

## STABILITY STUDY OF CANDIDATE FOR CERTIFIED REFERENCE MATERIAL FOR SKIMMED MILK MATRIX

### ESTUDO DA ESTABILIDADE DE CANDIDATO A MATERIAL DE REFERÊNCIA CERTIFICADO PARA MATRIZ DE LEITE EM PÓ DESNATADO

**MARINA ZUFFO**

<https://orcid.org/0000-0002-6658-6353/marinazuffo91@gmail.com>  
Universidade do Estado de Santa Catarina (UDESC), Chapecó, Santa Catarina, Brasil.

**ANDREIA ZILIO DINON**

<https://orcid.org/0000-0002-0676-5050/andreia.dinon@udesc.br>  
Universidade do Estado de Santa Catarina (UDESC), Chapecó, Santa Catarina, Brasil.

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#### ABSTRACT

The production of certified reference material (CRM) in stable powdered milk matrix is important to ensure metrological traceability and quality management in the validation of methods and routine laboratory analyzes to ensure the quality of this product. Thus, the objective of the present study was to evaluate the short-term stability of the candidate batch for certified reference material in skimmed milk powder matrix. The work was conducted through a classic study according to the recommendations of ISO Guide 35. The parameters of moisture, lipids and total titratable acidity were evaluated during 21 days of storage. The material was stored at three different temperatures (2-8 °C, 25 °C and 40 °C). The data obtained in the stability study were evaluated through simple linear regression analysis and verified against the criteria established by ISO Guide 35. It was verified that the batch of material produced remained stable for determinations of lipids, moisture and titratable acidity for 28 days at temperatures from 2 to 8 °C, 25 °C and 40 °C.

**Keywords:** Powdered milk; Physicochemical parameters; Quality control; Storage.

#### ABSTRACT

A produção de material de referência certificado (MRC) em matriz de leite em pó estável é importante para garantir a rastreabilidade metrológica e a gestão da qualidade na validação de métodos e análises laboratoriais de rotina que visam a garantia da qualidade desse produto. Assim, o objetivo do presente estudo foi avaliar a estabilidade a curto prazo de um lote candidato a material de referência certificado para matriz de leite em pó desnatado. O trabalho foi conduzido por meio de estudo clássico conforme as recomendações do ISO Guia 35. Os parâmetros de umidade, lipídios e acidez total titulável foram avaliados durante 21 dias de armazenamento. O material foi armazenado em três diferentes temperaturas (2-8 °C, 25 °C e 40 °C). Os

dados obtidos no estudo de estabilidade foram avaliados por meio da análise de regressão linear simples e verificados quanto aos critérios estabelecidos pela ISO Guia 35. Verificou-se que o lote de material produzido se manteve estável para as determinações de lipídios, umidade e acidez titulável por 28 dias nas temperaturas de 2 a 8 °C, 25 °C e 40 °C.

**Keywords:** Leite em pó; Parâmetros físico-químicos; Controle de qualidade; Armazenamento.

## 1 INTRODUCTION

The use of certified reference materials is necessary to ensure the quality of an analytical result issued by an analytical laboratory, and reference materials have been used analytically to provide quality and metrological reliability (LIU et al., 2020). However, when referring to materials in complex matrices, such as food, these criteria are more difficult to meet due to their instability, high cost, and low availability of suppliers (RODRIGUES et al., 2009). Thus, the development of reference materials is a time-consuming and costly process due to the various stages involved in their development (RODRIGUES et al., 2009).

Given the importance of using certified reference materials as a quality assurance tool for laboratories, it is necessary to study the development of new materials and seek alternatives to ensure greater stability, keeping them as close to their originality as possible (BRANDÃO et al., 2013). In this sense, skim milk powder is a matrix of regional interest due to its high production and demand, which reveals the importance of the availability of reference materials in this matrix, which is still incipient (STEIGER; PRADEL, 2015).

