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Method Validation and Calculation of Measurement Uncertainty for Salt Determination in Cheese by Mohr Method

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Abstract

Validation studies were conducted to verify the validity of the Mohr method, which is one of the most common methods for salt determination in the laboratory. Precision (repeatability and reproducibility) and accuracy are method validation parameters. Relative standard deviation (RSD) values obtained under reproducibility and repeatability conditions were compared with concentration-dependent precision values and the results were appropriate. The accuracy study was carried out using a standard reference substance. The t-test was used to determine whether the results were close to the true value and the results were found to be appropriate. As a result, the validation studies of the Mohr method were completed and are valid for our laboratory. A study on the measurement uncertainty of cheese salt was also conducted. Therefore, Urfa cheese and kashar cheese were preferred. The applied analysis method determined the uncertainty components known as weight, volume, precision, and accuracy. Then, the expanded uncertainty was calculated by combining the uncertainties resulting from these uncertainties. The expanded uncertainty is U(salt) =0.028.

Keywords: cheese, salt, validation, measurement, Mohr method

Mohr Metodu ile Peynirde Tuz Tayini İçin Ölçüm Belirsizliğinin Hesaplanması ve Metot Doğrulaması

Öz

Laboratuvarda tuz tayini için en yaygın kullanılan yöntemlerden biri olan Mohr yönteminin geçerliliğini doğrulamak için validasyon çalışmaları yapılmıştır. Kesinlik (tekrarlanabilirlik ve tekrar üretilebilirlik) ve doğruluk yöntem validasyon parametreleridir. Tekrar üretilebilirlik ve tekrar üretilebilirlik koşulları altında elde edilen Bağıl Standart Sapma (RSD) değerleri konsantrasyona bağlı kesinlik değerleri ile karşılaştırılmış ve sonuçlar uygun bulunmuştur. Doğruluk çalışması standart referans madde kullanılarak yapılmıştır. Sonuçların gerçek değere yakın olup olmadığını belirlemek için t-testi kullanılmış ve sonuçlar uygun bulunmuştur. Sonuç olarak Mohr yönteminin validasyon çalışmaları tamamlanmış ve laboratuvarımız için geçerlidir. Peynir tuzunun ölçüm belirsizliği üzerine bir çalışma da yapılmıştır. Bu nedenle Urfa peyniri ve kaşar peyniri tercih edilmiştir. Uygulanan analiz yöntemi ile ağırlık, hacim, kesinlik ve doğruluk olarak bilinen belirsizlik bileşenleri belirlenmiştir. Daha sonra bu belirsizliklerden kaynaklanan belirsizlikler birleştirilerek genişletilmiş belirsizlik hesaplanmıştır. Genişletilmiş belirsizlik U(tuz)=0,028'dir.

Anahtar Kelimeler: peynir, tuz, doğrulama, ölçüm, Mohr metodu

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Introduction

The primary purpose of chemical measurement is to make decisions based on the measurement result. It is taken into account when deciding on the compliance of a product with certain standards, quality standards, control of the production process, standards for legal decisions, commercial evaluation, and classification (Anderson et al., 1999). Chemical measurement results should be accurate, reliable, repeatable, and comparable on a national and international scale (Bulska & Lipiński, 2018). Reliable analysis is performed using methods and tools such as standard test procedures, internationally developed standard methods (ASTM-American Society for Testing and Materials, ISO-International Organization for Standardization, EPA-US Environmental Protection Agency, AOAC-American Official Analytical Chemist), calibration, performance tests, laboratory accreditation, proficiency tests and chemical metrology (Akdağ, 2004).

One or more organizations can accredit laboratories in a country. Law No. 4457 authorized the Turkish Accreditation Agency (TÜRKAK) to accredit laboratories in our country (Uras, 2009). Today, TS EN ISO/IEC 17025 General Conditions for the Competence of Testing and Calibration Laboratories are required for the accreditation of testing and calibration laboratories. This standard has been approved worldwide. ISO/17025 Laboratory accreditation standards consist of two main parts: technical conditions and quality management standards (Krismastuti & Habibie, 2022). Similar conditions to the quality management system specified in the ISO 9000 quality standard have been adapted for the laboratory. As a result, institutions or laboratories that implement a quality system in accordance with the ISO 9000 standard can spend less effort to meet these requirements (Douglas et al., 2003). Laboratory accreditation uses an evaluation system consisting of three basic issues (Alper, 2004). These are measurement uncertainty, method validation and traceability of results (Inal & Topkaya, 2010).

