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Standardization of Atomic Absorption Spectrometry Protocol for Calcium Analysis in Milk and Milk Products

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Abstract

Calcium analysis of calcium fortified milk is difficult due to high concentration of calcium and interfering elements phosphates and citrates. The International methods were analyzed but accuracy was low. Therefore, the calcium analysis method for milk and milk products using flame absorption atomic spectroscopy was standardized. The atomic absorption spectrometer was calibrated, and true values ranged between 99.90 to 101.13%. The initial calibration verification values were 100.09 to 100.52% which showed that equipment analysis accuracy is perfect. The wet and dry digestion methods were used for sample preparation. Spike and duplicate sample analysis were used to select the best digestion method. Dry digestion for 16 h at 600 °C showed the best sample preparation method for analysis of calcium. Lanthanum oxide and lanthanum chloride were used for minimizing chemical interference. It was observed that lanthanum chloride (0.05% w/v) gave better results for calcium analysis. The calcium content ranged between 4506 ppm to 5823 ppm for market *paneer* samples and 5735 ppm to 7773 ppm for market *khoa* samples, respectively.

Keywords

Calcium, Phosphates, Acid digestion, Dry digestion, Lanthanum chloride, Atomic absorption spectrophotometer

Introduction

Mineral fortification in milk is a challenging task as they alter the quality attributes [1]. Inorganic elements have several structural and metabolic functions in our body [2]. Milk contains most of the elements in sufficient amount except iron [3]. Milk has notable amount of calcium with highest bioavailability [4]. In milk, 2/3 of calcium is associated with casein micelles, whereas 1/3 is present as free form in milk serum [5]. The soluble calcium and added calcium reduce pH that reduce the stability of milk during heat treatments *viz.* pasteurization, sterilization, ultra-high temperature processing, drying and dehydration [6]. Several milk and milk products were fortified with calcium and analysis of calcium for quality control is important [7, 8].

Calcium can be analyzed in food samples by spectrophotometers, flame photometers, atomic absorption spectrophotometer (AAS) [9, 10], biosensors [11], inductively coupled plasma optical emission spectroscopy (ICPOES) [12]. AAS equipment is substantially utilized for quantification of inorganic elements in milk and milk products [13]. The use of microwave digestion is used for sample preparation for minerals analysis of complex food matrices. A microwave digestion system can reduce sample preparation time for proximate analysis by 80% [14, 15]. For instance, solid materials or those that are rich in fat usually require more drastic treatment [16].

Sample preparation is the most important step in mineral analysis. Accurate weighing of crucible and sample, charring and digestion are important for precision and accuracy of analysis method [17]. Dry digestion [18] or wet digestion [3] is applied to burn organic material in dairy products for sample preparation. In AAS, the sample was sucked by vacuum and pass-through acetylene flame. Every mineral absorbs a specific wavelength that reduces the intensity of that wavelength. The decrease in intensity is recorded and compared with standard curve concentration to determine the mineral content [19, 20].

The deviation in mineral concentration is observed due to some interfering compounds. It might be due to low temperature of flame which is not sufficient to segregate the mineral complexes or when the segregated elements are oxidized to a compound that will not segregate further at the flame temperature. In calcium analysis, lithium is used to reduce interference and increase the accuracy of analysis method [17]. Sometimes the recoded values are higher than the actual values due to absorption of similar wavelength by two or more molecules or light scattering caused by the suspended solid particles in flame. Background correction techniques can be used to obtain correct values [18, 21]. Precision and accuracy are very important for an analysis method [22]. The accuracy is analyzed by the deviation of the mean concentration measurement of the replicates versus the theoretical concentration value expressed as a percentage (% Bias) [23]. The precision is analyzed from the relative standard deviation of the concentration measurements and expressed as the percent coefficient of variation from the mean concentration of the replicates [24].

