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Study of the Stability and Homogeneity of Powdered Egg Matrix as a Candidate for Certified Reference Material for the Determination of Centesimal Composition

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The full process of fresh egg preservation presents limitations and is associated with high transportation costs, while processed powdered eggs offer numerous advantages, such as high stability against degradation, providing extended shelf life, easy production, transportation, and favorable storage conditions. Traditional methods for determining the proximate composition of eggs and derivatives lack the capability to eliminate the matrix effect and do not guarantee adequate precision of the results. Therefore, Certified Reference Material (CRM) are the most suitable and effective tool to achieve this purpose. However, in Brazil, CRMs for powdered egg matrix are currently unavailable, although it is not possible to eliminate matrix effects, these can be included in the measurement uncertainty of each CRM. This study aimed to develop a candidate CRM for the powdered egg matrix with the requirements for the competence of testing and calibration laboratories (17034:2016) for proximate composition analyses. The analysis of variance (ANOVA) results for stability and homogeneity indicated no significant variation among the data obtained in the tests and the storage time of the material. Consequently, it was concluded that the powdered egg matrix is stable under simulated transportation conditions (short-term stability) as well as storage conditions (long-term stability) and exhibits adequate homogeneity for a CRM candidate.

Keywords: candidate reference material, egg powder, proximate composition

Introduction

Certified reference materials (CRM) are used to ensure an adequate quality control of analytical tests, assess the accuracy and reliability of measurement results and demonstrate their metrological traceability.[1-3] They can be used at all stages of the measurement process, including evaluating the laboratory's quality control and validating the developed analytical method. Thus, to guarantee the accuracy and reliability of the results, the quality of the CRM is a decisive factor. The metrologically valid procedures for the production and certification of CRM, set forth in ISO 17034:2016 and ISO Guide 35:2017.[4-8]

Currently, there is a shortage in the world's supply of reference and certified reference materials, produced by national metrology institutes, ISO 17034 accredited institutions or globally recognized institutions. In addition, it can be seen that the world's supply of CRM is greater in meat and dairy matrices.[1-4]

The desirable characteristics for a CRM are homogeneous, stable, with low volatility and low toxicity, thus favoring simpler conditions for sampling and preparing the material. The powdered egg presents an adequate matrix to compose a certified reference material. In Brazil, powdered egg matrix CRM is not yet available for the centesimal composition analyzes in this matrix.[8,9]

An important premise for the reference material is to maintain its assigned characteristics and values at any time until the end of its validity period. The validity periods include stages ranging from production, transportation to the customer; rather, it gives a stability period given the proper use and storage by the final user. Regarding these mentioned periods, two of them have special relevance for the production of reference materials, which were evaluated

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in this work: long-term stability and short-term stability. In addition to these, another important factor is the study of between-bottle and within bottle homogeneities, and the minimum sample that could be used as confidence.[2,9]

Therefore, the present work aims to develop a CRM candidate in the powdered egg matrix that meets the production minimum requirements to become a reference material according to the ISO 17034:2016 standard,[7] for application in physical-chemical analysis of moisture and volatiles (total solids), fixed mineral residue and protein. These production minimum requirements evaluated in this work were production planning, material processing (bottling), measurement procedure, homogeneity and stability assessment, and data integrity and evaluation.

Experimental

Samples

The certified reference material (CRM) candidate was produced using whole egg powder, purchased from the company Naturovos, located in the southern of Brazil (Salvador do Sul, Rio Grande do Sul, 8°7'50'' S, 52°17'25'' W). For sampling, the total amount acquired was 2 kg of material from the same lot, and all particles of the material were smaller than 75 µm.

The acquired material was homogenized for 10 min in a food processor with a capacity of 4 L (Spolu, model SPL-201, Itajubá, Brazil). Then, the total content was packed in a Stand up Pouch plastic packaging (NZB Embalagens Plásticas, São Paulo, Brazil), weighing approximately 15 g, totaling 148 units. After filling, the material was destined for the study of homogeneity between units and also for short and long-term stability. For all tests, vial selection was done randomly. Environmental conditions (temperature and relative humidity) were monitored during the filling process using a digital thermo-hygrometer (Datalogger, Tzone model, São Paulo, Brazil).

