Flame Photometry, a Precise, Safe, and Reliable Method for Determining Sodium in Fried Corn-Based Snack Matrices

Fotometría de llama, un método preciso, seguro y fiable para la determinación de sodio en matrices de pasabocas fritos a base de maíz

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Abstract

In the food industry, it is crucial to validate analytical methods for the testing of nutrients and components in processed food products, especially with the recent inclusion of warning labels. This paper introduces a novel methodology for determining sodium levels in fried corn-based snacks. The main objective of this study was to determine the reliability of the method in consistently delivering accurate results. The methodology involved a thorough validation of the flame photometry analytical method to precisely measure the sodium content in corn-based snack foods. The equipment results were linearized for the specific analyte, and its accuracy was determined by calibration curves at four control points using two sodium standards. One standard was standardized control, and the second was prepared by directly diluting the food matrix of interest. The limits of the equipment for sodium detection and quantification were determined. Accuracy was evaluated using experimental recovery percentages. The results of the method's standardization were evaluated for 10 months by analyzing 150 corn-based snack samples and obtaining measurement data from stable sodium tests compared to the data reported on the packaging. The results indicated the accuracy and linearity of the method through a linear regression curve and analysis of variance, as well as homogeneity and homoscedasticity by applying the t-statistic and the Cochran Q test. Finally, the conclusion is that the method provides fast, safe, reliable, and accurate results. The sample analyzed is in compliance with the regulations for low-sodium foods without a warning label.

Keywords: Accuracy determination, calibration curves, corn-based snacks, flame photometry, sodium content, system validation.

Resumen

En la industria alimentaria es crucial validar los métodos analíticos para el análisis de nutrientes y componentes en productos alimenticios procesados, especialmente, con la reciente inclusión del sistema de etiquetado frontal de alimentos. En este artículo se presenta una metodología novedosa para determinar los niveles de sodio en pasabocas fritos a base de maíz. Esta investigación tuvo como objetivo determinar la fiabilidad del método a la hora de proporcionar resultados precisos de forma consistente. La metodología consistió en una validación exhaustiva del método analítico de fotometría de llama para medir con precisión el contenido de sodio en pasabocas a base de maíz. Los resultados del equipo se linealizaron para el analito específico, y su precisión se determinó mediante curvas de calibración en cuatro puntos de control utilizando dos estándares de sodio. Un estándar era un control normalizado, y el segundo se preparó diluyendo directamente la matriz alimentaria de interés. Se determinaron los límites del equipo para la detección y cuantificación de sodio. La precisión se evaluó utilizando porcentajes experimentales de recuperación. Los resultados de la normalización del método se evaluaron durante 10 meses analizando 150 muestras de aperitivos a base de maíz y obteniendo datos de medición de las pruebas de sodio estable comparados con los datos indicados en el envase. Los resultados indicaron la precisión y linealidad del método mediante una curva de regresión lineal y un análisis de varianza, así como la homogeneidad y homocedasticidad aplicando el estadístico t y la prueba Q de Cochran. Finalmente, se concluye que el método proporciona resultados rápidos, seguros, fiables y precisos. Adicionalmente, se encontró que la muestra analizada cumple la normativa sobre alimentos bajos en sodio sin etiqueta de advertencia.

Palabras clave: Determinación de precisión, curvas de calibración, aperitivos de maíz, fotometría de flama, contenido de sodio, validación del sistema.

Highlights

The method is used to determine compliance with the standards for low-sodium foods.
The correlation between theoretical and experimental concentrations confirms the accuracy of sodium measurement.
The test is applicable for detecting alkali metals in dairy products, meats, and processed foods.

Highlights

El método sirve para determinar el cumplimiento de normas para alimentos bajos en sodio.
La correlación entre concentración teórica y experimental confirma la precisión para medir sodio.
La prueba es aplicable para detectar metales alcalinos en lácteos, carnes y alimentos procesados.

1. INTRODUCTION

Salt (NaCl) is a crucial component in processed foods. Salt sodium is the main cation in extracellular fluids in animals and humans, like blood plasma [1]. Its accurate measurement is essential as it impacts both the flavor of products and human health [2]. Consuming a high amount of salt is connected to an increase in blood pressure, which in turn is a risk factor for developing cardiovascular diseases such as heart disease and stroke [3]. The individual sodium intake of adults and children usually exceeds dietary requirements in most countries [4]. The food labeling and advertising law enforcers warning labels restrict marketing and ban high-sodium foods and drinks from being sold in some sensible places [5].

