

Production and characterization of a bovine liver candidate reference material.

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Abstract: The preparation of a bovine liver candidate reference material and the steps taken to confirm its homogeneity, long and short term stabilities, and consensus values are described. Details of the sample preparation and the final collaborative exercise are presented. The material elemental composition was characterized by 17 elements (As, Ca, Cd, Co, Cu, Fe, K, Mg, Mo, Mn, Na, P, Pb, Se, Sr, V, and Zn) of nutritional and toxicological significance.

Keywords: reference material; bovine liver; proficiency test; analytical quality control.

1. INTRODUCTION

Brazil is one of the largest exporters of beef cattle in the world. According to the Ministry of Agriculture, in 2020 it is expected that domestic production of meat will supply 44.5% of the global marketplace [1]. Papers should be, preferentially, written in English. If your paper is written in Portuguese or Spanish, an English version of both, title and abstract, should be provided.

Increasing demands are being placed on the analytical scientific community to provide information on a growing range of inorganic and organic constituents in a vast array of commodities, including meat and meat products. Because of the rather wide variety and origins of such products and the wide range of elemental contents that are encountered, meats are subject to extensive routine monitoring for both toxic and essential elements. One of the ways to certify the quality and safety of meat for national consumer and exportation is through the determination of

nutrients and toxic elements. These analyses call for the need for development and validation of analytical methods able to determine such elements with appropriate precision and accuracy.

To evaluate some figures of merit for method validation, control the quality of analyzes and consequently the quality of the product, certified reference materials (CRM) are needed, preferentially with properties similar to the matrix to be analyzed [2]. The production of CRMs and reference materials (RM) in Brazil is still incipient [3,4].

With the view to attempting to fill some of the gaps, there is a need to contribute to the technological development of the country, increasing the availability of new reference material in various areas have become targets of researchers and institutions [5,6]. In this context, the preparation and characterization of a bovine liver candidate reference material were performed, according to with ISO series 30-35.

2. EXPERIMENTAL

2.1. Preparation of bovine liver

The liver used in this study was harvested from 57 Nelore breed (*Bos indicus*) cattle provided from breeding experiments developed at Embrapa Southeast Livestock (São Carlos SP, Brazil). The bovine livers were collected according to the Embrapa's "animal ethic commission", and were derived from clinically healthy animals, produced under sanitary conditions.

The cattle were fed on pasture and in the last 90 days in the feedlot. The animals were slaughtered in a commercial environment slaughtered, and livers were collected 24 h after slaughter. Approximately 320 kg fresh weight of liver was collected, washed with demineralized water and cutting in small pieces with ceramic knives to minimize contaminations. The livers were placed in polyethylene bags and frozen at -20°C until further processing. The frozen pieces of liver were then ground in a stainless steel comminuting machine (Skymesen, Brusque SC, Brazil), placed on polyethylene-lined trays, frozen, and lyophilized in a commercial food industry (Liofoods, Araras SP, Brazil). About 34 kg of lyophilized material was then ground in high-speed centrifuge mill (ZM 200, Retsch, Germany), by using 500 µm in the first step and 250 µm in a second one to obtain smaller particles sizes.

Afterward, the sample was divided into three buckets and homogenized using a Y homogenizer covered with inert resin (Model MA 201/5MO Marconi, Piracicaba, Brazil). About 3 kg of material was mixing during each run and stirred for 15 min. The homogenizing procedure was repeated three times to ensure homogeneity.

After homogenization, the sample was bottled in previously demineralized amber glass bottles. A total of 330 vials, containing 100 g of material was bulk sterilized with 25-30 kGy of cobalt 60
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y-radiation by IPEN, Institute of Energy Nuclear Research, (São Paulo SP, Brazil).

A bottle was subjected to particle size distribution test performed at the Nuclear Center Agriculture Energy (CENA, Piracicaba – SP, Brazil), using equipment Analysette 22 MicroTec Plus (Fritsch, Germany).

2.2. Certification Project

A series of preliminary experiments was undertaken to delineate the method of determination procedure. The sample was microwave assisted digested, and inductively coupled plasma optical emission spectroscopy (ICP OES) and inductively plasma coupled mass spectroscopy (ICP-MS) were using as measurement techniques. Certified reference material (CRM) of bovine liver (SRM® 1577b) from National Institute of Standards and Technology (NIST, USA) was used to check the accuracy of As, Ca, Cd, Co, Cu, Fe, K, Mg, Mo, Mn, Na, P, Pb, Se, Sr, V, and Zn mass fraction determinations.

