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Artigo Original

Performance verification and measurement uncertainty estimation for the determination of sodium chloride in butter

Verificação de desempenho e estimativa de incerteza de medição de metodologia de determinação de cloreto de sódio em manteiga

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Resumo

Os parâmetros mínimos de identidade e qualidade de manteigas no Brasil são regidos pelo Ministério da Agricultura Pecuária e Abastecimento. A partir deles é possível realizar a verificação de desempenho das metodologias para garantir a confiabilidade dos resultados. Assim, objetivou-se analisar o desempenho do método de determinação de NaCl em manteiga, baseado na ISO 1738:2004, avaliando-se precisão, exatidão e limite de quantificação (LQ). A precisão foi alcançada visto que os coeficientes de variação obtidos nos ensaios foram inferiores ao valor de Horwitz calculado. A exatidão foi aprovada como consequência da confirmação da proficiência tendo como provedor o FAPAS. O LQ de 0,41g de NaCl/100g de manteiga foi estabelecido avaliando-se a precisão e a recuperação em ensaio de determinação do teor de NaCl em uma solução preparada com Material de Referência Certificado. Como forma de garantia da qualidade, estimou-se ainda a incerteza associada a medição de NaCl por meio da avaliação das possíveis fontes de incerteza, dentre as quais: pesagem, analista, vidraria e o grau de pureza do reagente, verificando-se uma incerteza de medição total de 0,03g NaCl/100g de amostra. Por fim, constatou-se a conformidade do teor de NaCl em amostras comerciais de acordo com a legislação brasileira vigente.

Palavras-chave: Adulteração; Alimento; Metodologia; Confiabilidade

Abstract

The minimum parameters of identity and quality of butters in Brazil are governed by the Ministry of Agriculture, Livestock and Supply. From them it is possible to carry out the performance verification of the methodologies to guarantee the results reliability. Thus, the objective of this study was to analyze the performance of the method of determination of NaCl in butter, based on ISO 1738:2004, evaluating accuracy, precision and the limit of quantification (LOQ). The precision was obtained since the coefficients of variation found in the tests were lower than the Horwitz value. The accuracy was approved as a consequence of the confirmation of the proficiency having as provider the FAPAS. The LOQ of 0.41g of NaCl/100g of butter was established by evaluating the accuracy and the recovery in a NaCl content determination test, using a solution prepared with Certified Reference Material. To assure quality, the uncertainty associated with the NaCl measurement was also estimated, through the evaluation of the possible sources of uncertainty, among which were: weighing, analyzer, glassware and the reagent purity, with a measurement uncertainty of 0.03g NaCl/100g of sample. Finally, it was verified the conformity of the NaCl content in commercial samples according to the Brazilian legislation.

Keywords: Adulteration; Food; Methodology; Reliability; NaCl

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1 Introduction

The butter is a greasy product obtained from the batting and kneading (process used to acquire a creamy and uniform texture) of pasteurized cream. This originates only from cow's milk, with or without biological modifications. This product has a minimum composition, established by the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento - MAPA), of 82% of fat, 16% of water and 2% of non-greasy solids (NGS) (BRASIL, 1996), thus providing a basis for preventing the addition of low-value fats as a substitute for cow's milk fat and adulteration practices. These, in turn, can lead to the emergence of economic problems as well as compromising the health of those who may consume the product. In this way, it is necessary to inspect practices such as the addition of low-value fats as a substitute for fat derived from cow's milk (NTAKATSANE; LUI; ZHOU, 2013; TEMIZ *et al*., 2018).

Another parameter targeted for inspection in butter is sodium chloride, which is commonly used for food preservation. Its control is important since its presence in inadequate concentrations is a favorable factor to promote the oxidation of different types of food (FRANKEL, 2012). Méndez-Cid *et al.* (2017) carried out a study on the influence of the temperature and the presence of salt on butter, noting that its presence, as well as high temperatures, contribute to the oxidation process, which may lead to a decrease in color parameters. On the other hand, França *et al*. (2014), in a study on the contamination of butters and the effect of salt in microorganisms, affirm that the presence of this ingredient assists in the conservation of the product since it causes a decrease in water activity, making its presence scarce for the growth of the microbial population.

Another important factor on the presence of sodium chloride in butter is its influence on the processing of this one, since it affects the distribution of water in the product, making the malaxation process difficult (WALSTRA *et al*., 2006). The Brazilian legislation admits a maximum sodium chloride content of 2% for extra butter and first quality butter (BRASIL, 1996) and 3% for common butter (BRASIL, 2000), a presence of sodium chloride higher than these values is a cause of chemical changes (FRANÇA *et al*., 2014).