The salt-containing food products industry needs to obtain information quickly and easily. There must be methods to determine the safety of the products, and these methods need to be efficient, safe, and highly reliable. Therefore, choosing an accurate method for determining sodium content has become important for food and beverage manufacturers [6]. Methods used to determine sodium content in food could be, atomic absorption spectroscopy [7] and, inductively coupled plasma mass spectrometry (ICP-MS) [8]. These techniques demand substantial time for sample preparation and system calibration and necessitate significant capital investments and extensive training to operate proficiently.

On the contrary, Mohr titration involves the quantification of halides, particularly chlorides, utilizing a standardized solution of silver nitrate ($AgNO_3$) as the titrant and potassium chromate (K_2CrO_4) as the indicator [9]. Maintaining a pH range between 6.5 and 10.3 is crucial during the procedure; below pH 6.5, silver chromate becomes soluble, while above pH 10, silver reacts with hydroxyl ions to form oxides or hydroxides [10]. Furthermore, even slight fluctuations in pH within this range in the sample can result in inaccurate outcomes. Additionally, $AgNO_3$ is a highly toxic compound that can cause severe damage upon ingestion or exposure [11]. The toxicity of $AgNO_3$ is attributed to the silver ions (Ag+) which can bind to proteins, leading to denaturation and a caustic, corrosive effect [12]. Accidental ingestion of $AgNO_3$ can lead to severe burns in the oropharynx and esophagus, respiratory distress syndrome, and lung collapse [12]. In addition, it is highly water-soluble and strongly dissociated, resulting in uncomplexed cationic silver (Ag+), which is very toxic in standard laboratory water. The 96-hour LC50 value for fish ranges from about 3 µg/l to 70 µg/l of total silver [13].

The flame photometry method is based on the characteristic light emission that occurs when a gaseous atom in the flame is excited by heat [14]. It is based on the interaction between the atomized analyte and electromagnetic radiation using a flame that generates a solvation and vaporization process in the atomized sample generating the excitation of the atoms [15]. Specifically for sodium, a hydrogen and oxygen flame is used to create a reducing environment that induces light emission by the excited sodium atoms, emitting a specific wavelength that can be measured and used to determine the sodium concentration in the analyzed sample [16]. The excited atoms tend to radiate energy since they are not in equilibrium and when emitted they return to their fundamental state, the amount of energy emitted is proportional to the number of atoms in the excited state and proportional to the concentration of the substance [17].

The flame photometry equipment is composed of an atomizer that disperses the sample generating a cloud that when in contact with the flame generates a signal, this signal that leaves the atomizer contains the information of the analyte and other radiations produced that pass to a monochromator [18]. The monochromator eliminates the other radiations produced decreasing the noise in the analyzed sample, the signal that leaves the monochromator is an electromagnetic analytical signal that is translated into an electric current in a phototube, this electric current is translated into a signal processor and recorded in a meter obtaining the concentration measurement of the analyte studied, for the case of sodium these emissions are generated at a wavelength of 589 nm [19].

Flame photometry, a classic analytical technique, used in non-alimentary contexts, is widely used in the evaluation of alkali and alkaline soil metals, such as sodium, potassium, calcium, and magnesium. In the context of semiconductor sensors [20]. Water and soil decontamination [21], SARS-CoV-2 dissemination in epidemiology [22] among others. Flame photometry is regarded as a straightforward and cost-effective instrumental analytical method because it does not require complex instrumental components such as specialized nebulizers, radiation sources, and high-purity reagents and gases [23].

In the food industry, validating other analytical methods is crucial to ensuring safe, accurate, and reliable results for nutrient testing and components in processed food products. But it is also important how easy and fast these methods should be. [24], using flame photometry results, assess the impact of soaking foods in hot water on the potassium and phosphorus content in both plant- and animal-based foods.

The flame photometer has been utilized in some food-related applications. For instance, it has been employed to analyze minerals and heavy metals in *Syzygium cumini* fruit from different regions in Bangladesh [25], to quantitatively determine sodium ion levels in food sauces [26], and to analyze the calcium, sodium, and potassium content in vegetable-supplemented chicken soup in Vietnam [27]. Furthermore, the flame photometer has been used for elemental mineral analysis in three different Solanum spp. and to identify sulfur compounds in Laoshan green teas as well as in red wines associated with new oak wood [28]- [30]. [31] analyzed the changes in flavor substances in cooking and steaming shrimps. They measured the contents of sodium (Na^+) and potassium (K^+) by flame atomic absorption spectrophotometry. [23] used the same method to measure the sodium concentration in Brazilian light and non-light powdered instant soups. Both studies conclude that flame photometry is an effortless, safe, fast, and cost-effective technique for determining sodium.