Validation or validity is the whole of the operations performed to show that a device, method, or system works in accordance with the specified conditions (Çelebiler et al., 2011). The principles of method performance depend on the purpose and scope of the method to be applied (Menditto et al., 2007). Precision, accuracy, selectivity, linearity and measurement range, sensitivity, and robustness are the validity indicators of the methods (Araujo, 2009). The first application of a method in a laboratory, the development of a new method for analysis, the modification of a method in use, its application in another laboratory, or the change in the person applying or the device used are known as method validity (Ertaş & Kayalı, 2005).

Due to the uncertain effects of random effects, uncertain data determine the limits within which values can be encountered around the result. A measurement uncertainty consists of a series of uncertainties combined. Uncertainty calculations are necessary to improve the quality of food laboratories (AOAC 1998).

The Mohr method is simple, cheap and fast. To our knowledge, no validation or measurement uncertainty study has been conducted on cheese using this method. In this study, validation studies of the analytical method for the determination of salt in cheese according to the Mohr method were carried out and the measurement uncertainty was calculated.

Material & Method

Materials

Two different cheese samples taken from local markets were used to investigate the validation and measurement uncertainty of the salt determination method. One was kashar cheese with less salt content, the other was Urfa cheese with more salt. These samples were selected in two different varieties and different concentrations to cover all cheese types in the study. 0.1 N Silver nitrate ($AgNO_3$) solution and 5% potassium chromate (K_2CrO_4) solution were prepared. The $AgNO_3$ and K_2CrO_4 required to prepare these solutions were supplied by Sigma & Aldrich.

Analysis Method

In this study, the Mohr method, one of the most preferred methods, was used for method validation and measurement uncertainty calculation of salt determination in cheese. Approximately five grams of the homogenized sample was weighed into a conical flask, some hot pure water was added, and the mixture was shaken vigorously for five to ten minutes. The solution was filtered through the filter paper into a 500 mL volumetric flask. The conical flask was washed four to five times with hot water and then placed on the filter paper. This allowed the salt to remain in the conical flask and the filter paper to pass into the water. After the filtrate in the volumetric flask had cooled completely, it was combined with pure water up to the volume line. Then, 25 milliliters were taken into the conical flask and 2-3 drops of K_2CrO_4 solution were added. Burette was filled with $AgNO_3$ and zero was set. The sample was titrated with $AgNO_3$ (0.1 N) solution until a brick-red color developed (Sezey & Adun, 2019). Percentage of salt was calculated according to the equation 1 (Eq.1).

Salt % (g) = 0.00585xVxNxDFx100/m (Eq.1)

[V = Volume of $AgNO_3$ solution spent (mL), N = Concentration of adjusted $AgNO_3$ solution , m = Amount of sample taken (g), DF= Dilution factor (X g of sample was diluted into a 500 mL volumetric flask. 25 mL of this solution was also taken. In this case, the dilution factor is 500/25 = 20)].

Results & Discussion

Precision and Validation Studies

Validation studies were carried out in the laboratory to validate the Mohr method, one of the most widely used methods for salt determination in cheese. Kashar and Urfa cheese were preferred for the method's applicability to all cheese types. Certified reference material was used for the accuracy study. The method validation parameters were precision (repeatability, reproducibility) and accuracy.

Repeatability

For repeatability, the Mohr method performed ten studies on the selected cheese samples on the same day. Tables 1 and table 2 show the methods for calculating the mean, standard deviation, and relative standard deviation of the obtained data. Table 1 compares the relative standard deviation (RSD) values obtained under repeatability conditions with the concentration-dependent precision values. The Grubb test was applied to the within-group data to determine the different values in the analysis results. Since all % RSD values obtained under repeatability conditions were smaller than the 1.8 % RSD value given in the concentration-dependent precision values table, they were interpreted as being by the RSD values obtained under repeatability conditions.