Within-bottle and between bottle homogeneity

The within-bottle and between bottle homogeneity and the minimum sample that could be used were conducted as recommended by ISO Guide 35.[2] For the study of homogeneity between units, the number of units to be studied was defined according to equation 1:[2]

$$\text{No. units} = \sqrt[3]{\text{Nprod}} \quad (1)$$

where No. units is the number of units to be used in the homogeneity study between bottles and Nprod is the total number of bottles produced (148 bottles). According to equation 1, six units were randomly selected and the tests were carried out in triplicate for each unit.

As for the within-unit homogeneity, a CRM unit was randomly selected for each analysis, and the tests were performed in seven replicates in each vial. For the study of homogeneity between units, the first and last unist were selected, and the remaining four units needed for this study were randomly selected.[10]

To define the minimum sample size, one unit was randomly selected and the total solids, fixed mineral residue and total proteins were determined varying the sample size, and this size variation was analyzed according to method determination. The tests were carried out in triplicate.[10]

The standard deviation of homogeneity (heterogeneity) (Sbb) between units was calculated using the data obtained in the analysis of variance (ANOVA) of the homogeneity study between bottles, according to equation 2.[11]

$$\text{Sbb} = \sqrt{\frac{\text{MSamong} - \text{MSwithin}}{\text{n}}} \quad (2)$$

where the MSamong and MSwithin are the mean squares of among units, and n is the number of replicates analyzed in each bottle.

The data resulting from the study of homogeneity between units were evaluated using ANOVA with a confidence interval of 95% and evaluated using the *F* test.

Short- and long-term stability

The stability study was conducted as recommended by ISO Guide 35 and the vials were randomly selected.[2]

The short-term stability study was conducted at two temperatures: at 25 °C, simulating ambient conditions, and at 50 °C, simulating an elevated temperature that may occur during the process of transportation of the material to the customer.

The temperature of 25 °C was controlled by air conditioning and monitored by a digital thermo-hygrometer (Datalogger, model Tzone, São Paulo, Brazil). For the temperature of 50 ºC, the material was kept in a drying oven and monitored in its own temperature controller (Sterilifer, model SX 1.3 DTME, Diadema, Brazil).

The storage period for assessing the short-term stability was 0, 7, 14, 28, 45 and 60 days. At the end of the conditioning period, the units were submitted to tests of total solids, fixed mineral residue and total proteins. The tests were carried out in triplicate.[10]

For long-term stability, the storage condition was 25 °C, simulating room temperature. The temperature

was maintained by air conditioning and monitored by a digital thermo-hygrometer (Datalogger, model Tzone, São Paulo, Brazil).

The storage period for assessing long-term stability was 0, 28, 60, 120, 180 and 360 days. At the end of the storage period, the units were submitted to tests for total solids, fixed mineral residue and total proteins. The tests were carried out in triplicate.[10]

Total solids

For the total solids test, the metal capsules were initially placed in a vacuum drying oven (Solab, model SL-104/27, Piracicaba, Brazil) for 1 h at 98 to 102 °C. After this period, the capsules were removed and allowed to cool to room temperature in a desiccator. The initial weight of the capsule was determined, after weighing on an analytical balance (Ohaus, model NEW PIONEER PR224BR, calibration certificate accredited ISO 17.025 No. 26886 under accreditation CAL 0756, New Jersey, USA), the mass of 2.0 ± 0.0001 g of solid egg and the capsules were transferred to the vacuum oven, kept at 100 ± 2 ºC for 5 h, keeping a minimum vacuum of 25 mmHg.

After the drying period, the capsules were transferred to a desiccator and, after reaching room temperature, they were weighed on an analytical balance and the drying process was repeated approximately 3 times until obtaining a constant mass using drying time in an oven for 1 h.[10]

Fixed mineral residue

For the fixed mineral residue test, the crucibles were heated in a muffle furnace (SPLabor, Presidente Prudente/SP, model SP-1200DM/F) at a temperature of 550 ± 25 °C for 30 min. After this period, the crucibles were removed and allowed to cool at room temperature in a desiccator. The initial crucible weight was determined and then 2.0 to 3.0 ± 0.0001 g of the sample were weighed in the crucible.