In general, few previous studies have used this technique for food characterization. Specifically, in products such as extruded and fried snacks made from corn, where the sodium level can vary considerably due to the manufacturing processes and the reactions of ingredients used at high fried temperatures, it is important to rely on techniques such as flame photometry. Based on this, this study aimed to validate the flame photometry method for determining sodium content in extruded and fried corn-based snack matrices for the first time.

2. METHODOLOGY

2.1 Instrumentation

A Digimed® flame photometer (DM-64-4E, Sao Paulo, Brazil) was used for the present study; measurements were performed based on the recommendations of the manufacturer, precision level of 1 % or 0.1 ppm, response time 8 s, volume per analysis 2 mL. The photometer is programmed to aspirate 7.00 ± 0.20 mL min-1 of the standards and samples. An analytical balance (±0.0001 g, Sartorius Entris® II, Advanced

BCE224I-1S, Goettingen, Germany), was used for weighing the samples. Micropipettes (Transferpette®, D-10000, Germany) with adjustable volumes were utilized during the standards preparation. A food processor (Oster®, FPSTFP1455, China) was used to homogenize the samples before testing.

The glassware was washed with deionized water before each analysis and all the glassware was washed weekly with 10 % (v/v) HNO3 for 30 min and then rinsed with plenty of deionized water to remove traces of acid.

2.2 Reagents and samples

High-purity deionized water obtained from the Elix Advantage® 3/5/10/15 F3DA70491A system (resistivity 0.275 µS cm-1) was used to prepare the analytical standards and samples. The standard reference solution for the calibration curves was a sodium solution of Digimed® DM-513KC-64-A (Sao Paulo, Brazil).

2.3 Sample preparation procedure

Samples were collected monthly at 15 samples per month for 10 months. The samples were 30 g of fried corn-based snack matrixes (SNK-MN).

The SNK-MN samples were deposited in the food processor and the product was homogenized at maximum power at 30 s intervals, ensuring that the entire sample was completely homogenized. A clean and dry beaker was tared in the analytical balance until 100 g of sample was obtained. From the initial sample, 30 g were selected, using clean and dry watch glass. The sample of 30 g was placed in a balloon and was made up to 250 mL with constant agitation. The sample was allowed to settle for 3 min and 10 mL was withdrawn with the pipette. The 10 mL of diluted sample was again volumed to 100 mL, and stirred constantly for 5 min, from the second dilution 10 mL were removed and transferred to a beaker to feed the aspiration system of the flame photometer. The photometer was previously turned on and calibrated for sodium reading, the sample was atomized and the reading in ppm Na was generated from 0.00 ppm to 100 ppm. This procedure was performed in triplicate (n = 3) and with an analytical blank. The official AOAC method 963.13-1964, [32], and [33] were used as guides for the determination of sodium and potassium [34].

To determine the concentration in mg Na / 100 g in the finished product from the flame photometer reading data in ppm was used (1)

$$\text{mg Na} = \left(\frac{X_1 * \frac{V_1}{V_2} * V_3}{M}\right) * (100\text{g}) \qquad (1)$$

Where X_1 is sodium result, photometer measurement (mg/L), V_1 is volume dilution (L), V_2 is volume of the aliquot taken to make the dilution (L), V_3 is aliquot volume (L), and M is sample mass of finished product (g).

2.4 Instrumental parameters

The data obtained in the experimental tests is quantitative. The data was handled using descriptive statistics. The standard calibration curve was performed based on specific concentration standards of 2, 5, 10, 20, 30, 50, 80, and 100 ppm of standard solution, with wavelength-specific readings for sodium in 589 nm.

The linearity was evaluated by elaborating two calibration curves using the standard standards and the solution of the SNK-MN samples at dilutions of 0.02, 0.05, 0.10, 0.20, 0.30, 0.50, 0.80, 1.00 % (v/v) and determining the linearity of the photometer reading by implementing linear regression statistics, analysis of variance for the regression curve and statistical t-test. The limits of quantification (LOQ) and detection (LOD) were calculated using the equation of the linear regression line [35].

2.5 Assessment of the precision and accuracy of the proposed method

2.5.1 Control tests

For the precision of the method, four controls corresponding to calibration points for sodium were taken from the standard calibration curve with concentrations of 10, 30, 50, and 80 ppm of the standard solution, performing four measurements for each standard. A “Q test” of Cochran was conducted to assess homogeneity among four groups of calibration curve measurements [36]. A chi-square distribution with k-1 degrees of freedom for a significance level of 0.05 was used. We used (2) for the implementation of the test.

$$Q = \frac{(k-1) * \sum_{i=1}^{k} * (X_i - \bar{X})^2}{\sum_{j=1}^{n} * (X_{ij} - X_i)^2} \qquad (2)$$

Where Q is Cochran's statistic, k is the number of groups, n is the total number of observations, Xi is the mean of the variances of groups, is the overall mean of the variances, and Xij is the variance of the jth element of the i^{th} group. Equation (2) was simplified and converted into (3)

$$Q = \frac{(K-1)SSbetween}{SSwithin} \qquad (3)$$

Where k is the number of groups, $\sum^{k}_{i=1} * (X_i - X^{-})^2$ is expressed as the sum of squares between the groups (SSbetween), $\sum^{n}_{j=1}{}^{*} (X_{ij} - X_i)^2$ is expressed as the sum of squares within the groups (SSwithin).

