Proficiency - testing scheme for histamine detection in fishery product

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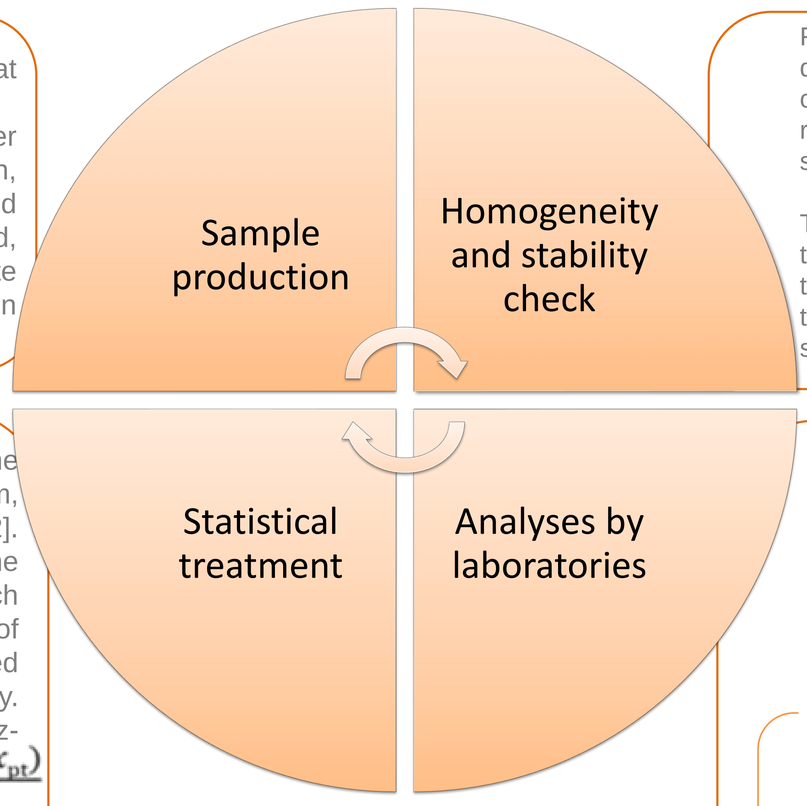
INTRODUCTION

Histamine, a biogenic amine, is a toxin generated by bacteria in the fish's tissues. Histamine fish poisoning results from the consumption of inadequately preserved and improperly refrigerated fish. Thus, this metabolite is an indicator of fish quality and a biomarker for quality control during the food production and transportation.
The number of laboratories performing the analyses of histamine has gradually increased in recent years. There are various analytical methods available for quantifying histamine in food samples, with most relying on physicochemical analyses. These latter are considered more conventional compared to enzymatic method wich is a valuable alternative for laboratories.
Bipea set up a regular proficiency-testing scheme intended to the detection and quantification of histamine in fishery products. Homogeneous and stabilized samples of naturally or artificially contaminated fishes were prepared and shipped to the laboratories that were required to return their results indicating the applied methods. The statistical treatment of the data was performed by BIPEA according to ISO 13528 standard [1]. Assigned (consensus) values were calculated from the participants' results according to comparable methods and the performances of the laboratories could then be evaluated individually and collectively according to ISO 17043 standard [2]. The collected results enable a comparison of histamine quantification based on the analytical method performed.

METHODOLOGY

Manufacturing involved contaminating fresh fish with histamine at given levels of concentration.
A defined quantity of fish was ground using the Stephan grinder until a paste-like product was obtained. Then, the spiking solution, which consisted of histamine diluted in acetone, was added and mixed again with the grinder to ensure even distribution. Afterward, the mixture was transferred into a pneumatic dispenser to distribute it into 100g jars via the conveyor. The samples were then frozen and stored at -20°C ± 2°C.



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.

The stability of the samples was checked during the statistical treatment through the follow-up of the robust standard deviation and the examination of participants' results consistency with previous trials on similar products, which demonstrated that they were sufficiently stable for the duration of the test.