In this context, it is necessary to have methodologies capable of identifying the substances that make up the butter, as well as the possible compounds that are used in the food adulteration processes. However, it is not enough to have a methodology available, but its reliability must be verified, which makes verification of performance essential when the method used is standardized or validation when the method is not standardized. This step is of great importance especially when using non-standardized methodsor developed by the laboratory or normalized outside its scope (UYSAL, 2013; RIBANI *et al*., 2004).

When verifying a methodology, that is, the performance of the method, the following parameters should be monitored: work range, precision (repeatability, reproducibility and intermediate precision), accuracy, recovery, limit of detection (LOD) and limit of quantification (LOQ), when applicable. All such parameters shall be calculated taking into account the requirements of the standard or regulated specifications (INMETRO, 2017).

Ribeiro *et al.* (2018) conducted the performance verification of the spectrophotometric method for determination of nitrite ion in drinking water. For this purpose, they evaluated the linearity with a correlation coefficient (r) value equal to 0.999, as well as the precision, with studies of repeatability and intra-laboratory reproducibility obtaining coefficient values of variation lower than 2.52%, and the accuracy assessed for the concentration levels of 5.0 mgN·L^{-1} and 10.0 mgN·L^{-1} with a relative error result of 15.8 and 8.1%, respectively. The study also determined the limits of detection and quantification with values of 0,56 and 1,70 mgN·L^{-1}, respectively; making it possible to verify the method proposed by the authors.

Several others validation/verification studies of analytical methodologies involving animal products are found in the literature. Takemoto *et al.* (2009) carried out the validation of a methodology for the simultaneous determination of synthetic antioxidants in vegetable oils, margarines and hydrogenated fats. The authors obtained a good recovery efficiency (greater than 96±10%) of antioxidants in two concentration levels, linearity (with r superior to 0.993) and values of the limits of detection and quantification similar to those found in the literature.

Marthe *et al.* (2010) developed an analytical methodology for the determination of pyrethroids (cypermethrin and deltamethrin) in butter and to ensure the reliability of the results, they also performed the validation process. For this, the authors analyzed parameters such as accuracy (with coefficients of variation lower than 2.9% in the repeatability study and less than 6% for reproducibility), recovery (results greater than 85.2%), linearity (with correlation coefficients higher than 0.996%) and selectivity. They also determined the limit of detection, with values of 0.082 μg·g^{-1} for cypermethrin and 0.11 μg·g^{-1} for deltamethrin and the limit of quantification, with values of 0.28 μg·g^{-1} for cypermethrin and 0.32 μg·g^{-1} for the deltamethrin.

In addition to performance verification studies, another important and necessary point to express a value reliably is the measurement uncertainty, since the result of a measurement without its uncertainty is only an estimate of the value of the measurement. In this way, it is possible to use the values obtained to compare them with reference values provided in a standard or specification, in addition to quantitatively indicating their quality to guarantee the reliability of the results, thus resulting in a complete result (BRASIL, 2003; CRUZ *et al*., 2010).

In order to estimate the measurement uncertainty of a method, it is necessary to determine the sources of uncertainty associated with analytical determination, which can be divided basically into two types: A and B. Those that are characterized as type A, as they are obtained by a statistical analysis, have value equivalent to the standard deviation of the mean. On the other hand, the uncertainties associated with calibrated measurement standards and certified reference materials, for example (type B), are obtained by assessing the uncertainty value mentioned in either the calibration certificate or in the technical manual or specification. Once the input values are determined, the input standard uncertainty is obtained by dividing the input values and their respective dividers by the type of distribution (EURACHEM/CITAC, 2002; CRUZ, 2010).

Burin *et al.* (2008), after the validation study of a calcium determination method in meat products, estimated the measurement uncertainty according to the Eurachem/citac Guide (2002).The study was carried out by specifying the measurement (concentration of calcium), identification of the sources of uncertainty (sample mass, volume spent, dilution volume and volume of the aliquot, concentration of potassium permanganate solution) and calculation of the combined uncertainty, obtaining a final uncertainty value of ± 0.00904 g, with a confidence level of approximately 95% and k = 2.87, so that the final result for analysis of calcium in sausage was 0.108 ± 0.009 g of calcium/100 g of the sample.

In view of the above, this work had as objective to verify the performance of the analytical method of determination of sodium chloride in butters based on ISO 1738: 2004 and to estimate the uncertainty of measurement of the method in question in order to evaluate whether different brands of first quality butter meet this parameter.