For the data stability test, the X-S control plot was used to evaluate the variability of the process versus its mean (X) and standard deviation (S), Determining the upper and lower limits, as well as the central limits, using 15 observations for 10 months with a total of 150 data. To determine X, (4), (5) and (6) were used:

$$LCS = \bar{\bar{X}} + 3\frac{\bar{S}}{c4\sqrt{n}} \qquad (4)$$

$$Central\ limit = \bar{\bar{X}} \qquad (5)$$

$$LCI = \bar{\bar{X}} - 3\frac{\bar{S}}{c4\sqrt{n}} \qquad (6)$$

To determine S, (7), (8) and (9) were used:

$$LCS = \bar{S} + 3\frac{\bar{S}}{c4}\sqrt{1 - c4^2} \qquad (7)$$

$$Central\ limit = \bar{S} \qquad (8)$$

$$LCI = \bar{S} - 3\frac{\bar{S}}{c4}\sqrt{1 - c4^2} \qquad (9)$$

Where is the average of the average of the observations, is the average of the standard deviations, C4 is a specific variable for the creation of control charts, and n is the number of observations.

2.5.2 Accuracy

For the accuracy determination, the recovery percentages were calculated and compared to the theoretical value of the standard solution at the calibration points in sodium (ppm).

3. RESULTS AND DISCUSSION

3.1 Instrumental parameters

Table 1 shows the calibration data and recovery percentages. For the calibration curves, an average percentage recovery R of 88 % was obtained, which is within the range of 80 % and 120 % as determined by

[37], to demonstrate the accuracy of a method during method validation [38]. The linearity of the method was also evaluated by using two calibration standards (standard pattern DM-513KC-64-4) and saline standard from dilutions of the product under analysis (Table 1).

Table 1

Calibration data and their recovery percentages using DM-513KC-64-4 standard solutions

Theoretical concentration (ppm)	Experimental concentration (ppm)*	Recovery (%)
0.02	0.022 ± 0.0025	108 %
0.05	0.053 ± 0.0036	106 %
0.10	0.113 ± 0.0144	113 %
0.20	0.212 ± 0.0335	106 %
0.30	0.281 ± 0.0915	94 %
0.50	0.493 ± 0.0815	99 %
0.80	0.792 ± 0.0695	99 %
1.00	0.981 ± 0.0583	98 %

Source: own elaboration.

* Mean ± standard deviation, n = 3

A calibration graph with practically overlapping data was obtained as shown in Figure 1 with a coefficient of determination of 0.993 for standard calibration and 0.9985 for saline calibration (Data in Table 1s of the supplementary material). These coefficients demonstrate the fit of the experimental data versus the photometer reading points for the concentrations analyzed and the absence of systematic reading error [39]. The values of the recovery percentage are in all cases higher than those obtained by [6], for meat products.