The crucibles were placed in the muffle at 550 ± 25 ºC for 4 h and after this period, the crucibles were transferred to a desiccator and, after reaching room temperature, they were weighed on an analytical scale and the drying process was repeated approximately 4 times until obtaining a constant mass using a drying oven for a period of 1 h.[12]

Protein

0.250 ± 0.0001 g of sample was weighed and transferred to a micro Kjeldahl digestion tube, along with 10 mL of P.A. sulfuric acid (Qhemis, São Paulo, Brazil), and approximately 8 g of catalytic mixture (1 g of sodium sulfate and 7 g of hydrated copper sulfate both from Synth, Diadema, Brazil). Then, the samples were heated in a nitrogen digester block (Marconi, model MA402, Piracicaba, Brazil), initially at 150 °C and subsequently at 420 °C for 2 h.

The samples were distilled and titrated with a 0.10 mol L^{-1} hydrochloric acid solution (Synth, Diadema, Brazil). The titrant solution had its concentration checked with sodium carbonate type certified reference material (BAM certified, Certificate No. U8-1218 for reference material, Sigma-Aldrich, Darmstadt, Germany). The operating conditions of the nitrogen distiller were verified using certified reference material type ammonium sulfate (CPA chem, Bogomilovo, Bulgaria, lot 690322).

Results and Discussion

Limit of quantification (LOQ), precision and combined uncertainty

The limit of quantification (LOQ) represents the lowest concentration of an analyte that could be determined with confidence, and it was verified that the LOQ of total solids was 5%, and the fixed mineral residue and protein was 0.5%. The combined uncertainty, in relative terms, was 0.21% for total solids, 3.10% fixed mineral residue and 3.51% for protein.

Intra-flask and inter-flask homogeneity

The results obtained in the evaluation of within-unit homogeneity were 3.57 ± 0.02% for fixed mineral residue, for protein 46.37 ± 0.17% and for total solids 95.36 ± 0.10%.

CRM has no standardized reference values for standard deviation or measurement uncertainty; however, it is observed that the relative standard deviation values obtained for homogeneity between units are below the precision criteria for tests conducted under repeatability conditions, because according to the Ministry of Agriculture, Livestock and Food Supply (MAPA),[13] the limit values of relative standard deviation in terms of the concentration of the analyte in the sample are 2.7% for fixed mineral residue and 1.3% for protein and for total solids, and the observed value was 0.56 and 1.3% for protein and for total solids, and the values obtained were 0.37 and 0.10%, respectively. Such comparison can be performed since the study of homogeneity between units is conducted in the same way as the evaluation of the repeatability of the methods and was compatible with the standard deviation observed in other works.[10,14]

The minimum amount of sample that did not show a significant difference when compared with the amounts recommended by the test method, was 0.05 g for protein, 1 g for fixed mineral residue and 1.5 g for total solids.

The results of the homogeneity study between units are shown in Figure 1.

The values obtained in the homogeneity study between units were: 3.54 ± 0.07% for fixed mineral residue, 46.77 ± 0.22% for total protein and 95.25 ± 0.09% for total solids. The data resulting from the evaluation of homogeneity between units did not show a significant difference, according to the ANOVA study conducted at 95% confidence rate. This result shows that the material presents adequate homogeneity for a CRM candidate, as it is compatible with the uncertainty obtained by NIST in CRM 1845a. The uncertainties values of NIST CRM 1845a are 0.37% for total solids, 0.078% for ash, and 0.47% for protein.[15]

In addition, the values of fixed mineral residue, protein and total solids are compatible with the technical regulation of identity and quality for powdered egg.[16]

The values obtained for the standard deviation of homogeneity were 0.03% for fixed mineral residue, 0.04% for protein and 0.03% for total solids. Considering that the approach to estimate the variance was one-factor, it can be considered that the square of the standard deviation of homogeneity (Sbb^2) is identical to the uncertainty of homogeneity between units $(\mu bb^2)^2$ and will be used to estimate the total uncertainty of the CRM.[2]

Short- and long-term stability (ubb)

The results obtained for the ubb, containing the respective determination coefficients and straight-line equations, are shown in Figure 2.

Figure 2 shows the angular coefficient (slope) very close to zero, suggesting the stability of the material under simulated transportation conditions. The ANOVA analysis shows, with a confidence of 95% in the results, that there was no significant difference between the means obtained in each vial. The results referring to the long-term stability study are shown in Table 1.