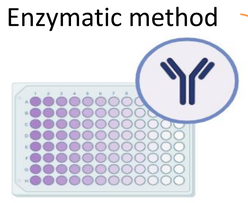
The participants were required to return their results on histamine quantification and the method used through an online reply form, Statistical treatment was conducted according to ISO 13528 [2]. Assigned values (x_{pt}) was estimated using the robust means of the results from application of robust 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**

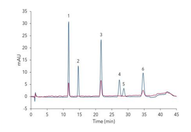
Once the homogeneity and the stability has been demonstrated, the sample were shipped frozen to all participants who are invited to analize the samples as soon as possible after reception,, In this study, laboratories were asked to quantify histamine in fish:

Enzymatic method

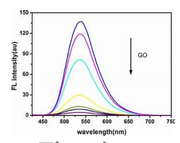


or

Physicochemical methods



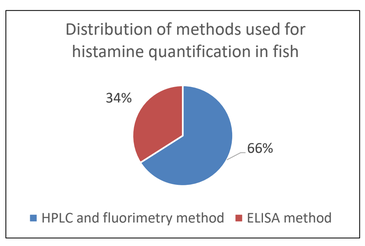
HPLC



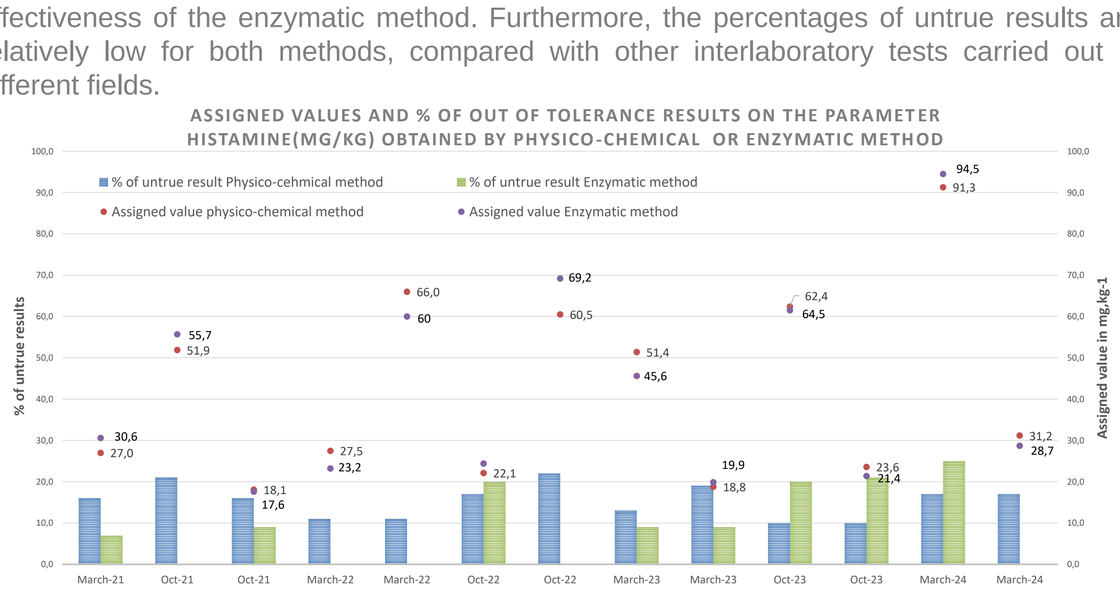
Fluorimetry

RESULTS and DISCUSSION

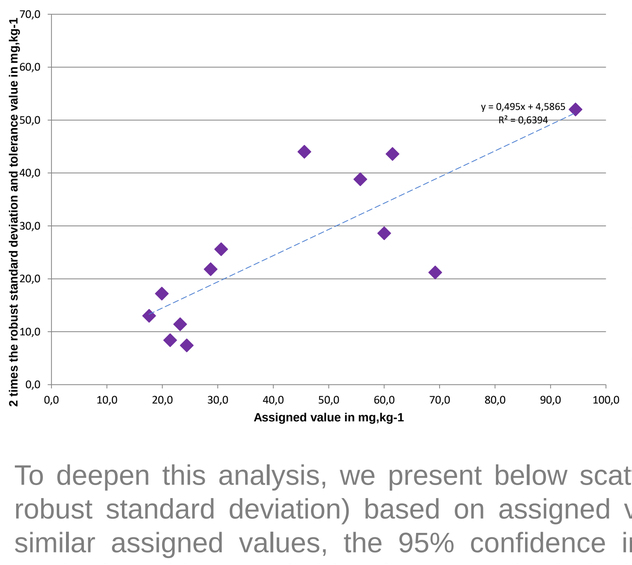
In this study, we have collected and analyzed the results submitted by laboratories on the 13 interlaboratory tests carried out since March 2021. An average of 36 laboratories reported results for all these tests, with the repartition between physicochemical and enzymatic methods shown opposite. Physicochemical methods such as HPLC or fluorimetry remain the most common among the participating laboratories.