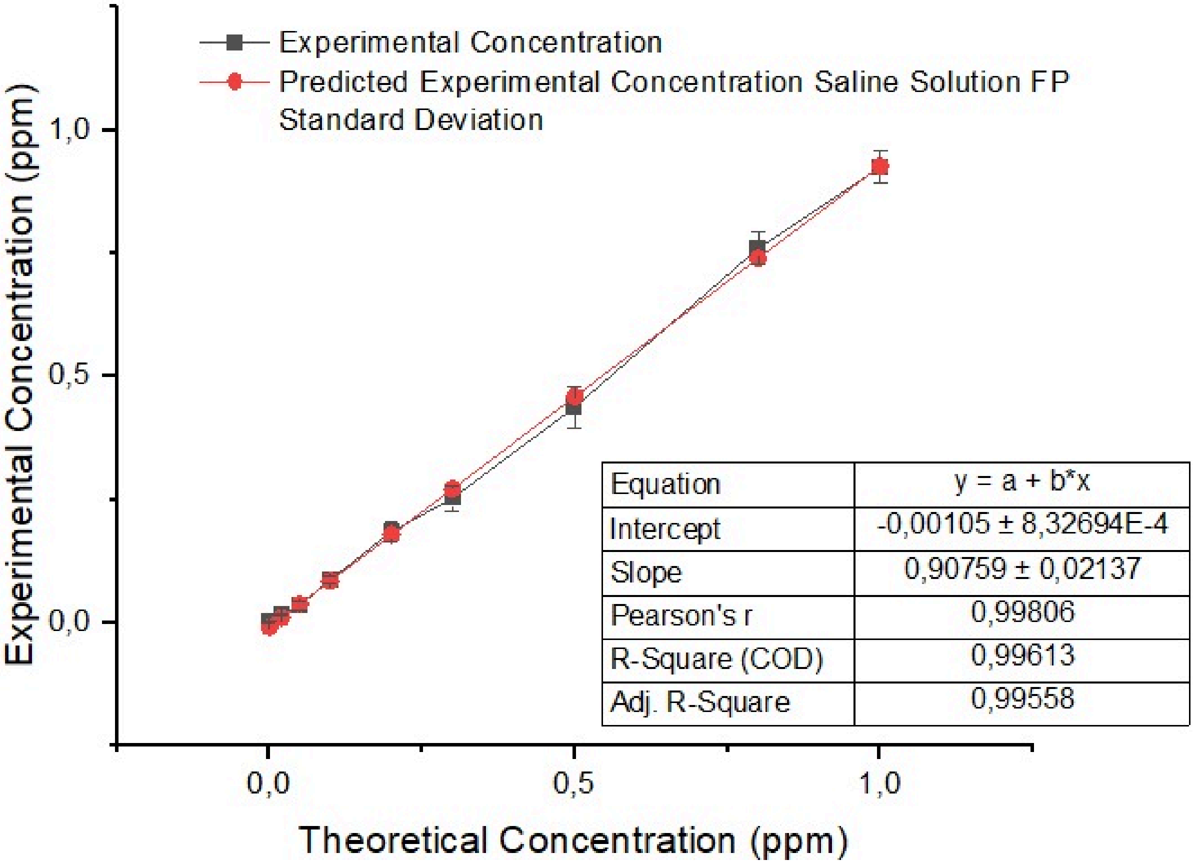

Figure 1.

Fitted regression curve

Source: own elaboration.

Curve regression statistics indicate a positive correlation in the linear regression model with a 0.999 correlation coefficient. Based on the above, the closeness of the data obtained by flame photometry versus the measurement points established for the analyzed concentrations of sodium in ppm can be demonstrated. The coefficient of determination indicates the fit of the regression line against the analyzed data obtaining a value of 0.998; the determined standard error obtained is 0.0142 demonstrating the fit of the model [40]. The low standard deviation of the residuals, and the high ratio of readings of the samples analyzed of sodium evidence the linearity of reading of the method for SNK-MN product analysis, with a limit of detection LOD 0.015 ppm and a limit of quantification LOQ of 0.0526 ppm for sodium. Taking into account that LOD and LOQ are defined as the lowest concentration of the analyte that can be reliably detected and quantified [41], the low values obtained are desirable. The LOD and LOQ values of the present work are lower than those reported by [23] of 0.16 ppm and 0.49 ppm respectively, for its study on instant soups. And similar results to those obtained by [6], for meat products.

3.2 Assessment of the precision and accuracy of the proposed method

The analysis of variance obtained an F value of 4545.87 and a critical F value of 4.15x10-11 indicating that the regression model has a high statistical significance since the critical F value is lower than the calculated F, evidencing the importance of the relationship between variances for the reading data in the flame photometer for sodium in ppm and the correlation of the values, a strong relationship is determined that is statistically significant between the theoretical concentration in ppm and the experimental measurements, determining the linearity of the method as demonstrated by the high F value and the low P value for the theoretical concentration of measurement; the upper and lower limits of the intercept include 0 and therefore the line is compatible to pass through the intercept (Table 2S of the supplementary material).

The agreement between the experimental measurements and the theoretical calibration points in the flame photometer is evidenced by observing an overlap in the points of the fitted regression curve (Figure 1), demonstrating precision in the method and reliability of the obtained results in the determination of sodium by flame photometry of the intensity measured versus the concentration.

The experimental values often do not match the mathematical model, leading to differences between observed and predicted values [42]. Analysis of the residuals (Figure 2) of the present study shows a random distribution on the zero line with dispersion, indicating that linear calibration explains the variance of the calibration data.