Table 1. Studies on Salt Determination in Kashar Cheese of the 1st and 2nd Analysts under Repeatability Conditions

Number of Repeatability	1st Analyst	2nd Analyst
1	2.75	2.68
2	2.70	2.72
3	2.60	2.77
4	2.65	2.72
5	2.77	2.72
6	2.75	2.68
7	2.65	2.80
8	2.75	2.72
9	2.70	2.78
10	2.67	2.77
Average	2.70	2.74
Standard deviation	0.054	0.042
RSD	0.020	0.015
RSD %	1.56	1.55
Grubb top	1.297	1.418
Grubb down	1.853	1.418
Grub criterion	2.29	2.29
Evalution	Suitable	Suitable

Table 2. Studies on Salt Determination in Urfa Cheese of the 1st and 2nd Analysts under Repeatability Conditions for 3rd Day

Number of Repeatability	1st Analyst	2nd Analyst
1	7.09	7.12
2	7.05	7.03
3	7.14	7.12
4	7.10	7.12
5	7.14	7.07
6	7.00	7.03
7	7.14	7.12
8	7.14	7.17
9	7.14	7.07
10	7.18	7.12
Average	7.11	7.10
Standard deviation	0.053	0.045
RSD	0.007	0.006
RSD %	0.74	0.64
Grubb top	1.329	1.544
Grubb down	2.088	1.544
Grub criterion	2.29	2.29
Evalution	Suitable	Suitable

Reproducibility

For reproducibility, ten replicates were performed on selected cheese samples using the Mohr method on various days. The mean, standard deviation, and relative standard deviation of the data obtained in Tables 3, 4, 5, and 6 were calculated. The table shows the agreement between the relative standard deviation (RSD) values obtained under reproducibility conditions and the concentration-dependent precision values. The RSD values were compared with the RSD values. According to the analysis results stated in the tables, all RSD values obtained under reproducibility conditions were interpreted as suitable since the concentration-dependent precision values were less than 1.8 % RSD.

Table 3. Reproducibility Study on Kashar Cheese Sample (1st Analyst)

Number of Repeatability	a	b	X	a-b	(a-b)/X	((a-b)/X)²
1 (1st day)	7.138	7.080	7.109	0.058	0.008	0.000
2 (1st day)	7.138	7.100	7.119	0.038	0.005	0.000
3 (1st day)	7.046	7.060	7.053	-0.014	-0.002	0.000
4 (1st day)	7.138	7.100	7.119	0.038	0.005	0.000
5 (2nd day)	7.000	7.060	7.030	-0.060	-0.009	0.000
6 (2nd day)	7.138	7.120	7.129	0.018	0.003	0.000
7 (2nd day)	7.184	7.100	7.142	0.084	0.012	0.000
8 (3rd day)	7.138	7.040	7.089	0.098	0.014	0,000
9 (3rd day)	7.138	7.100	7.119	0.038	0.005	0.000
10 (3rd day)	7.184	7.120	7.152	0.064	0.009	0.000
Total						0.001
RSD						0.006
RSD %						0.6

a: First analysis result, b: Second analysis result and X: Mean value.

Table 4. Reproducibility Study on Kashar Cheese Sample under Different Days (2nd Analyst)

Number of Repeatability	a	B	X	a-b	(a-b)/X	((a-b)/X)²
1 (1st day)	7.138	7.100	7.119	0.038	0.005	0.000
2 (1st day)	7.138	7.120	7.129	0.018	0.003	0.000
3 (1st day)	7.138	7.120	7.129	0.018	0.003	0.000
4 (1st day)	7.000	7.060	7.030	-0.060	-0.009	0.000
5 (2nd day)	6.954	7.000	6.977	-0.046	-0.007	0.000
6 (2nd day)	7.138	7.120	7.129	0.018	0.003	0.000
7 (2nd day)	7.046	7.080	7.063	-0.034	-0.005	0.000
8 (3rd day)	7.138	7.080	7.109	0.058	0.008	0.000
9 (3rd day)	7.092	7.120	7.106	-0.028	-0.004	0.000
10 (3rd day)	7.046	7.080	7.063	-0.034	-0.005	0.000
Total						0.000
RSD						0.004
RSD %						0.4

Table 5. Reproducibility Study on Urfa Cheese Sample (1st Analyst)

