

Development of reference material in a fish matrix for physicochemical proficiency testing

Desenvolvimento de material de referência em matriz pescado destinado a ensaio de proficiência físico-químico

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Abstract

For the fishery chain's growth and development to occur sustainably, it is necessary to develop solid scientific and technological bases that help guarantee the quality of this raw material. Analytical results provide one of the main ways to prove the quality of these products. In this context, developing reference materials for proficiency testing in fish matrix contributes to this raw material's scientific, technological, and quality advancement. This study describes the production steps of a reference material destined for proficiency assays in a fish matrix. The study covers material selection, preparation, and development of homogeneity and short-term stability studies simulating transport conditions. The results were evaluated using Horwitz standard deviation and standard deviation by experience from previous rounds of proficiency testing programs, and comparisons were made between the two. It was found that the batch of material produced was homogeneous and stable during the stability study, enabling its use in proficiency tests.

Keywords: fish; proficiency testing; quality.

Resumo

Para que o crescimento e desenvolvimento da cadeia pesqueira aconteça de maneira sustentável, é necessário o desenvolvimento de bases científicas e tecnológicas sólidas, que auxiliem na garantia da qualidade desta matéria-prima. Uma das principais maneiras de comprovar a qualidade destes produtos é fornecida por meio de resultados analíticos. Neste contexto, o desenvolvimento de materiais de referência destinados a ensaios de proficiência em matriz de pescado contribui para o avanço científico, tecnológico e de qualidade desta matéria-prima. Este trabalho descreve as etapas de produção de um material de referência destinado a ensaios de proficiência em matriz pescado. O estudo abrange as etapas de seleção do material, preparação e desenvolvimento dos estudos de homogeneidade e estabilidade a curto prazo, simulando condições de transporte. A avaliação dos resultados foi apresentada por meio de desvio padrão de Horwitz e desvio padrão pela experiência de rodadas anteriores de programas de ensaio de proficiência, realizando-se comparações entre ambos. Verificou-se que o lote de material produzido apresentou-se homogêneo e se manteve estável durante o estudo de estabilidade, possibilitando sua utilização em ensaios de proficiência.

Palavras-chave: peixe; ensaios de proficiência; qualidade.

1. INTRODUCTION

The hygienic sanitary control of food is a preponderant factor in preventing foodborne diseases, which highlights the work developed in testing laboratories, which perform quality control of food to ensure the safety of these products (GERMANO, 2022; AUGUSTIN, 2006). It is recommended that laboratories that perform quality control of food use internationally recognized methods and submit them to internal and external quality controls (KOCH et al. 2011). One of the tools that help to ensure the quality and reliability of analytical results is participation in proficiency testing (PE), a quality control tool in which characterized, sufficiently homogeneous, and stable test items are sent to different laboratories. Thus, the PE allows comparing the performance obtained by a laboratory in the analysis of the same test item in specific parameters (THOMPSON et al. (2006).

The availability of reference materials (RM) in a fish matrix intended for proficiency testing, is incipient due to the high complexity of developing these materials. With the increase of competitiveness in the market, industries have adopted more rigid quality norms as standard behavior. Thus, laboratories must present a quality program that ensures the traceability of all operations involved in the process (ULRICH, 2011).

Among the various quality parameters evaluated in fish, sorbic acid and total volatile bases are highly relevant given the peculiar chemical constitution of this raw material, which gives it a nutritional richness and high deterioration potential. The total volatile bases parameter is one of the most used tests to determine the freshness of fish. The total volatile bases are nitrogenous compounds formed when the fish is deteriorating (SCHERER, 2004). Endogenous enzymes produce them of bacterial origin, responsible for the loss of freshness and the appearance of the first signs

of putrefaction in fish (OLIVEIRA, 2014). Brazilian legislation determines the maximum value of total volatile bases for fish (except for some species with different levels) of 30 mg of nitrogen per 100 g (BRASIL, 2017).

