Comparison of interlaboratory test results for sulfite analysis in food: influence of the matrix

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INTRODUCTION

BIPEA (Bureau Interprofessionnel d'Études Analytiques) is a European non-profit organization gathering more than 2900 member laboratories in the world throughout 130 countries. It offers more than 220 regular proficiency testing schemes (PTS) in various fields including food industry.

Sulfites are chemical compounds containing the sulfite ion (SO_3^{2-}), commonly used as additives in a wide range of food products. Valued for their preservative properties, they prevent oxidation reactions and inhibit microbial growth. However, despite their effectiveness, sulfites are also recognized as allergens. Their consumption can cause allergic reactions in sensitive individuals. Therefore, sulfites are listed among the 14 regulated allergens in the European Union [1], and their presence must be clearly indicated on product labeling when concentrations exceed 10 ppm, in accordance with EU and FDA regulations [2].

To ensure compliance with these requirements, laboratories are required to implement reliable methods for the detection and quantification of sulfites in various food matrices. In response to this demand, the BIPEA established a PTS in 2015 to assess the analytical performance of laboratories in sulfite determination according to ISO 17043 standard [3]. This program includes two trials per year with different matrices and currently involves approximately 40 laboratories worldwide. In this study, data from two interlaboratory tests will be analyzed: the first, conducted in April 2024 on raisins, and the second, conducted in November 2024 on shrimp. The aim is to evaluate the influence of the food matrix on the variability of sulfite quantification results at comparable concentration levels.

METHODOLOGY

Sample preparation

The protocol depends on the matrix:

Shrimp

Bipea purchased a commercial shrimp batch whose composition explicitly indicated the presence of sulfite:

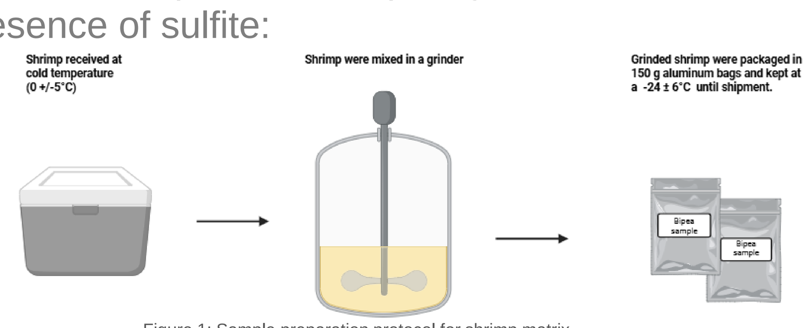

Figure 1: Sample preparation protocol for shrimp matrix

Raisins

Bipea purchased a commercial raisins batch which do not contain sulfite for spiking it:

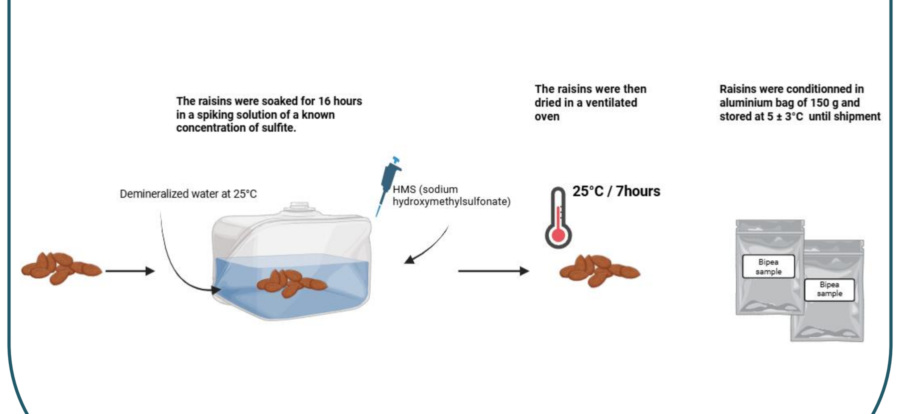

Figure 2: Sample preparation protocol for raisins matrix

Homogeneity and stability

Homogeneity

For those trials, the homogeneity between samples was checked during the step of statistical treatment of laboratories' data by comparison between the robust standard deviation of the laboratory results of the studied trial compared with previous ones on similar samples, produced according to the same procedure.

Stability

To assess the stability of shrimp samples, analyses were carried out by a subcontracting laboratory over a 2 months storage period at -24 ± 6°C. Three samples were analyzed in duplicate two months after production and compared with initial homogeneity control. Analyses showed a sulfite decrease, but samples remained stable within the subcontractor's uncertainty.

The same study was conducted on samples of spiked raisins stored at 5 ± 3°C for 6 weeks. The results showed that the samples were stable over this period.

Analysis by laboratories

Samples were shipped refrigerated to all participants who are invited to analyze the samples as soon as possible after reception. In this study, laboratories were asked to quantify sulfite in food matrix.

Below are the methods commonly used by laboratories during trials.