The development, preparation and certification of a certified reference material for skimmed milk powder in Brazil, to guarantee the quality of measurements made on dairy-based foods, both from a nutritional and safety point of view, is an important step in national metrological development (D E NONI, 2004).

Thus, the present work aimed to evaluate the short-term stability of the candidate batch for certified reference material for skimmed milk powder matrix.

## 2 MATERIAL AND METHODS

The pre-production and production stages of the material were developed in the laboratories of the Senai/SC Institute of Technology (IST). All stages of the material batch processing followed

the requirements of the ABNT NBR ISO 17034 standard (ABNT, 2017). All equipment and utensils used during processing were previously cleaned and sanitized with 70% alcohol and, in the case of equipment, operated according to the respective operational procedures.

## 2.1 Material Preparation

A total of 13.2 kg of skim milk powder, purchased from a single batch of 400 g commercial packages, was used. The powdered milk sample was then classified using a decreasing mesh size system. The portion retained on the 0.5 mm mesh sieve was quantified and discarded. The raw material passing through the 0.5 mm mesh sieve was homogenized in a cutter for 5 minutes and then transferred to aluminum cans in individual amounts of approximately 40 grams. The cans were sealed with a seamer. The batch produced was stored at room temperature (25°C) until the studies began.

## 2.2 Analytical Methods

The parameters of moisture, lipids and total titratable acidity were determined according to the methodology proposed by the Ministry of Agriculture, Livestock and Supply (MAPA, 2019) for powdered milk.

Moisture content of the material was determined by the oven drying method at 85 °C . For acidity analysis, a 10 mL aliquot of the sample was suspended in deionized water and titrated with 0.1 mol/L sodium hydroxide solution. The lipid content was determined by the Roese - Gottlieb method using ester extraction after acid hydrolysis (MAPA, 2019).

## 2.3. Homogeneity Test

Batch homogeneity was assessed for each property of interest in this study. Ten samples were randomly selected using Microsoft Visual Basic's random number function and measured in duplicate under random repeatability conditions. The results were assessed for outliers using the Cochran test, according to ISO 5725-2 (ISO, 2019). Homogeneity was assessed according to the model described in ISO 13528:2015, by calculating the between-sample standard deviation (between-sample standard deviation ). The Horwitz standard deviation was used as the criterion for

evaluating the between-sample standard deviation, in which the standard deviation must be less than or equal to 0.3 times the limiting standard deviation (ISO, 2015).

## 2.4. Stability Test

The stability and properties of the certified reference material candidate were monitored in the short term to assess the influence of time and temperature. The total Forty-five individual samples weighing 40 g each were randomly selected and divided into three groups of 15 samples each. They were stored at different temperatures: Fifteen samples at 25 °C (temperature-controlled room); 15 samples at 2–8 °C (Brastemp upright refrigerator); and 15 samples at 40 °C (7Lab forced circulation heating oven). Every seven days, five samples were taken from each temperature, kept for a few minutes at room temperature, and evaluated in duplicate. Stability was assessed according to criteria established in ABNT ISO Guide 35 (ABNT, 2020) by detecting trends over time, followed by simple linear regression analysis at the different working temperatures. Linear regression analysis was performed as a function of time and temperature, and the slopes obtained were tested for significance using the t-test.

$$t_b = \frac{b}{s(b)} \quad \text{Eq.(1)}$$

Being,

$b$ : angular coefficient obtained in linear regression;

$s(b)$ : standard error of the slope.

According to ABNT ISO Guide 35 (ABNT, 2020), the value of  $p > 0.05$  and  $t_b < t(0.05;n-2)$  is not significant. The value of  $p$  ( $p > 0.05$ , that is, probability greater than 5%), indicates that there is a high probability that the difference observed between the groups is due to chance, therefore, it is considered that there is no significant difference between the groups.