The ANOVA analysis with a confidence rate of 95% for the results indicates that there was no significant difference between the means obtained for each parameter. The equation of the straight line was calculated according to equation 3, where a corresponds to the linear coefficient, b to the angular coefficient, Y to the concentration of the

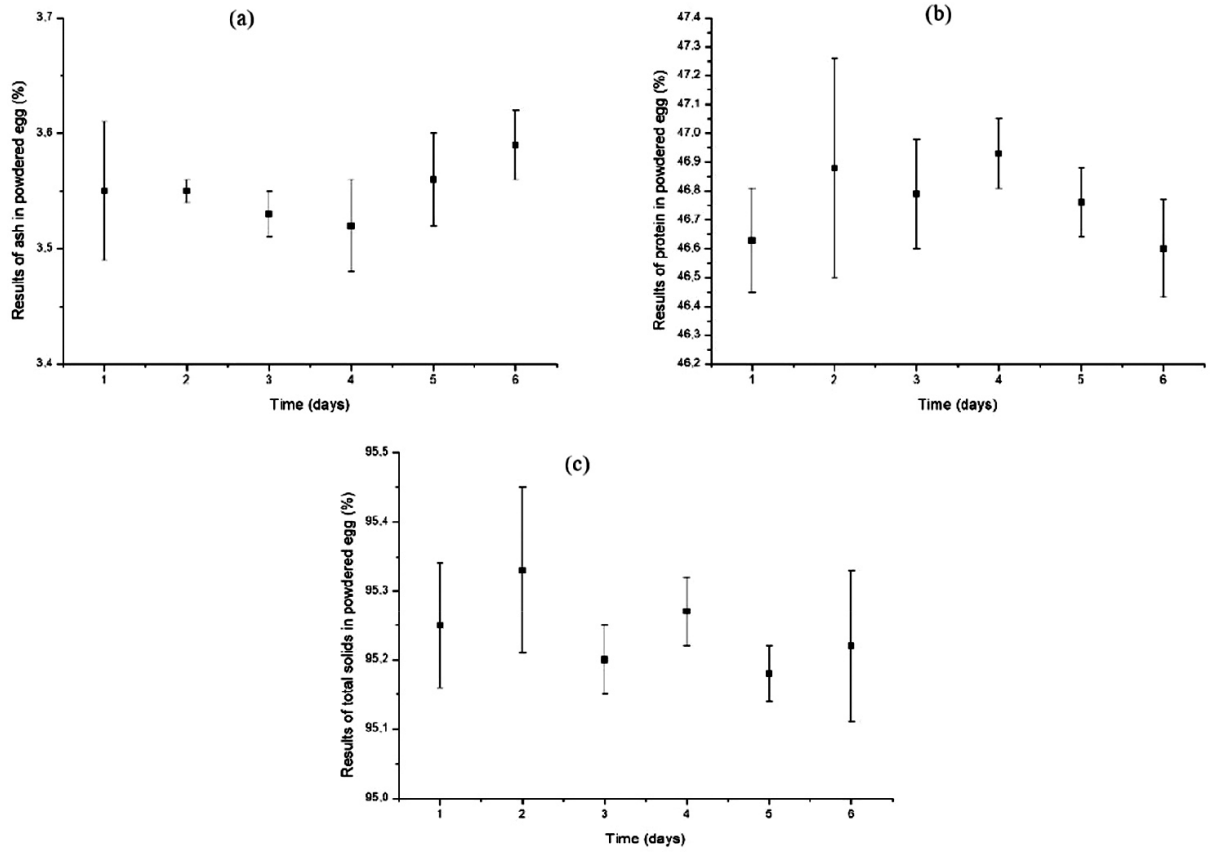

Figure 1. Homogeneity results between units, (a) fixed mineral residue, (b) total protein and (c) total solids. Error bars refer to the standard deviation of 3 measurements from each vial.