2 Material and methods

2.1 Determination of sodium chloride

Initially 5.0 ± 0.5 g of butter samples were weighed into 250 mL beakers. Then 50 mL of water was added, at a temperature of approximately 50 °C, together with 2 mL of 5% potassium chromate, with the mixture being homogenized and titrated with 0.1 mol.L^{-1} silver nitrate. For the blank determination, ultrapure water titration was conducted at 50°C under the same conditions as the assay performed with the sample. The percentage of sodium chloride contained in the butter samples was obtained from the Equation 1, expressed in grams of sodium chloride per 100 grams of sample.

$$g\ of\ NaCl/100g\ of\ butter = \frac{5,844 \cdot 0,1 f \cdot (V_3 - V_{blank})}{m_{sample}}\% \tag{1}$$

In which, V_3 is the volume of $AgNO_3$ spent on titration of the sample, in mL, V_{blank} is the volume of $AgNO_3$ spent on blank titration, m_{sample} is the mass of the sample weighted, in grams, and f is the correction factor obtained in the standardization of the $AgNO_3$.

2.2 Determining Performance Verification Parameters

The performance verification step of the method under analysis was conducted based on the determination of the following parameters: precision, accuracy and the limit of quantification. The precision was assessed by the study of repeatability and intermediate precision. The first one was expressed by the calculation of the coefficient of variation (CV) (Equation 2), obtained based on the average of 6 replicated performed by the same analyst. While the coefficient of variation for intermediate precision was obtained from the average of 14 replicates performed by two analysts on different days (BRASIL, 2003; INMETRO, 2017).

$$CV\ (\%) = \frac{s}{\bar{x}} \cdot 100\% \tag{2}$$

In which, S is the absolute standard deviation and $\bar{x}$ is the arithmetic mean of the number of repetitions.

To evaluate the CV, the Horwitz was used (Equation 3), in which C is the concentration of analyte present in the sample or standard (BRASIL, 2015).

$$Horwitz = 2^{(1-0.5\log C)} \tag{3}$$

The determination of the limit of quantification (LOQ) was performed by determining the sodium chloride content in 5 mL of aqueous sodium chloride solution with a concentration of 4 g.L^{-1} and prepared from a certified reference material (CRM). Then, titration with silver nitrate was carried out, as described in item 2.1, to define de LOQ of NaCl in 100 g of sample.

The criteria for the LOQ approval were precision, comparing the coefficient of variation with the Horwitz value, and the recovery, which is estimated from the percentage relation between the observed and expected value (Equation 4) (INMETRO, 2017).

$$Recuperation(\%) = \frac{observed\ value}{expected\ value} \cdot 100\ (\%) \tag{4}$$

From the data obtained through Equation 4, a comparative analysis was performed based on the acceptance criterion established by the Official Methods of Analysis International (AOAC) (AOAC, 2016).

The accuracy was assessed by participating in a proficiency testing conducted by the Proficiency testing from Fera (FAPAS), an internationally recognized provider of food chemistry proficiency testing programs. As acceptance criterion, the evaluation of the z-score test was performed (ABNT NBR ISO/IEC 17043 *apud* INMETRO, 2017). Finally, the measurement uncertainty associated with the analytical method was determined.

2.3 Estimation of measurement uncertainty

In order to carry out the estimation of method uncertainty, from the equation that defines the measurand (Equation 1, described in item 2.1), the main sources of uncertainty were related through the construction of an Ishikawa diagram. Once these sources were defined, the input value for each of them was determined by the type of distribution presented. For type A sources, the standard deviation of the mean was considered as the input value; while those of type B had as input the uncertainty value mentioned in the calibration certificate, technical manual or specification.

Since the sources of uncertainty and the measurand were mathematically related, the variation of the measurand (y) in relation to the variation of a given input source (x_i) was estimated by calculating the coefficient of sensitivity (Equation 5) (MOURA *et al.*, 2011).

$$C_i = \frac{\partial y}{\partial x_i} \tag{5}$$

Subsequently, the uncertainty component was estimated, with the same unit of the measurand, multiplying the sensitivity coefficient and the standard uncertainty of the input estimate. Thus, the combined standard uncertainty was determined by combining the results obtained with the multiplication (Equation 6).

$$u_c(y) = \sqrt{\sum_{i=1}^{N} \left(\frac{dy}{dx_i} u(x_i)\right)^2} \tag{6}$$

In which, $u_c(y)$ is the combined standard uncertainty and $u(x_i)$ is the standard uncertainty of the input estimate.