- Monier-Williams AOAC 99.28
- Chemical method according to NF EN 1988-1
- Enzymatic method according to EN 1988-2
- ISO 5522
- Other method

Statistical treatment

The participants were required to return their results on sulfite quantification and the method used through an online reply form. Statistical treatment was conducted according to ISO 13528 [4]. Assigned values (x_{pt}) was estimated using the robust means of the results from application of robust algorithm A. Performances of each laboratory were evaluated using a tolerance value (VT) of 50% of x_{pt}. This value is used to identify an interval around the assigned value. Results in this range are considered as satisfactory. Moreover, laboratory results (x_i) were also evaluated through z-scores (z). The z-score for a result x_i is calculated as :

$$z_i = \frac{(x_i - x_{pt})}{\sigma_{pt}}$$

Where σ_{pt} is the standard deviation for proficiency assessment (VT/2).

The results can then be classified as follows:

- $z_i \leq |2|$: **satisfactory**
- $|2| \leq z_i < |3|$: **questionable**
- $z_i > |3|$: **unsatisfactory**

RESULTS and DISCUSSION

In this study, we collected and analyzed the results submitted by laboratories for two interlaboratory tests: one conducted in April 2024 on raisins, and another in November 2024 on shrimp. These tests allowed the evaluation of laboratories' performance in determining sulfite concentration in the respective matrices. A total of 32 laboratories reported results for the raisin analysis, and 23 laboratories for the shrimp analysis.

The table below describes the key statistical parameters for each of the two tests, and figures 3 and 4 are histograms illustrating the distribution of the results obtained for each trial.

The dispersion is lower for raisins (robust s = 14.4 mg/kg; CV ≈ 21%), as indicated by histograms clustered around the assigned value. Conversely, shrimp show greater variability (robust s = 39.1 mg/kg; CV= 47%), with a wider spread of results.

Statistical parameter	Trial of April 2024 – Raisin matrix	Trial of November 2024 - Shrimp matrix
Assigned value		
Number of returned results	32	23
Assigned values for proficiency testing (x_{pt}) (in mg/kg)	281,5	303,6
Number of results taken into account for x_{pt} estimation	26	21
Robust standard deviation of the results s(x_{pt})	14,4	39,1
Coefficient of variation (%) CV (x_{pt})	21	47
Proficiency		
Tolerance value (VT)	117,2	286,6