If the slope of the obtained line is not significant for the specified confidence level (95%), there is no correlation between the property value and time and, therefore, the property value of interest of the material is considered stable.

## 3 RESULTS AND DISCUSSION

### 3.1. Homogeneity

The results obtained in the homogeneity study (Table 1), using the Cochran test ( $C_c \leq C_t$ ), demonstrated that there were no trends for any of the physical-chemical parameters evaluated according to the criteria established by the Harmonized International Protocol (THOMPSON et al., 2006).

Table 1 – Results obtained in the homogeneity test.

Parameter	Average	Detour Standard ( $S_s \leq 0.3 \times \bar{O}$	$S_s \leq 0.3 \times \bar{O}$	
Lipids (g/100g)	1.5	0.0014	0.0170	Homogeneous
Moisture (g/100g)	3.5	0.0046	0.0351	Homogeneous
Acidity titratable (g/100g)	0.17	0.0026	0.0027	Homogeneous

Source: prepared by the author (2025).

The standard deviation estimate (  $S_s$  ) with values close to zero “0.00” (Table 1) occurs because the estimate of the variance between the samples is relatively smaller than the variance within the samples. This can be expected when the items produced are highly homogeneous. In these cases, the variance between the samples is expressed as zero (ABNT, 2020).

Thus, it can be observed that the candidate for certified reference material for skimmed milk powder matrix was homogeneous, considering the Horwitz standard deviation as a criterion. (ISO, 2015). This indicates that the material production process was effective in ensuring the homogeneity of the analytes.

### 3.1. Stability

In the results obtained in the stability study (Tables 2 to 4), no trends were detected over time and the slopes and regressions were insignificant in all cases, according to the criteria established by ABNT ISO Guide 35 (ABNT, 2020).

Table 2 – Results obtained in the short-term stability study at a temperature of 25 °C.

Statistic	Parameter		
	Lipids	Humidity 25 °C	Acidity Titrable
Average (g/100g)	1.5	3,5439	0.17
$R^2$	0.0300	0.0089	0.1142
Angular coefficient	0.0002	0.0001	0.0002
Error Standard	0.0003	0.0002	0.0001

p-value	0.5213	0.7287	0.2005
Also	0.6579	0.3538	1,3434
n-2	14	14	14
t(0.05;n-2)	2,1448	2,1448	2,1448
Conclusion	Stable	Stable	Stable

Source: prepared by the author (2025).

Table 3 – Results obtained in the short-term stability study at a temperature of 2 to 8 °C.

Statistic	Parameter		
	Lipids	Humidity	Acidity Titrable
	2 to 8 °C		
Average (g/100g)	1.5	3,5429	0.17
R <sup>2</sup>	0.0201	0.0086	0.0322
Angular coefficient	0.0001	0.0001	0.0000
Error Standard	0.0002	0.0003	0.0000
p-value	0.6003	0.7329	0.5063
Also	0.5361	0.3481	0.6821
N	14	14	14
t(0.05;n-2)	2,1448	2,1448	2,1448
Conclusion	Stable	Stable	Stable

Source: prepared by the author (2025).

Table 4 – Results obtained in the short-term stability study at a temperature of 40 °C.

Statistic	Parameter		
	Lipids	Humidity	Acidity Titrable
	40 °C		
Average (g/100g)	1.5	3,5413	0.17
R <sup>2</sup>	0.0047	0.0115	0.2056
Angular coefficient	0.0001	0.0001	0.0001
Error Standard	0.0003	0.0002	0.0000
p-value	0.8008	0.6929	0.0777
Also	0.2571	0.4031	1,9037
N	14	14	14
t(0.05;n-2)	2,1448	2,1448	2,1448
Conclusion	Stable	Stable	Stable

Source: prepared by the author (2025).