Finally, the expanded uncertainty (U) was determined in order to obtain the range that could cover a large fraction of the value distribution, which can reasonably be attributed to the measurand. This value, obtained by Equation 7, is a result of the multiplication of the combined standard uncertainty and the coverage factor (EURACHEN/CITAC, 2002).

$$U = k u_c(y) \tag{7}$$

The coverage factor for the defined probability level was obtained on the basis of Student's t distribution, where the degree of freedom equals the number of effective degrees of freedom of the combined standard uncertainty of the measurand determined by the Welch- Satterthwaite (Equation 8). On the other hand, when treating a Tybe B distribution, the effective number of degrees of freedom was considered as infinite (ABNT, 2008).

$$V_{eff} = \frac{{u_c}^4(y)}{\sum_{i=1}^{N} \frac{{u_i}^4(y)}{v_i}} \tag{8}$$

2.4 Evaluation of commercial butters

The conformity of the sodium chloride content in samples of commercial butter was evaluated, taking into account the current Brazilian legislation. The assay was performed with 10 replicates according to the methodology described in item 2.1. The samples were acquired in supermarkets and were kept under cooling until the moment of the analysis, being named in alphabetical order: sample A; sample B; sample C; sample D.

3 Results and Discussion

3.1 Validation Parameters

The evaluation of precision with the study of repeatability and intermediate precision was carried out by means of tests for the determination of the sodium chloride content in butter. Thus, it was possible to obtain the results of the coefficient of variation and the Horwitz values calculated for the concentration levels worked. For the repeatability test, the mean of the replicates was 1.54% and the CV was 1.26%. While in the evaluation of the intermediate precision, the average of the results of the two analysts was equal to 1.52% and the CV equal 1.91%. For the level of work concentration the Horwitz value was equal to 3.76. In this way, it can be affirmed that the results of the coefficients of variation obtained in the analyzes were inferior to the value of Horwitz, comproving the precision of the method.

A similar evaluation was performed by Goscinny *et al.* (2011) for the method of analysis of melamine residue in milk. In this study the Horwitz value was also used for the approval of repeatability and intermediate precision in tests performed with waffle samples. The CV results obtained for the concentrations of 1 mg of melamine / kg of waffles and 30 mg of melamine / kg of waffles were 0.9% and 3.2% in the repeatability test and of 1.2% and 3.3% for determination of reproducibility. The values calculated with the Horwitz equation were 10.7% for repeatability and 16.1% for reproducibility.

In the accuracy study, conducted from the Food Chemistry Proficiency Test 25160 roundabout promoted by FAPAS (Proficiency testing from Fera), according to ISO 1738: 2004, gave a result of 1.00 %, higher than the indicated value by FAPAS (0.99%), resulting in a z-score equal to 0.5.In this way, the proficiency of the method of determination of chloride in butter is verified, since the obtained results presented satisfactory results with $|z| \leq 2$, according to ABNT NBR ISO/IEC 17043 (ABNT NBR ISO/IEC 17043 *apud* INMETRO, 2017).

Similar result was obtained by Bilandzic *et al.* (2014) in participating in the proficiency test with the method evaluation of the determination of aflatoxin M1 in raw milk and UHT milk, obtaining a satisfactory result with a z-score of 0.9. Therefore, once the precision and accuracy of the methodologies used were verified, the limit of quantification was determined.

In order to determine the limit of quantification of the method, tests were carried out with 5 mL of a 4 $g.L^{-1}$ sodium chloride solution. The mean of the replicates was 0,41g of NaCl/100 g of butter, with a standard deviation of 0.01 and CV equal to 1.48%. Considering the experimental data, a recovery of 103% was obtained, which meets the recovery criteria whose acceptance range is from 95 to 105%, as established by the Official Methods of Analysis International (AOAC) present in Table 1 (AOAC, 2016).

Since the results of the coefficient of variation and reco-

Table 1 — Acceptance criteria for recovery

Analyte (%)	Analyte ratio	Unity	Recovery (%)
100	1	100%	98 – 102
10	10^{-1}	10%	98 – 102
1	10^{-2}	1%	97 – 103
0.1	10^{-3}	0,1%	95 – 105
0.01	10^{-4}	100 mg.kg^{-1}	90 – 107
0.001	10^{-5}	10 mg.kg^{-1}	80 – 110
0.0001	10^{-6}	1 mg.kg^{-1}	80 – 110
0.00001	10^{-7}	100 mg.kg^{-1}	80 – 110
0.000001	10^{-8}	10 mg.kg^{-1}	60 – 115
0.0000001	10^{-9}	1 µg.kg^{-1}	40 – 120

Source: Adapted from AOAC, 2016

very are lower than their acceptance criteria, a satisfactory accuracy and percentage of recovery at the assessed concentration is ascertained. Thus, the value of 0.41 g of NaCl/100 g of butter was established as the limit of quantification of this method, them it proceeded to the step of estimating the uncertainty associated with the method employed.