Sorbic acid is a widely used food additive for food preservation due to its ability to slow and/or inhibit microbial growth [8]. The use of sorbic acid in fish and fish products is permitted by RDC No. 329 of December 19, 2019, which establishes which food additives and technology adjuvants are authorized for use in foods, determining the maximum limit of 0.2 g per 100 g, thus reinforcing the importance of controlling this additive (BRASIL, 2019).

Due to the lack of available RM and its necessity in the quality assurance and reliability of the assays performed in food analysis laboratories, this study aimed to develop a batch of RM in a fish matrix to be used in a PE to determine Sorbic acid and total volatile bases.

2. MATERIAL AND METHODS

2.1 Material

The RM was prepared in the Matrix Production Laboratory of the Proficiency Testing Provider of the SENAI Institute of Technology in Chapecó (Santa Catarina State, southern Brazil). The batch of 113 material items was prepared from cooked fish filet, water, fat, and additional constituents purchased in Chapecó. The homogenization of the material was done in a cutter for 10 min. After homogenization, the material was filled into aluminum cans and presented with 40 g of the material in wet homogenized form. These were then sterilized by moist heat at 121 °C for 55 min. After the sterilization process, these were stored under a controlled temperature of 25 ± 3 °C.

2.2 Physicochemical determinations

Determinations were made in the Physicochemical Laboratory of the SENAI Institute of Food and Beverage Technology in Chapecó. The material was evaluated by determining sorbic acid and total volatile bases according to methods standardized by the Ministry of Agriculture, Livestock, and Supply (MAPA). The determinations were performed in duplicate under random repeatability conditions.

2.3 Homogeneity analysis

For the homogeneity evaluation, 12 samples were selected using the random number function of Microsoft Visual Basic. These samples were stored under a controlled temperature of 25 ± 3 °C until the moment of the material measurement. The results obtained were evaluated for the presence of Outliers through Cochran's test according to ISO 5725-2 [5]. Homogeneity was evaluated according to the model described in ISO 13528:2015 [6] by calculating the between-samples standard deviation. The Horwitz standard deviation and the standard deviation obtained from experience with previous programs were used as criteria for evaluating the between-sample standard deviation. In both models, the between-sample standard deviation should be less than or equal to 0.3 times the limiting standard deviation.

2.4 Stability analysis

The stability of the material and its properties were monitored in the short term. In order to evaluate the influence of temperature, during transport, on the values of material properties, three units of the material were sent by reverse transport to a company in the city of Belém (Pará State, north Brazil), which had temperatures during transport ranging from 10 to 41 °C. The difference between the mean of the homogeneity test and the stability test for each parameter was used to evaluate the stability of the material according to ISO 13528:2015 [6]. The Horwitz standard deviation and the standard deviation obtained from experience with previous rounds were used as criteria to evaluate the difference between the means. In both models, the difference between the means should be less than or equal to 0.3 times the limiting standard deviation.

3. RESULTS AND DISCUSSION

3.1 Homogeneity study

The results obtained in the homogeneity study are listed in Tables 1 and 2. According to criteria established by the International Harmonized Protocol [11], no outliers were detected, employing Cochran's test ($p > 0.05$) in all cases.

Table 1 - Results obtained in the homogeneity test considering the Horwitz standard deviation as SD

ASSAY	AVERAGE	STANDARD DEVIATION (Ss)*	%CV ^a	0.3xσ	SS ≤ 0.3xσ
Total volatile bases (mg N/100 g)	51.24	0.00	22.64	6.79	In accordance
Sorbic acid (g/100 g)	0.0348	0.0006	0.0023	0.0007	In accordance

*Standard deviation between samples. ** Horwitz standard deviation.

Source: From the authors (2021)

Table 2 - Results obtained in the homogeneity test considering as SD, the standard deviation obtained from the experience of previous rounds of proficiency testing programs.