The criteria established by ABNT ISO Guide 35 (ABNT, 2020) were met in all cases, where p-value > 0.05 and  $t_b < t(0.05;n-2)$  were obtained, i.e., the slope and regression are insignificant at the

95% confidence level. Consequently, there is no instability or trend; therefore, the material's property value of interest is considered stable for all physicochemical parameters evaluated.

Values close to zero for  $R^2$  (Tables 2 to 4) indicate that  $y$  does not depend on  $x$ , that is, the analyte concentration does not depend on temperature under the conditions evaluated in the present study.

Through the results obtained, it is possible to ensure that the material is stable in the short term, that is, the material can be stored for 28 days and transported at temperatures of 2 to 8 °C, 25 °C and 40 °C evaluated in this work, without significant influence on the evaluated parameters due to the sample storage temperature.

#### 4 CONCLUSIONS

It is concluded that the candidate for certified reference material for skim milk powder matrix showed stability within 28 days of storage at temperatures of 2 to 8°C, 25°C, and 40°C for the determination of lipid, moisture, and total titratable acidity parameters. Thus, it is ensured that the material is stable in the short term and can proceed to characterization analysis studies for certification purposes.

#### REFERENCES

ABNT ISO 17034. *General requirements for the competence of reference material producers*. 2017.

ABNT ISO GUIDE 35. *Reference materials — Guide for characterization and evaluation of homogeneity and stability*. 2020.

Brandão MLL et al., Development of reference material for microbiology of foods containing coagulase-positive staphylococci in cheese matrix. **Brazilian Journal of Food Technology**, Campinas, v. 16, n. 1, p. 73-79, Jan. / Mar. 2013. DOI: <https://doi.org/10.1590/S1981-67232013005000006>. Available at:

<https://www.scielo.br/j/bjft/a/5LWqKkz6KnX6sLX76RQGjSx/?lang=pt>. Access on : July 5 , 2021.

DE NONI, I. Reference material needs for quality assessment of milk and dairy products. **Accreditation and Quality Assurance** , vol. 9, p. 226-231, 2004. <https://doi.org/10.1007/s00769-004-0768-3> . Available at : <https://link.springer.com/article/10.1007/s00769-004-0768-3> . Access on : Feb 25 , 2022.

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results – Part 2: basic method for the determination of repeatability and reproducibility of a standard measurement method*. 2019.

ISO 13528, *Statistical methods for use in proficiency testing by interlaboratory comparison*. 2015.

LIU, J. et al. Determination and uncertainty estimation of tangeretin purity certified reference material. **Microchemical Journal**, China, v. 158. 2020. DOI: <https://doi.org/10.1016/j.microc.2020.105205>. Available at: <https://www.sciencedirect.com/science/article/abs/pii/S0026265X20311784>. Accessed on: 06 Jul. 2021.

Ministry of Agriculture, Livestock and Food Supply. *Manual of official methods for the analysis of foods of animal origin*. Secretariat of Agricultural Defense. 2nd ed. Brasília. 2019.

RODRIGUES, T.; ALBANO, F.; SILVA, M. *Preparation and certification of reference materials through proficiency testing: a case study of the RS metrological network*. In: 49th Congress Brazilian Chemistry Association. Porto Alegre: Brazilian Chemistry Association. 2 p, 2009.

STEIGER, T.; PRADEL, R. COMAR: the international database for certified reference materials—an overview. **Accreditation and Quality Assurance**, v. 20, n. 1, 2015. DOI: <https://doi.org/10.1007/s00769-014-1079-y>. Available at: <https://link.springer.com/article/10.1007/s00769-014-1079-y#citeas>. Accessed on: February 25, 2022.

THOMPSON, M.; ELLISON, SLR A review of interference effects and their correction in chemical analysis with special reference to uncertainty. **Accreditation and Quality Assurance**, 2005. DOI: <https://doi.org/10.1007/s00769-004-0871-5>. Available at: <https://link.springer.com/article/10.1007/s00769-004-0871-5#citeas>. Accessed on: 25 Feb. 2022.