3.2 Estimation of measurement uncertainty

For the estimation of measurement uncertainty, the data from the standardization of the $AgNO_3$ (0,1 mol.L^{-1}) with NaCl and from the determination of the sodium chloride content in a sample of butter (Sample I) present in laboratory routine were used. The data from these respective assays are presented in Table 3.

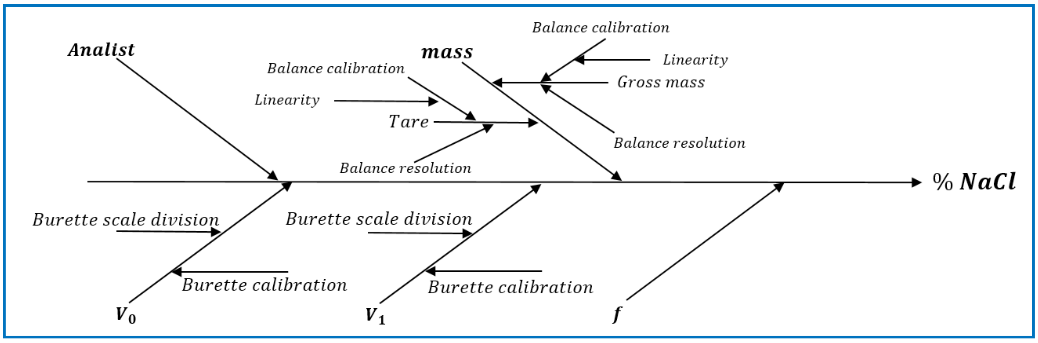
After the data collection presented in Table 3, the estimation of measurement uncertainty followed. The first step was to express mathematically the relationship between the measurand and the input quantities, using as base the Equation 1, described in item 2.1. From the components of the formula for obtaining the sodium chloride content, it was possible to point out the probable sources of uncertainty, which were analyzed in detail from the construction of the Ishikawa Diagram (Figure 1).

With the analysis of Figure 1, it was observed that the correction factor of the $AgNO_3$ (f) solution is considered

Table 3 — Data on the standardization tests of the AgNO3 solution with NaCl and determination of the sodium chloride content in Sample I

Parameter	Value
Mass of NaCl (g)	0.05580
Volume of $AgNO_3$ spent in the determination of the blank standardization (mL)	9.61667
Volume of $AgNO_3$ spent on standardization (mL)	0.05000
Purity of standard (%)	0.99835
Real concentration obtained (mol.L^{-1})	0.09965
Sample I mass (g)	5.00390
Volume of $AgNO_3$ used in the determination of the NaCl content in Sample I	0.20000
Volume of $AgNO_3$ used in the determination of the blank content in Sample I	14.4000
NaCl contente in sample I (g de NaCl/100 g de manteiga)	1.66000

Figure 1 — Ishikawa diagram to evaluate the sources of uncertainty for the determination of NaCl in butter



V_0 = volume of $AgNO_3$ spent on titration of the blank, V1 = volume of $AgNO_3$ spent on the titration of the butter sample and f = correction factor obtained in the standardization of the $AgNO_3$ solution.

as one of the possible causes of measurement uncertainty. Such a parameter can be obtained by the relation between de concentration of the real solution and the concentration of the desired solution. Since the desired concentration is constant, only the real concentration interferes with the uncertainty relative to the factor, which is obtained from Equation 9.

$$C_{Real} = \frac{m \cdot 1000 \cdot T}{(V_1 - V_0) \cdot MM} \tag{9}$$

In which, m is the mass of NaCl used, in grams, T is the purity of the salt, dimensionless, V_1 is the volume of $AgNO_3$ used in the titration of the salt, in mL, V_0 is the volume spent in the titration of the blank in mL and MM is the molar mass of the salt ($g.mol^{-1}$).

In order to complement the possible causes of uncertainty, the sources of uncertainty contribution of the real $AgNO_3$ concentration were analyzed, by constructing a new Ishikawa Diagram (Figure 2).