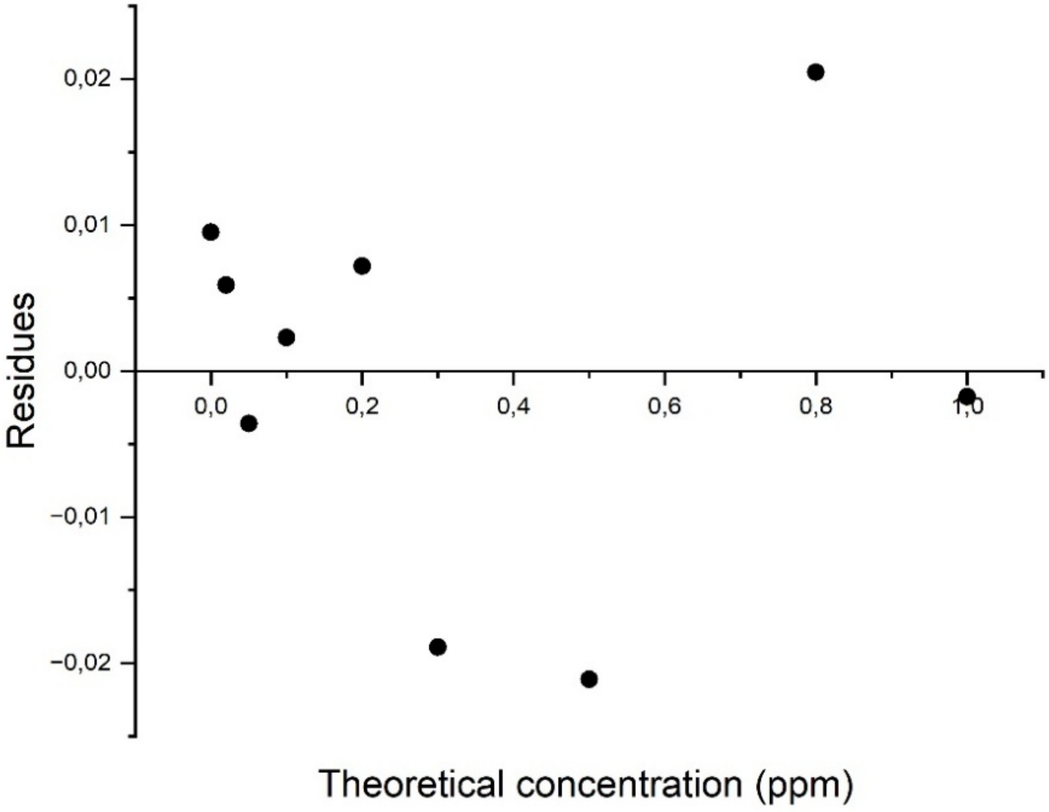

Figure 2.
Residual of linear calibration data
Source: own elaboration.

When performing the statistical t-test (Table 3S of the supplementary material) it was determined that there is no statistical difference between the concentrations of the two samples; the mean and variance of the two samples are comparable and the statistical analysis does not indicate sufficient evidence to reject the null hypothesis of hypothetical difference of the means different from zero, this shows that the flame photometry method is efficient and also demonstrates the accuracy it has for the determination of sodium in ppm in the analyzed solutions.

The test statistical analysis of Cochran was used to validate the homogeneity of variances demonstrating homoscedastic behavior using a probability of 0.05. A Q value of 0.0000221 was obtained (Table 2), which is below the critical value analyzed by the Chi-square table (in the original Cochran´s paper) with (k-1) which is 7.815 for three degrees of freedom [36].

Table 2

Cochran Q test

Calibration curve	Curve data				Media	Total mean	SSbetween	SSwithin	Q
1	0.010	0.299	0.493	0.802	0.401	0.401	0.00003859	1.7434875	0.0000221
2	0.010	0.301	0.502	0.796	0.402				
3	0.010	0.298	0.491	0.794	0.398				
4	0.010	0.296	0.494	0.809	0.402				

Source: own elaboration.

Media: Group average; Total Mean: average of the average of the groups; SSbetween: sum of squares between groups; SSwithin; sum of squares within groups.

To determine the stability of the samples during the study, a control X plot was made to determine the behavior versus the mean of the data (Figure 3), showing a distribution of the data above the mean control line and a peak outside the established limits, indicating that the experimental control data are stable for the mean during the 10 months of testing for the 150 samples analyzed.

