PROFICIENCY-TESTING SCHEME FOR ALLERGENIC PROTEINS ANALYSIS IN WINE

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INTRODUCTION

Fining agents are commonly used in the winemaking process to clarify stabilized wines. They have different origins (animal, vegetal or mineral) and are added to wines to remove or reduce elements that would cloud the wine or affect its aroma, color and/or bitterness. These agents should not be present in the final product but even the presence of low amount of residual fining proteins can represent a risk for allergic consumers [1,2]. Reliable detection and quantification of the residual allergenic agents is necessary to ensure compliance with food labelling, as the EU Regulatory Framework (Regulation No. 579/2012 of 29 June 2012 establishes that wines treated with allergenic additives or processing aids are subjected to specific labelling if their presence can be detected in the final product [3,4]). According to the OIV-COMET 502-2012 resolution, wines are considered free of presence of residues if allergens are not detected using techniques with a detection and quantification limits of 0.25 mg/L and 0.50 mg/L respectively [5,6]. To meet this requirement, different analytical approaches such as immunological tests, genomics tests (PCR) and several chemical tests based on mass spectrometry were developed. Among them, ELISA test (Enzyme-Linked-Immunosorbent) assay is routinely used to detect allergens in wines, because of its specificity and sensitivity, easy application and since the equipment is not required. However, commercial kits available are likely to estimate diverse forms of the researched protein. To respond to increasing demand of laboratories that need to evaluate their performances, BIPEA organizes regular proficiency testing schemes to encourage the detection and quantification of residual fining proteins in wines. The aim of this study is to describe the setting up of the tests and show the results obtained in 3 different trials for casein, ovalbumin and lysozyme analyses on white, red and rosé wines.

EXPERIMENTAL

Sample production and shipment

From October 2019 to June 2020, three different wines (white, red and rosé) were spiked with casein, ovalbumin and lysozyme at different spiking levels (from 0 to 1 mg/L).
- White wine: Graves blanc, alcoholic strength by volume: 13.5%.
- Rosé wine: IGP Sable de Camargue, alcoholic strength by volume: 12.5%.
- Red wine: IGP Pays d’Occ, Cabernet Sauvignon, alcoholic strength by volume: 13.5%.

The procedure for the preparation of the samples varies according to the added allergens in wine. For lysozyme and ovalbumin, a batch of wine was spiked with the target allergens and then homogenized and divided into series. This operation was performed using a homogenization tun. The principle of a quick successive production, which involves a quasi-simultaneous filling, ensures the homogeneity of the product between all the samples. Concerning casein, samples of wine were individually spiked using a calibrated solution.

Nine batches of samples were prepared at different concentrations (see Table 1). The homogeneity and stability of the samples were verified according to the requirements of ANXII-B of the ISO 13528 standard [7].

Results collection and data statistical treatment

From October 2019 to June 2020, the results obtained in 3 proficiency testing schemes (PTS) for detection and quantification of residual fining proteins in wines were collected. The results are compiled in three tables (see Table 1).

RESULTS & DISCUSSION

Results of the proficiency tests of October 2019, February and June 2020 (Rosé, red and white wine respectively) are examined. Table 1. summarizes the statistical data of each test for each allergen. Assigned values (x*) were estimated for all tests except for not spiked wines, for which most of the results were expressed as quantification limits. Standard uncertainties (u(x)) that allow quantification of the confidence intervals that can be given to the assigned value, were calculated as indicated in paragraph 7.7 of the ISO 13528 standard [7].

Lab results are acceptable, with only few unsatisfactory ones, however, data examination allowed to note that, in general, results are dispersed, as coefficients of variation are ≥ 21% for all PT. These data are not startling, considering the uncertainty of every technique, but are useful as method validation data to the method of analysis and the variety of ELISA kits used by the laboratories that may differ in operating method for allergen extraction and quantification. For the casein analyses, some improvements could be done to get more meaningful results, like likely to estimate diverse forms of casein and, likewise, commercial kits for ovalbumin cannot be specific for this protein but take into account other forms of albumin.

Nevertheless, dispersion may be caused also by other factors as the presence of low amount of residual fining proteins. To respond to increasing demand of laboratories that need to evaluate their performances, BIPEA organizes regular proficiency testing schemes to encourage the detection and quantification of residual fining proteins in wines. The aim of this study is to describe the setting up of the tests and show the results obtained in 3 different trials for casein, ovalbumin and lysozyme analyses on white, red and rosé wines.

Histograms in Figures 1 to 3 show the distribution of quantitative laboratories’ results of 3 PT. On these graphs, assigned value and tolerance interval are indicated in the x-axis and the results of the laboratories are shown in different colors as a function of the performed Elisa kit. Some statistics by kit are also shown in Tables 2 to 4. These data show that the lower standard deviation is observed for major kits used by laboratories, even if a lack of agreement is observed for some results. The parameters of the estimated means, standard deviation and z-scores for a result x are calculated as:

\[
\bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i
\]

\[
\sigma = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2}
\]

\[
z = \frac{x - \bar{x}}{\sigma}
\]

Laboratories with a “z score ≤ │2│” or “z score > │3│” are considered having reported “unsatisfactory” results, while the remaining laboratories (which z score is between │2│ and │3│) reported “Questionable” results. Results are published in a specific interlaboratory comparison report distributed to all participants who can then classify their results and implement some corrective and/or preventive actions if necessary.

CONCLUSION

These PTS enable the participating laboratories to draw up a general inventory of their analytical skills and improve their analytical performances in detection and quantification of residual fining proteins in wines. This program, approved and accredited by COFRAC (Comité Français d’Accréditation / French Accreditation Body), has been further developed to include beta-lactoglobulin and gluten analyses to allow laboratories to demonstrate their performances for analyses of these allergens too. Laboratories can now monitor punctually and/or continuously through time the reliability of their results and correspond to the detection and quantification procedures by the accreditation bodies according to ISO/IEC 17025 [8] for allergens analyses in wines.

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