In figure 2 the sub-cause “linearity” represents the maximum difference between the actual mass of the vessel and the reading of the balance (EURACHEM/CITAC, 2002). In this diagram, it was chosen to not list the temperature, since it presents a low contribution to the global uncertainty, related to the fact that its variations can be considered very small and, therefore, despised.

Still analyzing Figure 2, it was found that the contributions referring to the “analyst” cause are equivalent to the standard deviation resulting from the determinations of the measurand and are classified as being of Type A. While the contributions related to the measuring equipment (scale and burette) and to the purity of the reagents, whose input values have been withdrawn from their certificates, are classified as Type B (ABNT, 2008).

By evaluating the sources of uncertainty for the determination of the real concentration of $AgNO_3$ (C_{real}), it was possible to determine the type of distribution of each source and consequently the value of its divisor and them calculate the standard uncertainty. The values of each of these parameters are shown in Table 4.

Figure 2 — Ishikawa diagram with sources of uncertainty for the calculation of the real concentration of $AgNO_3$

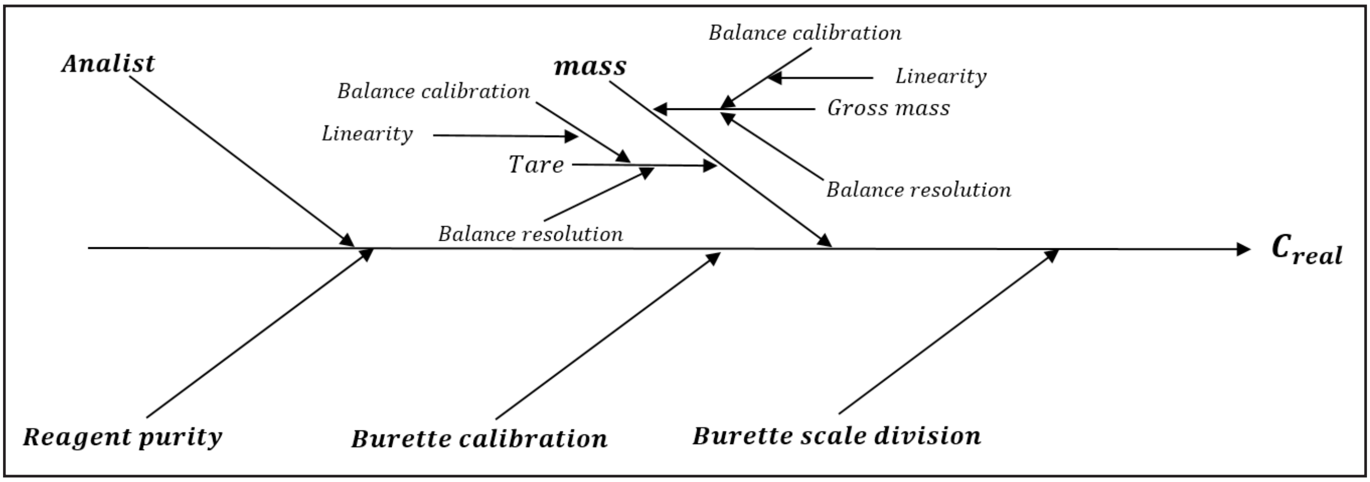


Table 4 — Description of the types, divisors and standard uncertainties of the sources of uncertainty regarding the calculation of the real $AgNO_3$

Origin	Source	Type	Divisor	Standard uncertainty
m	Maximum balance error	Rectangular	√3	2.29×10^{-5}
m	Balance resolution	Rectangular	√3	5.77×10^{-5}
V_1	Maximum error for the burette	Student	√n	7.07×10^{-3}
V_1	Burette Scale Division	Triangular	√6	4.08×10^{-2}
V_0	Maximum error for the burette	Student	√n	7.07×10^{-3}
V_0	Burette Scale Division	Triangular	√6	4.08×10^{-2}
Analyst	Standard deviation of repetitions	Student	√n	3.28×10^{-4}
Purity of the standard(T)	Uncertainty of the purity	Student	√n	7.07×10^{-5}

V_0 = volume of $AgNO_3$ spent on titration of the blank, V_1 = volume of $AgNO_3$ spent on the titration of the butter sample and f = correction factor obtained in the standardization of the $AgNO_3$ solution.

Table 5 — Equations and values of the sensitivity coefficients of the input sources of the uncertainty estimation of the correction factor determination in the standardization of the $AgNO_3$ solution with NaCl.