Table 1 – Statistical treatment of the results

Dispersion of raisins matrix results < ***Dispersion of shrimp matrix results***

No significant effect of the method was observed

Results suggest a possible matrix effect

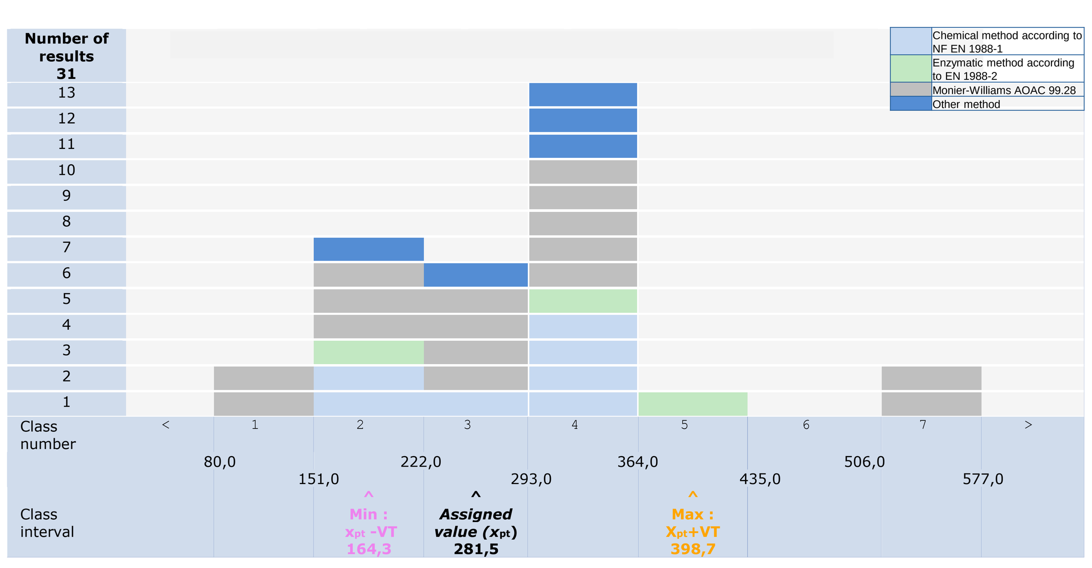

Figure 3: Histogram of results on raisins matrix trial

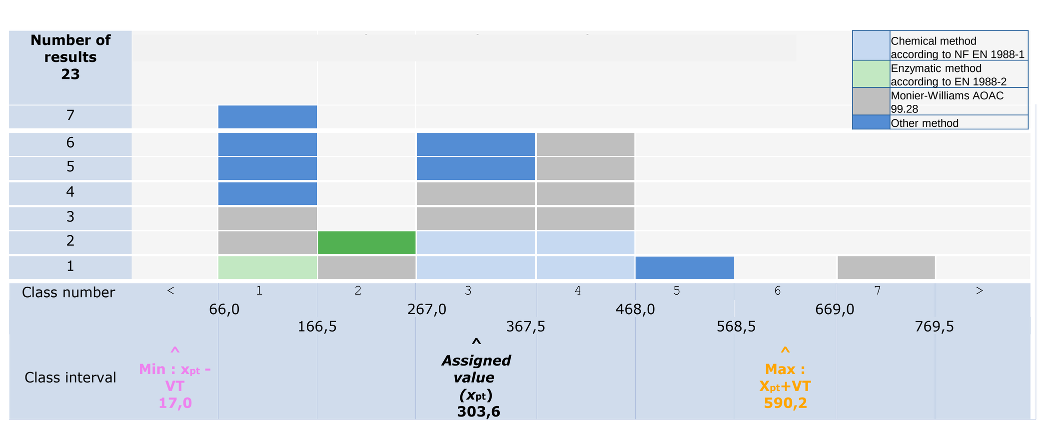

Figure 4: Histogram of results on shrimp matrix trial

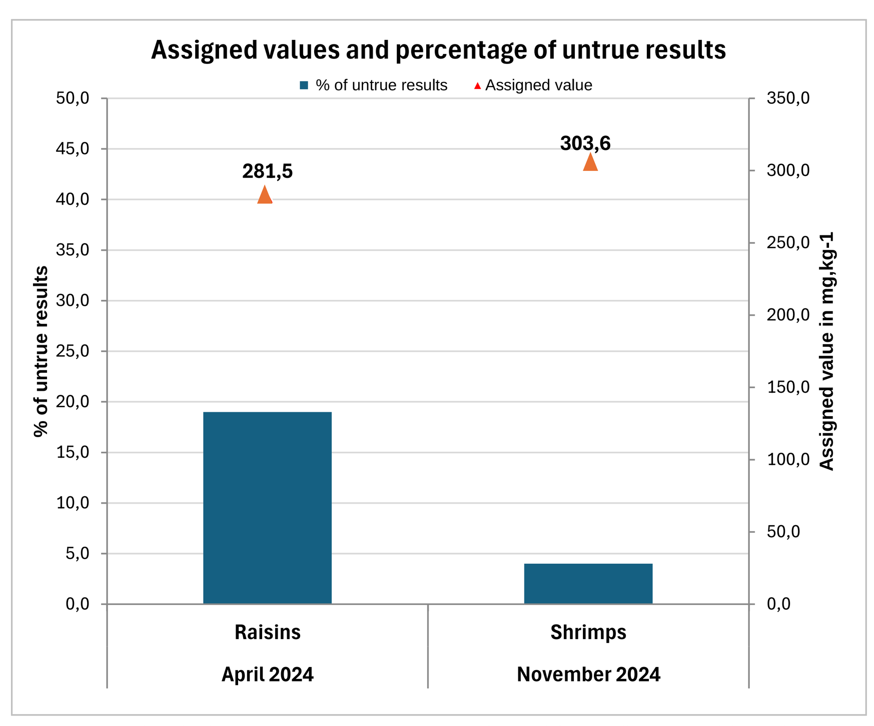

Figure 5: Assigned values and percentages of untrue results for trials of April 2024 on raisins and November 2024 on shrimp

❖ This figure illustrates the proportion of questionable and unsatisfactory results by assigned value and matrix for each trial.

Figure 5, which illustrates the proportion of questionable and unsatisfactory results and assigned value by matrix, shows a higher proportion for raisins compared to shrimp at comparable concentration level. This difference is partly explained by the higher VT calculated for shrimp, showing that the matrix not only affects analytical results but also influences statistical treatment and the evaluation of laboratory performance during PTS.

The complex composition including proteins, lipids, and higher water content makes extraction and accurate measurement more difficult, resulting in greater dispersion across laboratories results.

These differences highlight that laboratories must be more careful and rigorous in choosing and implementing methods for complex matrices. Adapting and optimizing analytical methods according to the nature of the matrix is therefore essential to reduce the dispersion of results and ensure their reliability.

CONCLUSION

The results of the trials highlight the influence of the matrix on the determination of sulfite concentrations. The development and implementation of reliable and robust methods are therefore essential to ensure precise quantitative data, directly contributing to food safety and regulatory compliance. Finally, participation in interlaboratory studies proves to be an indispensable tool for assessing method performance, identifying potential sources of variability for laboratories.

[1] European Union. Regulation (EU) No 1169/2011 on the provision of food information to consumers. Off J Eur Union. 2011;L 304:18–63.
[2] U.S. Food and Drug Administration. *Food labeling: declaration of sulfiting agents in foods*. Code of Federal Regulations Title 21, Part 101.100(a)(4). 2023.
[3] ISO 17043 - Conformity assessment - General requirements for proficiency testing.
[4] ISO 13528 - Statistical methods for use in proficiency testing by interlaboratory comparison