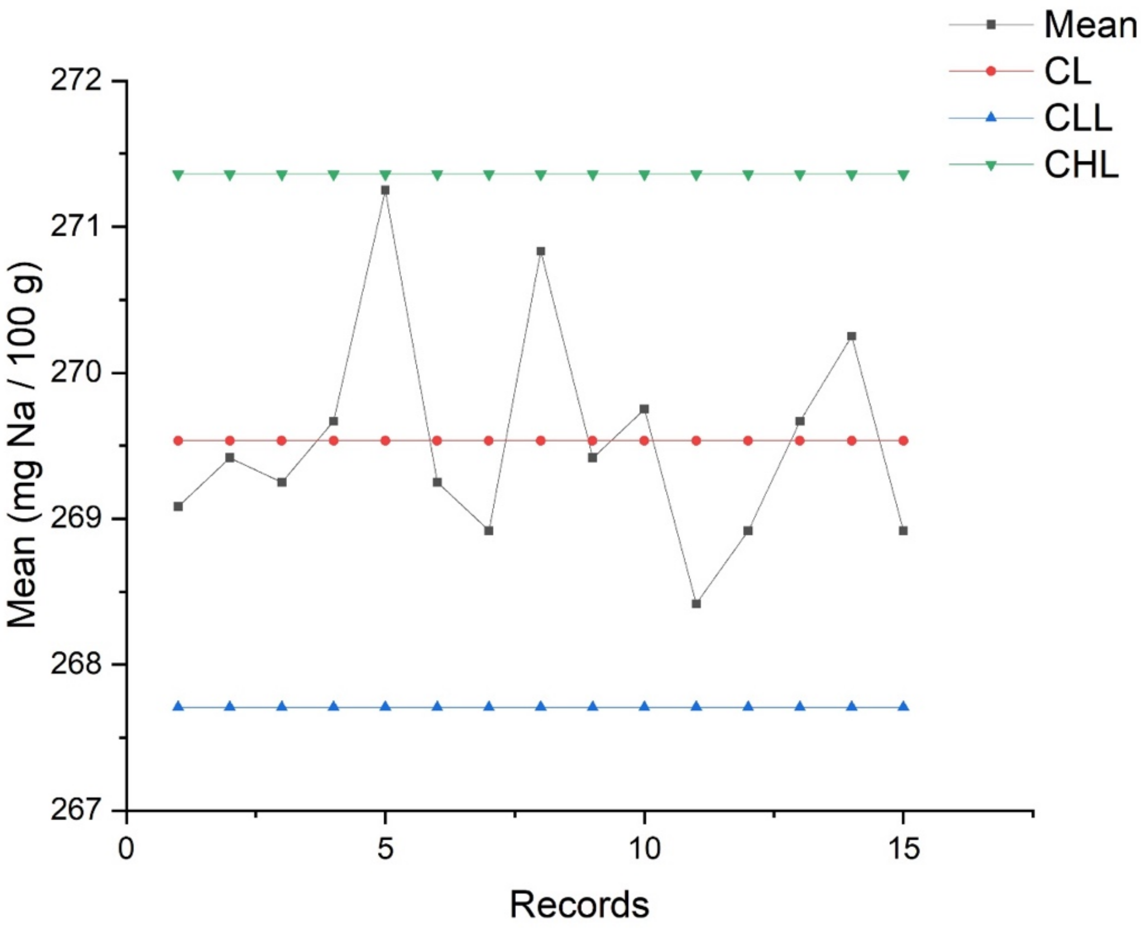

Figure 3.

Graphic X for control of means for sodium mg in 100 g of SNK-MN. CL= control line; CLL= critical lower limit; CHL= critical higher limit

Source: own elaboration.

Figure 4 for the control of the stability of the samples shows that the process is controlled for the standard deviation demonstrating the stability of the 150 samples with scattered data on the control line demonstrating a controlled trend of the data without exceeding the maximum and minimum limits established. Figure 4 and Figure 5 demonstrate the stable behavior of the product analyzed during the ten months of study and follow-up, evidencing control over the process and stability of the product, generating reliability of the data obtained in the sodium analysis. The concentration in mg Na/100 g of the finished product reported on the packaging is 269. For the data analyzed to the reported value of 269 mg Na/100 g, an average sample mean of 269.53 was obtained using (1) and an average standard deviation of 1.87, determining, as shown in the X-S graphs, stability in the samples over time and experimental sodium reading values with low deviation in comparison with the value reported in the product. The results are lower than the limits established by Resolution 2013 of 2020 in Colombia, which establishes the technical regulation that defines the maximum sodium content of processed foods. [43]. Per the technical regulation, the permissible sodium level for this food category (corn snack) is 497 mg Na/100 g. At this sodium level, the processed food tested would not need to use the warning label.