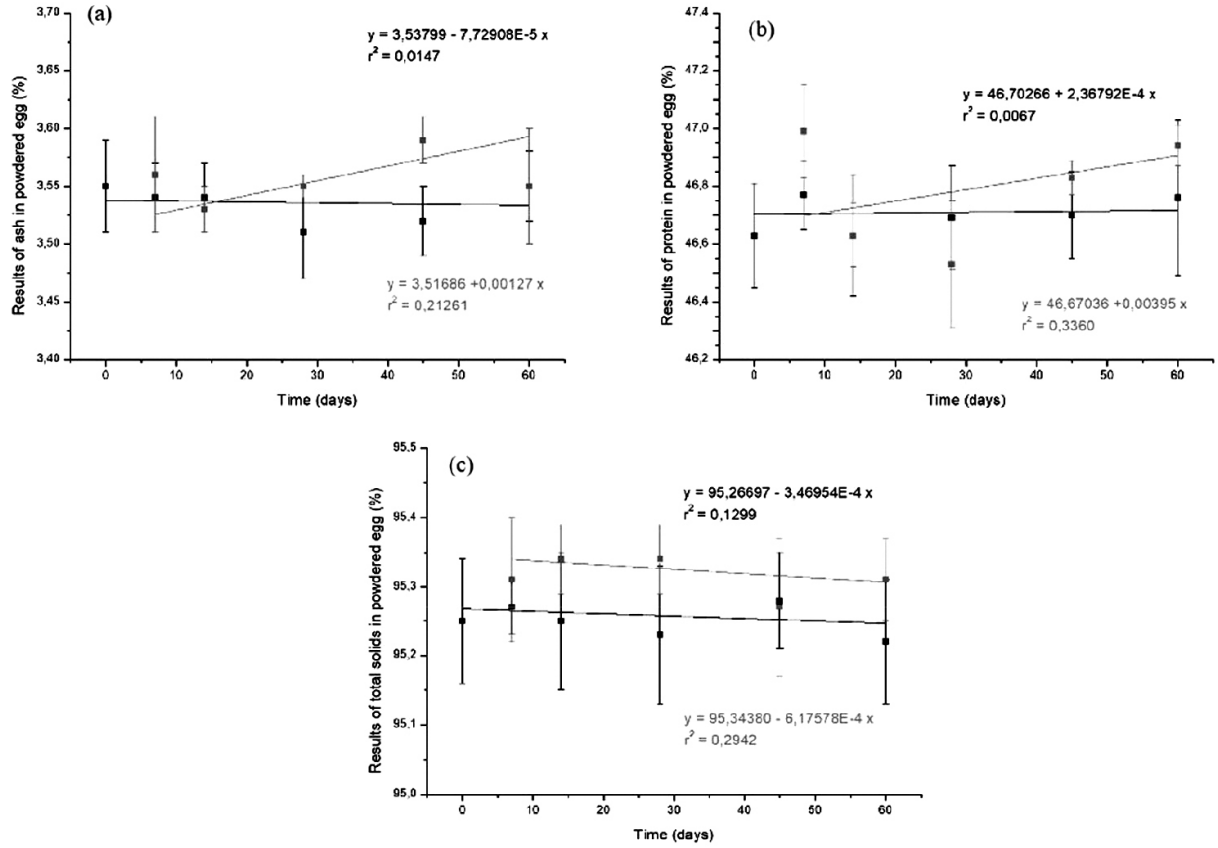

Figure 2. Short term stability results at 25 °C (black) and 50 °C (grey), (a) fixed mineral residue, (b) protein and (c) total solids. Error bars refer to the standard deviation of three measurements from each vial.

Table 1. Calculated *t* values for the short-term stability study

Parameter	Average / %	Standard deviation / %	Storage temperature / °C	Angular coefficient	Standard deviation of the angular coefficient	*t* calculated (t_b)	μ_{lts}
Fixed mineral residue	3.52	0.04	25	-8.26×10^{-5}	8.49×10^{-5}	0.97	0.031
Total protein	46.73	0.16	25	2.51×10^{-4}	1.74×10^{-4}	1.44	0.063
Total solids	95.28	0.09	25	2.44×10^{-4}	2.88×10^{-4}	0.84	0.105

μ_{lts}: long-term stability measurement uncertaint.

analyte and X to the elapsed time of the study in days.[2,11,16]

$$Y = a + bX \quad (3)$$

From the angular coefficient data (b) and the angular coefficient standard deviation (s(b)), the calculated *t* (t_b) was calculated for each equation of the line, as described in equation 4 and are contained in Table 1:[2,11,16]

$$t_b = \frac{|b|}{s(b)} \quad (4)$$

The calculated *t* values were compared with the two-tailed Student's *t*-value with 95% with 3 degrees of freedom, $t_{0,95,3} = 3.18$. Of the calculated data, no value exceeded the Student's *t*-reference value ($t_{0,95,3}$), evidenced that there is no significant variation between the results obtained in the tests and the storage time of the material, concluding that the CRM is stable both under simulated transportation conditions (short-term stability study) and under storage conditions (long-term stability study).[11,16]

