from proficiency-tests. 2022; Accred Qual Assur 27: 289-

294.

[5].-Technical Annex Nº 183/LE407 https://www.enac.es/documents/7020/ce0aa84a-c087-4580-8a41-d535fa25e54a.