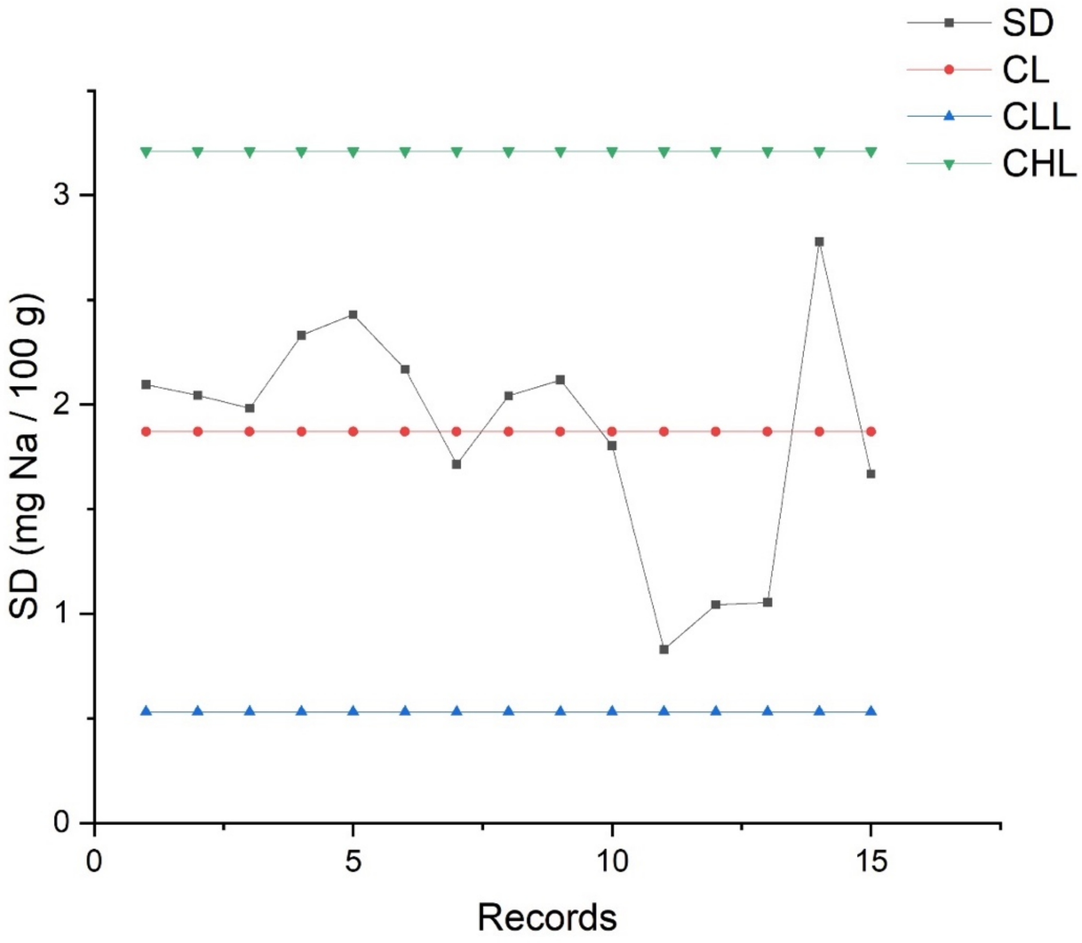

Figure 4.

Graphic S for Standard deviation control for sodium mg in 100 g of SNK-MN.SD Standard deviation; CL Control line; CHL critical higher limit; CLL critical lower limit.

Source: own elaboration.

4. CONCLUSIONS

The results obtained show that the analytical method of determination by flame photometry offers safe, precise, reliable, and accurate data for the determination of sodium in corn-based food matrixes. Preparing sample dilutions reduces analysis time.

Using the implemented statistics, it was possible to determine that the parameters of linearity, accuracy, specificity, and repeatability are acceptable for quantifying sodium in the snack sample by flame photometry with a limit of detection LOD of 0.015 ppm and a limit of quantification LOQ of 0. 0526 ppm for sodium. Similarly, compliance with the acceptance criteria is evident in all the tests performed. This guarantees the efficiency and effectiveness of implementing the flame photometry method for determining sodium in corn-based snack foods. However, this test is not limited to this type of food matrix, as it could also be applied for alkali metals detection in dairy products, meats, beverages, and other processed foods.

The accuracy of the method was assessed by comparing the recovery percentages with the theoretical values of the standards utilized. The findings revealed a strong correlation between the theoretical and experimental concentrations, indicating the precision and accuracy of the flame photometry method in determining sodium. Determining sodium in processed foods is essential for compliance with high sodium regulations.

The analyzed sample complies with regulations for low-sodium foods without a warning label. However, the main limitation of the method is the requirement for frequent calibration since, to maintain accuracy, it is necessary to calibrate the equipment constantly with known sodium standards and with the specific standards for the equipment, which have an average cost of USD 3700 and a useful life of six months.

Supplementary materials

suppl3.zip (zip) SUPPLEMENTARY MATERIAL
(Data in Table 1s of the supplementary material).

(Table 2S of the supplementary material).

(Table 3S of the supplementary material).

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