2.3. Homogeneity Studies

The between-bottle homogeneity test aims to check for variability among bottles. Ten bottles of the candidate material were randomly selected, and to each flask were carried out three replicates of 250 mg, the elements were determined and subjected to analyses of variance (ANOVA).

For achievement of within-bottle homogeneity testing, a flask was also randomly chosen (7 replicates) and the elements were evaluated in triplicates.

2.4. Test of Minimum Mass

The minimum mass study was conducted according to the standards recommended by the ISO GUIDE 34. This study was carried out using three different masses of beef liver candidate material: 100, 200, and 250 mg ± 0.001 mg. A

total of 7 digestions for each mass were performed. The results were evaluated based on the standard deviation values, and it was concluded that the minimum sample intake should be 250 mg, that present lower values of RSD, defined as the minimum mass. Otherwise, INAA analyses were performed using test portions in the range of 150-180 mg, and were observed homogeneity for distribution of Co, Cu, Fe, K, Mo, Mn, Na, Se, and Zn within the uncertainty of the method.

2.5. Determination of stability

Short term stability was assessed over a total of 30 days by using ICP OES determination method. Three bottles were randomly selected and subjected to a storage regime, including two-time points (0 and 30 days) in the controlled condition of high humidity $95\pm 5\%$ and temperature (40°C). Luminance was not evaluated, considering the samples were bottled in amber glasses and individually sealed in aluminum-nylon pouches.

The determination of the elements was performed in triplicate in the time 0, in the beginning of experiment, and after 30 days.

Long-term stability study was also performed using ICP OES method. Three vials of the reference material candidate were also randomly selected, storage in a cold chamber with controlled temperature and humidity (10°C and 25%). The determinations of elements were made every 8 weeks for 12 months and then the results were available by ANOVA single factor.

2.6. Collaborative study

The collaborative study beginning with the chosen and an invitation to laboratories specialized that performed biological analysis. Each laboratory received a bottle containing 100 g of the candidate material.

The laboratories were invited to apply the methods they considered most reliable in their hands for the elements they are used to do in bovine liver. The contents of the bottles were required to be mixed before each analytical sub-sample was taken. The measurements for the elements were made in six (6) steps, and all the independent results were reported, expressed on a dry mass basis. The dry mass correction factor being determined on a separate portion of the sample, according to a specified procedure (oven drying at 105°C).

3. RESULTS

3.1. Uncertainty evaluation

Ten separated vials were statistically evaluated, using analysis of variance (ANOVA) with single factor, where the F-critical was compared to F-calculated to determine the homogeneity of the available material.

The bottles presented average values within the 95% confidence interval, and the material has its homogeneity ensured for all evaluated elements, except for Zn. With results statistically different (F-calculated (3.70) > F-critical (2.39)) the material was not considered homogeneous for Zn.

3.2. Short and long-term stability

The single factor ANOVA results with a significance level of 5% ($\alpha = 0.05$) to check the short-term stability. All evaluated elements proved to be stable, with no statistically significant differences comparing the values recorded on the first day and after 30 days period.

The long-term stability data were evaluated during 12 months, and the single factor ANOVA with a significance level of 5% ($\alpha = 0.05$) and no significant differences were found in the samples ($F_{\text{critic}} > F_{\text{anal}}$). A regression analysis of the plotted data was also performed, indicating slope

(b) close to zero. Thus, the samples were stable even with open vials and stored at 10°C and 25% humidity. The obtained values were considered in the evaluation of expanded uncertainty.

3.3. Assignment of property values and uncertainties obtained

The uncertainty implies confidence in the validity of the measurement result. A value with its uncertainty allows us to evaluate the precision or accuracy of an analytical method.

It was calculated the uncertainty associated with the characterization pattern (ucar), homogeneity (ubb), the long-term stability (ults) and the expanded uncertainty of the candidate reference material (UMR).

3.4. Characterization

The characterization was based on results from either a primary analytical technique carried out by instrumental nuclear atomic atomization (INAA), or the combined results from two or more chemically independent analytical techniques obtained at Embrapa and collaborating expert laboratories, according proposed by ISO series 30-35.

4. CONCLUSIONS

The produced Brazilian bovine liver reference material is available to the laboratories and is particularly useful in the investigation of contaminants and nutrients in food analysis.

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