Number of Repeatability	a	b	X	a-b	(a-b)/X	((a-b)/X)²
1 (1st day)	2.68	2.70	2.69	-0.02	-0.01	0.00
2 (1st day)	2.77	2.82	2.80	-0.05	-0.02	0,00
3 (1st day)	2.74	2.72	2.73	0.02	0.01	0,00
4 (1st day)	2.68	2.68	2.68	0.00	0.00	0,00
5 (2nd day)	2.75	2.72	2.74	0.03	0.01	0,00
6 (2nd day)	2.70	2.68	2.69	0.02	0.01	0.00
7 (2nd day)	2.69	2.74	2.72	-0.05	-0.02	0.00
8 (3rd day)	2.72	2.73	2.73	-0.01	0.00	0.00
9 (3rd day)	2.68	2.70	2.69	-0.02	-0.01	0.00
10 (3rd day)	2.72	2.75	2.74	-0.03	-0.01	0.00
Total						0,001
Total						0.001
RSD						0.008
RSD %						0.8

a: First analysis result, b: Second analysis result and X: Mean value.

Table 6. Reproducibility Study on Urfa Cheese Sample under Different Days (2nd Analyst).

Number of Repeatability	a	b	X	a-b	(a-b)/X	((a-b)/X)²
1 (1st day)	2.77	2.72	2.74	0.05	0.02	0.00
2 (1st day)	2.72	2.70	2.71	0.02	0.01	0.00
3 (1st day)	2.77	2.70	2.73	0.07	0.02	0.00
4 (1st day)	2.77	2.72	2.74	0.05	0.02	0.00
5 (2nd day)	2.70	2.72	2.71	-0.02	-0.01	0.00
6 (2nd day)	2.74	2.70	2.72	0.04	0.02	0.00
7 (2nd day)	2.75	2.74	2.74	0.01	0.00	0.00
8 (3rd day)	2.75	2.76	2.75	-0.01	0.00	0.00
9 (3rd day)	2.66	2.70	2.68	-0.04	-0.01	0.00
10 (3rd day)	2.71	2.75	2.73	-0.04	-0.01	0.00
Total						0.002
RSD						0.010
RSD %						1.0

a: First analysis result, b: Second analysis result and X: Mean value.

All RSD values obtained under reproducibility conditions since it is smaller than the RSD value of 1.8% given in the table of precision values as a function of concentration (Table 7), the RSD values obtained under reproducibility conditions are interpreted as appropriate.

Table 7. Comparison of Concentration-Dependent Precision Value (AOAC, 1998).

Percent of Analyst Concentration (%)	Analyst Ratio	Unit	RSD %
100	1	100 %	1.3
10	10^{-1}	10 %	1.8
1	10^{-2}	1 %	2.7
0.1	10^{-3}	0.1 %	3.7
0.01	10^{-4}	100 ppm	5.3
0.001	10^{-5}	10 pp	7.3
0.0001	10^{-6}	1 ppm	11
0.00001	10^{-7}	100 ppb	15
0.000001	10^{-8}	10 ppb	21

Analysis of Accuracy

For the accuracy parameter, 10 replicate analyses were performed using the reference material (white cheese) and the mean, standard deviation and systematic error of the values obtained were calculated (Table 8). The t-test was performed to check whether the systematic error calculated according to the certificate value was significantly different from the actual value and the t-value was calculated.

Table 8. Standard Reference Material (white cheese) Analysis Results

Number of Repeatability	Salt (%)
1	3.34
2	3.29
3	3.33
4	3.33
5	3.29
6	3.22
7	3.24
8	3.2
9	3.33
10	3.12
Real value	3.31
Found value	3.27
Relative error	0.012
Standard deviation	0.072
t value	1.789
t critical value	2.262

From the t-test table in the literature, the critical t value at 9 degrees of freedom (n-1) in the 95 % confidence interval was found to be 2.262. Since the calculated value was 1.789<2.262, it was interpreted as there was no significant difference between it and the certificate value.

Sezey and Adun (2019) carried out validation studies of the Mohr titration method for the determination of salt content in olive and/or olive brine. The method was successfully validated as its accuracy (70-120%) and precision (RSD 5%) were within acceptable ranges.