ASSAY	AVERAGE	STANDARD DEVIATION (Ss)*	%CVA	0.3 \times σ	SS \leq 0.3 \times σ
Total volatile bases (mg N/100 g)	51.24	0.00	22.5%	3.47	In accordance
Sorbic acid (g/100 g)	0.0348	0.0006	25%	0.0026	In accordance

^a Coefficient of variation (CV) based on the provider's experience from proficiency testing in previous programs, expressed as a % relative to the mean value of the analyte.
*Standard deviation between samples.

Source: From the authors (2021)

The estimate of standard deviation for the total volatile bases measurement in Tables 1 and 2 was expressed as zero "0.00" because the estimate of between-sample variance (Ss) is relatively smaller than the within-sample variance (Sw). This can be expected when the proficiency test items are highly homogeneous, and in these cases, the between-sample variance is expressed as zero.

The results presented in Tables 1 and 2 indicate that the Horwitz standard deviation criteria and the standard deviation based on experience with previous rounds of proficiency testing programs were met for both parameters, indicating that both approaches are applicable for assessing material homogeneity. Adopting the standard deviation with experience from previous rounds of proficiency testing, according to ISO 13528:2015(E), presents as the main advantage in determining the mean and standard deviation based on reasonable estimates of performance, includ-

ing the varied behavior of the different methodologies applied for the determination of the analyte. As a premise, it is expected that the values are based on runs with matrices similar to the one studied, with similar values for the parameters, and where laboratories use similar techniques.

Hence, we can conclude that the material in question was homogeneous for both parameters considering both the Horwitz standard deviation and standard deviation obtained through experience in previous rounds of the proficiency testing program as criteria, indicating that the production process of the material was effective in ensuring the homogeneity of the analyte.

3.2 Stability study

The results obtained by evaluating the stability under transport conditions are shown in Tables 3 and 4.

Table 3 - Results obtained in the stability test, considering the Horwitz standard deviation as SD.

ASSAY	AVERAGE*	STANDARD DEVIATION (Ss)	%CV ^a	0.3 \times σ	SS \leq 0.3 \times σ
Total volatile bases (mg N/100 g)	54.56	3.32	22.64	6.79	In accordance
Sorbic acid (g/100 g)	0.3220	0.0025	0.0023	0.0007	In accordance

^aAverage value obtained from samples submitted to transport conditions.
^{**} Horwitz standard deviation.

Source: From the authors (2021)

Table 4 - Results obtained in the stability test, considering as SD the standard deviation obtained from the experience of previous rounds of proficiency testing programs.

ASSAY	AVERAGE*	STANDARD DEVIATION (SS)	%CVA	0.3 X Σ	SS \leq 0.3 X Σ
Total volatile bases (mg N/100 g)	54.56	3.32	22.5%	3.46	In accordance
Sorbic acid (g/100 g)	0.3220	0.0025	25%	0.0026	In accordance

* Coefficient of variation (CV) based on the experience of the provider of proficiency testing programs. Expressed in %, relative to the analyte value.

* Average value obtained from samples submitted to transport conditions.

Source: From the authors (2021)

Tables 3 and 4 show that the sorbic acid parameter in the matrix in question was unstable when the Horwitz standard deviation was used as a decision criterion. Nonetheless, assuming the criterion of experience from previous rounds of proficiency testing programs, both parameters were stable in the matrix simulating transport conditions.

This fact indicates that during the performance of a proficiency testing program for the sorbic acid parameter, a more dispersed behavior of the values found is expected, depending on the methods used by the laboratories participating in the program, decreasing the significance of the instability in the material. Thus, we can conclude that the material has acceptable stability under reverse transport conditions to be used as reference material in a proficiency testing program.

4. CONCLUSIONS

Based on the results obtained, one can conclude that the production process described in this article has generated a homogeneous and stable reference material. In the short term, this propitiates its use in proficiency testing programs in the fish matrix for total volatile bases and sorbic acid parameters.

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