The long-term stability measurement uncertainty (μ_{lts}) was calculated according to equation 5, where (s(b)) corresponds to the standard deviation of the angular coefficient and *t* to the duration of the long-term stability study, in days:

$$\mu_{lts} = s(b) \times t \quad (5)$$

The long-term stability measurement uncertainty results are presented in Table 1 and was used to estimate the total uncertainty of the CRM and can be considered adequate for a CRM candidate, as they are compatible with the uncertainty obtained by NIST in CRM 1845a.[15]

Conclusions

This work presented the preparation of a candidate for CRM, where it was possible to observe that the selected material, egg powder, met the conditions established by ISO Guide 35, for homogeneity and stability, presenting conditions for shipment to customers (short-term stability) and storage (long-term stability), being able to be a candidate for certified reference material.

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References

1. Lu, Z.; Deng, F.; He, R.; Tan, L.; Luo, X.; Pan, X.; Yang, Z.; *Microchem. J.* **2019**, *151*, 104213. [Crossref]
2. ISO Guide 35: *Reference Materials-Guidance for Characterization and Assessment of Homogeneity and Stability*, ISO: Geneva, 2017.
3. Yang, M.; Liu, F.; Wang, M.; Zhou, J.; Wei, R.; Wang, R.; *Anal. Bioanal. Chem.* **2020**, *412*, 635. [Crossref]
4. Wise, S. A.; *Anal. Bioanal. Chem.* **2022**, *414*, 7015. [Crossref]
5. ISO Guide 31: *Reference Materials-Contents of Certificates, Labels and Accompanying Documentation*, ISO: Geneva, 2015.
6. Yang, M.; Li, S.; Wang, M.; Zhou, J.; Liu, Q.; Zhang, L.; Wang, T.; *Microchem. J.* **2021**, *168*, 106330. [Crossref]
7. ISO 17034:2016: *General Requirements for the Competence of Reference Material Producers*, ISO: Geneva, 2016.
8. Yang, M.; Liu, F.; Wang, M.; Zhou, J.; Zhang, J.; Wang, T.; *Food Chem.* **2020**, *309*, 125253. [Crossref]
9. Abeyrathne, E. D. N. S.; Lee, H. Y.; Ahn, D. U.; *Poult. Sci.* **2013**, *92*, 3292. [Crossref]
10. Gab-Allah, M. A.; Lijalem, Y. G.; Yu, H.; Lee, S.; Baek, S.; Han, J.; Choi, K.; Kim, B.; *Food Chem.* **2023**, *404*, 134542. [Crossref]
11. Zhou, J.; Yang, M.; Li, F.; Wang, M.; Zhang, Y.; Wei, M.; Li, X. Qi, X.; Bai, X.; Chai, Y.; *Food Chem.* **2023**, *406*, 135012. [Crossref]
12. ISO 936:1998: *Meat and Meat Products - Determination of Total Ash*, ISO: Genebra, 1998.
13. Ministério da Agricultura, Pecuária e Abastecimento (MAPA); Portaria (SDA) No. 728, de 26 de dezembro de 2022, Dispõe a *Aprovação do Regulamento Técnico de Identidade e Qualidade de Ovo Integral Pasteurizado e de Ovo Desidratado*, Diário Oficial da União (DOU), Brasília, No. 246, de 30/12/2022, p. 24. [Link] accessed in November 2023
14. Prasad, A. D.; Thangavel, S.; Rastogi, L.; Soni, D.; Dash, K.; Kumar, S. J.; *Microchem. J.* **2022**, *172*, 106926. [Crossref]
15. National Institute of Standards & Technology; *Certified Reference Materials, 1845*, https://tsapps.nist.gov/srmext/certificates/1845a.pdf, accessed in November 2023.
16. Yang, Y.; Fan, Q.; Wang, Q.; Tian, W.; Qiu, P.; Gao, B.; Du, J.; *Microchem. J.* **2022**, *183*, 107935. [Crossref]

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