Calculation of Measurement Uncertainty

The uncertainty components of the applied analysis method are weighing, volume, precision and accuracy.

The uncertainty of the precision balance used in the studies was given as ± 0.001. The standard uncertainty was found as 0.001/√3 = 0.0006 with a rectangular distribution.

The 5 mL pipette calibration certificate value is given as ± 0.05 ml. The standard uncertainty is found as 0.05/√3 = 0.028 with a rectangular distribution. The 10 mL pipette calibration certificate value is given as ± 0.1 mL. The standard uncertainty is found as 0.1/√3 = 0.057 with a rectangular distribution. In the studies conducted, the accuracy relative error was calculated as 0.012 (Table 8). Volume uncertainty values are given in Table 9.

Table 9. Volume Uncertainty Values

Compound	Value	Standard Uncertainty	Relative Standard Uncertainty
Volume of pipette (ml)	5	0.028	0.0056
Volume of pipette (ml)	10	0.057	0.0057
Total volume uncertainty	-	-	0.008

The combination of RSDs obtained from repeatability and reproducibility studies in Urfa and kashar cheese should be used to determine the uncertainty of precision. The following eqution 2 (Eq.2) was used to achieve this. The uncertainty due to precision was found to be U_{total} = 0.0008.

$$RSD_{(compound)} = \sqrt{(\sqrt{(RSD_1)^2 \times \sqrt{df_1} + \sqrt{(RSD_2)^2 \times \sqrt{df_2} + ... + \sqrt{(RSD_n)^2 \times \sqrt{df_n}}}}) / \sqrt{(df_1+df_2df_3+...+df_n)}} \quad \text{(Eq.2)}$$

[RSD: Relative standard deviation, df: Degrees of freedom (df=n-1; n: Number of repetitions)].

Total Uncertainty

The total uncertainty was calculated by combining the uncertainty results from weighing, volume, precision and accuracy using the formula below.

Total uncertainty (U) was calculated as 0.014 according to equation 3 (Eq.3).

$$U = \sqrt{(U_{weigh})^2 + (U_{volume})^2 + (U_{certainity})^2 + (U_{truth})^2} \quad (Eq.3)$$

Expanded Uncertainty

The expanded uncertainty was calculated according to equation 4 and 5 (Eq.4; Eq.5) and found to be 0.028. At this point, it is possible to give the values of our study as the result of the report in the form of equation 6 (Eq.6).

Expanded Uncertainty (U_{top})= *Total standard uncertainty* (U*top*) *x k* (Eq.4)

k= 2 (%95 confidence interval)

U_{salt}=*k x* $U_{(Protein\text{-}Relative\ Std.Uncertainity)}$ (Eq.5)

U_{salt} = 2 x 0.014

U_{salt} = 0.028

Result (%) = Analysis Result ± (Analysis Result x Expanded Uncertainty) (Eq.6)

Conclusion

Validation studies were conducted to verify the validity of the Mohr method, which is one of the most common methods for salt determination in the laboratory. Therefore, Urfa cheese and Kashar were preferred. Precision (repeatability and reproducibility) and accuracy are the method validation parameters. The relative standard deviation (RSD) values obtained under the conditions of reproducibility and repeatability were compared with the concentration-dependent precision values and the results were found to be appropriate. The accuracy study was carried out using a standard reference substance. The t-test was used to determine whether the results were close to the true value and the results were found to be appropriate. As a result, the validation studies of the Mohr method were completed and are valid for our study. A study on the measurement uncertainty of cheese salt was also conducted. The applied analysis method determined the uncertainty components including volume, precision, weight, and accuracy. Then, the expanded uncertainty was calculated by combining these components. The expanded uncertainty is U(salt) = 0.028

Author Contributions

Yasin Yakar contributed to data collection, writing, and analysis. *Elif Esra Altuner* participated in data collection and writing. *Yener Tekeli* and *Tuba Tekeli* contributed to writing and manuscript review. *Merve Özdemir* and *Tuğçe Çelik* were involved in data collection and analysis. All authors have read and approved the final version of the manuscript.

Ethic

There is no any ethical issue in publishing this article.

Conflicts of Interest

The authors declare no conflicts of interest.

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