Source	Equation for obtaining the sensitivity coefficient	Sensitivity coefficient
Maximum balance error	$\frac{\partial C_{real}}{\partial m} = \frac{1000.T}{(V_i - V_0).MM}$	1.786
Balance resolution	$\frac{\partial C_{real}}{\partial m} = \frac{1000.T}{(V_i - V_0).MM}$	1.786
Maximum burette error (V_1)	$\frac{\partial C_{real}}{\partial V_i} = \frac{-m_{sal}.1000.T}{V_i^2.MM}$	-0.01
Burette Scale Division (V_1)	$\frac{\partial C_{real}}{\partial V_i} = \frac{-m_{sal}.1000.T}{V_i^2.MM}$	-0.01
Maximum burette error (V_0)	$\frac{\partial C_{real}}{\partial V_0} = \frac{m_{sal}.1000.T}{V_0^2.MM}$	0.01
Burette Scale Division (V_0)	$\frac{\partial C_{real}}{\partial V_0} = \frac{m_{sal}.1000.T}{V_0^2.MM}$	0.01
Standard deviation of repetitions	1	1
Purity of the standard (T)	$\frac{\partial C_{real}}{\partial T} = \frac{1000.m}{(V_i - V_0).MM}$	0.1

Based on the values of the standard uncertainties, it was possible to determine the sensitivity coefficients, which were calculated through the derivative of the function for the real concentration of $AgNO_3$ as a function of each source. The equations for obtaining these coefficients as well as the results obtained for the standardization of an $AgNO_3$ solution are presented in Table 5.

Based on the values of the sensitivity coefficients, the uncertainty contributions of each source were obtained by multiplying these coefficients with the standard uncertainty obtained previously. Finally, the combination of the uncertainty contributions, by calculating the square root of the quadratic sum of the contributions, resulted in the combined uncertainty.

For the calculation of the coverage factor k, the degree of effective freedom was determined by the Welch-Satterthwaite (Equation 8, described in item 2.3). Then the multiplication between the combined uncertainty and the coverage factor was performed and the expanded uncertainty was obtained. Applying the values obtained from the standardization of an $AgNO_3$ solution with NaCl, the real concentration was equal to 0,0996 ± 0,0014 $mol.L^{-1}$, in which 0,0014 $mol.L^{-1}$ is the expanded uncertainty. The contribution portions, in percent, of each source of uncertainty were obtained considering as 100% the combined uncertainty (Table 6).

Table 6 — Contribution share of the sources of uncertainty related to the actual concentration of $AgNO_3$

Source of uncertainty	Contribution of uncertainty (%)
Maximum balance error (m)	0.35
Maximum balance error (m)	2.16
Maximum error for the burette (V_1)	1.10
Burette Scale Division (V_1)	36.73
Maximum error for the burette (V_0)	1.10
Burette Scale Division (V_0)	36.73
Standard deviation of repetitions	21.82
Uncertainty of purity (T)	0.01

The value of the expanded uncertainty and the value of the sensitivity coefficient associated with the real concentration of $AgNO_3$ (C_{real}) were used to estimate the uncertainty related with the factor f (Equation 10). Such that the input value equals the expanded uncertainty of the C_{real} and the divisor to the coverage factor of C_{real}. The sensitivity coefficient (Equation 11) was obtained by calculating the derivative of the factor equation as a function of the real concentration. The contribution of the desired concentration was not related since it is a constant.

$$f = \frac{C_{real}}{C_{pretendido}} \tag{10}$$

$$\frac{\partial f}{\partial C_{real}} = \frac{1}{m_{pretendido}} \tag{11}$$

The effective degree of freedom, the coverage factor and the expanded uncertainty were calculated in a manner analogous to the calculations performed in estimating the uncertainty for the real concentration. Once the measurement uncertainty associated with the standardization correction factor in the $AgNO_3$ was estimated, it was followed by the estimation of the measurement uncertainty of the determination of sodium chloride content. The type of distribution of each source and consequently the value of its divisor for the calculation of the standard uncertainty are presented in Table 7.

Table 7. Description of the types, divisors and standard uncertainties of the sources of uncertainty regarding the calculation of the NaCl content in a butter sample.

Origin	Source	Type	Divisor	Standard uncertainty
V_3	Maximum burette error	Student	√n	4.08×10^{-3}
V_3	Burette Scale Division	Triangular	√6	7.07×10^{-2}
V_{blank}	Maximum burette error	Student	√n	4.08×10^{-3}
V_{blank}	Burette Scale Division	Triangular	√6	7.07×10^{-2}
F	Uncertainty of factor f	Student	$K_{C_{real}}$	7.02×10^{-3}
m_{sample}	Maximum balance error	Rectangular	√3	6.37×10^{-5}
m_{sample}	Balance resolution	Rectangular	√3	5.77×10^{-5}
Type A	Standard deviation of the duplicate	Student	√n	6.95×10^{-4}

V3 = volume of AgNO3 spent on sample titration, Vblank = volume of AgNO3 spent on blank titration, msample = mass of the butter sample weighted.