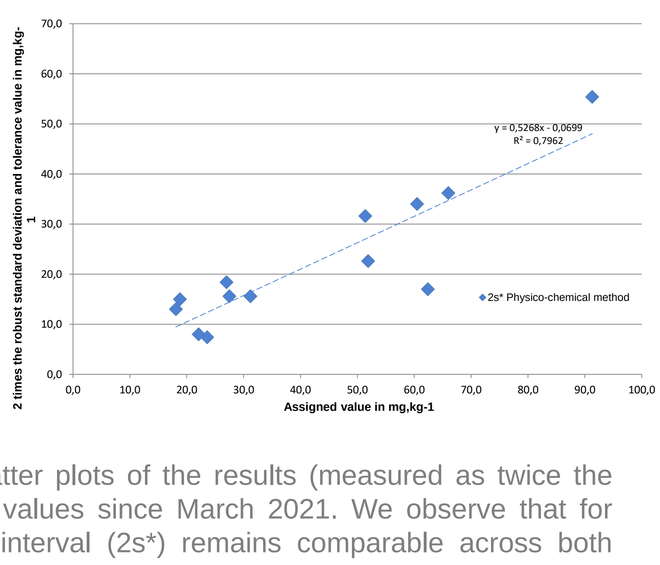
The graph below presents a comparison of the assigned values obtained for each test according to the different methods, as well as the percentage of out-of-tolerance results for each method. It is observed that the results are generally similar. In some cases, enzymatic methods yield higher values, while in others, it is the physicochemical methods. No consistent trend can be identified. It is observed that the percentage of incorrect results, meaning the values provided by the laboratories that are not accounted into the statistical treatment, is generally higher for physicochemical methods. This suggests a greater effectiveness of the enzymatic method. Furthermore, the percentages of untrue results are relatively low for both methods, compared with other interlaboratory tests carried out in different fields.



***Scatter plot: 2s** of the histamine parameter as a function of the assigned values obtained by enzymatic method**



***Scatter plot: 2s** of the histamine parameter as a function of the assigned values obtained by physicochemical method**



To deepen this analysis, we present below scatter plots of the results (measured as twice the robust standard deviation) based on assigned values since March 2021. We observe that for similar assigned values, the 95% confidence interval (2s*) remains comparable across both methods, with remarkably close standard deviations. In other words, despite differences in analytical approach, both methods yield results with similar statistical variability. This is a significant observation, as it suggests that neither method offers a distinct advantage in terms of overall result accuracy.
This analysis thus concludes that, notwithstanding theoretical differences in expected accuracy, both methods demonstrate broadly similar performance when assessed through their 95% confidence intervals. This finding has important implications for practical application, as it underscores that tolerances and statistical accuracy are equivalent. Therefore, factors such as method complexity, cost, resource availability, and ease of implementation should guide the choice of the most suitable method.

CONCLUSION

In conclusion, this study suggests that both methods yield equivalent results. This provides laboratories with a wider range of options, allowing them to choose a method for histamine quantification based on their resources and technical expertise. The role of interlaboratory testing is crucial, as it enhances the robustness of these findings and ensures consistency across different laboratories. Further studies are needed to confirm these trends and assess the influence of various factors on method effectiveness.

REFERENCES

[1] **ISO 13528** - Statistical methods for use in proficiency testing by interlaboratory comparison
[2] **ISO 17043** - Conformity assessment - General requirements for proficiency testing.