Table 8. Determination of the sensitivity coefficient of the input sources of uncertainty estimation of the determination of the sodium chloride content in a sample of butter.

Source	Equation for obtaining the Sensitivity Coefficient	Sensitivity Coefficient
Maximum burette error (V_3)	$\frac{\partial\ NaCl}{\partial V_3} = \frac{MM.10^{-1}.f}{m_{amos}}$	0.117
Burette Scale Division (V_3)	$\frac{\partial\ NaCl}{\partial V_3} = \frac{MM.10^{-1}.f}{m_{amos}}$	0.117
Maximum burette error (V_0)	$\frac{\partial NaCl}{\partial V_{branco}} = \frac{-MM.10^{-1}.f}{m_{amos}}$	-0.117
Burette Scale Division (V_0)	$\frac{\partial NaCl}{\partial V_{branco}} = \frac{-MM.10^{-1}.f}{m_{amos}}$	-0.117
Factor Uncertainty	$\frac{\partial NaCl}{\partial V_{branco}} = \frac{-MM.10^{-1}.f}{m_{amos}}$	1.658
Maximum Balance Error	$\frac{\partial\ NaCl}{\partial m_{amos}} = \frac{-MM.10^{-1}.f.(V_{amos}-V_{braco})}{{m_{amos}}^2}$	-0.331
Balance Resolution	$\frac{\partial\ NaCl}{\partial m_{amos}} = \frac{-MM.10^{-1}.f.(V_{amos}-V_{braco})}{{m_{amos}}^2}$	-0.331
Standard Deviation of Readings	1	1

The uncertainty estimation of the $AgNO_3$ concentration was similarly estimated, alongside with the sensitivity coefficients of the uncertainty of the NaCl content. The results obtained are shown in Table 8.

After the uncertainty estimation for the correction factor of the AgNO3 solution, it was calculated the uncertainty contribution, the combined uncertainty, the effective degree of freedom, the coverage factor and the expanded uncertainty, in the same way as in the uncertainty estimation of the real concentration of $AgNO_3$. Finally, with the results of the determination of sodium chloride in a sample of butter from the laboratory routine, a sodium chloride content of 1.66 ± 0.03 g of NaCl/100 g of sample was obtained, in which 0.03 is the expanded uncertainty. The contribution shares of each source of uncertainty obtained, considering 100% as the combined uncertainty, are presented in Table 9.

Table 9 — Contribution share of sources of uncertainty related to determining the content of sodium chloride in a sample of butter

Source of uncertainty	Uncertainty contribution (%)
Maximum burette error (V_3)	0.08320
Burette Scale Division (V_3)	24.95898
Maximum burette error (V_{blank})	0.08320
Burette Scale Division (V_{blank})	24.95898
Factor f uncertainty	49.73807
Balance Maximum error (m_{sample})	0.00016
Balance resolution (m_{sample})	0.00013
Standard deviation of the duplicate	0.17728

3.3 Evaluation of commercial butters

The sodium chloride content was evaluated in samples of first quality type commercial butters, in order to verify if they comply with the legislation described in Ordinance No. 146 of the Ministry of Agriculture, Livestock and Supply which determines the sodium chloride content. This ordinance states that for this type of butter the NaCl content should not exceed 2% (m/m) in its salt form (BRASIL, 1996). At the end of the analyzes the sodium chloride content obtained was equal to 1.51, 1.65, 0.98 and 1.94 g of NaCl/100 g of sample, for the samples A, B, C, and D. Thus it is evident that the sodium chloride content of the samples were in compliance with the current legislation.

4 Conclusions

The analytical methodology presented in this work for the determination of sodium chloride in butter, based on ISO 1738:2004, presented a satisfactory performance, since the validation parameters stablished by INMETRO (accuracy, precision and limit of quantification) for performance verification were confirmed. In order to verify the analytical reliability, the measurement uncertainty associated to the method was estimated, verifying that the burette error is the source that presents the greatest contribution to the uncertainty. Regarding the analysis of the sodium chloride content in 4 first quality commercial samples, it was observed that the NaCl contents were in accordance with the recommended by the Brazilian legislation in force.

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