For several years no work was conducted to standardize calcium content of milk. The old AOAC method is still used for calcium analysis. During analysis of milk and milk products, we observe some limitations which are rectified by standardized of AAS protocol. The calcium determination in milk and dairy products by AAS after digestion of the sample using wet digestion and dry digestion (600 °C) methods were carried out, to check the accuracy and trustworthiness of the sample preparation techniques.

Materials and Methods

Materials

The analytical method was standardized using AAS (AA-7000, Shimadzu, Tokyo, Japan). Wet digestion was carried out in digestion assembly (Vapodest 40 C. Gerhardt, UK Ltd., Brackley, Northants), dry digestion was performed in electric furnace (Tempsens Instruments, Udaipur, India) used for dry digestion, Water double distillation machine (Bhanu Scientific Instrument company, Bangalore, India). High quality Borosil glass wares and crucibles were used for sample preparation and volume makeup. Acetylene was used as source of flame in AAS.

Reagents

Analytical reagent grade calcium salts, chemicals and acids were bought from Loba Chemie Pvt. Ltd. (Colaba, Maharashtra, India). Water with resistivity below ten MΩ cm was used to dilute and samples preparation.

Hydrochloric acid (36.5% minimum assay)
Nitric acid (69.71% minimum assay)
Perchloric acid (70.0% minimum assay)
Sulfuric acid (98.0% minimum assay)
Tricarboxylic acid (TCA): 24% m/v aqueous solution.
$CaCO_3$ (99.9% pure)

LaO_3 solution: Five grams of lanthanum oxide was mixed with concentrated nitric acid (5 ml) and made up to 100 ml with water.

$LaCl_3$ solution: Five grams of lanthanum chloride was mixed with concentrated nitric acid (5 ml) and made up to 100 ml with water.

Stock standard solution: Exactly 2.538 g calcium carbonate (99.9%) was weighed and added 25 ml nitric acid to it. Dilute it with double distilled water to final volume of 500 ml.

Standard solutions: 5 different concentrations *viz.* 1, 2, 4, 6, and 8 ppm calcium standards were prepared. Lanthanum was added at concentration of 500 mg/L to each standard solution. Using the above solutions, a calibration curve was drawn.

Instrument calibration

The instrument was daily calibrated before calcium estimation and the standard solutions were prepared just when analysis was made. A blank sample and all standard solutions were analyzed. The permissible deviation between known concentration and determined value was ±5% maximum.

Initial calibration verification

The 3 standard calcium solutions were prepared with concentration fall within the range of calibration curve. Therefore, 3, 5, and 7 ppm calcium solutions were prepared and analyzed. The permissible deviation between known concentration and determined value was ±10% maximum.

Spike sample analysis

Milk is the main sample of analysis. Control (water and milk) and calcium spiked (500, 600, and 700 ppm calcium) water and milk samples were processed by 5 different methods of digestion. The samples were analyzed for calcium by AAS. The permissible deviation between known concentration and determined value was ±25% maximum. The formula used to determine spike sample recovery ratios is given below:

$$\%\ recovery\ = \frac{SSR - SR}{SA} \times 100$$

Where,

SSR: calcium content of sample + calcium Spike (conc.)
SR: calcium content of sample
SA: calcium Spike (conc.)

Duplicate sample analysis

One sample analyzed in duplicate (same sample weight/volume, same chemical and physical treatment, same final sample preparation and analysis). From a pooled milk tank, the same amount of milk was digested and analyzed for calcium. The calcium values of duplicate samples were determined

and compared using following equation:

$$RPD = \frac{[S - D]}{[S + D]/2} \times 100$$

Where,

S = calcium content of sample 1
D = calcium content of sample 2

Milk, *paneer*, and *khoa* samples

The fresh milk samples of equally mixed cow and buffalo milks were collected from the milking section of National Dairy Research Institute, Karnal. This milk was used for preparation of *khoa* and *paneer*. 15 *khoa* and 11 *paneer* samples were also procured from local vender of Karnal for calcium analysis.

Khoa preparation

For preparation of *khoa*, 4 kg milk was placed in a steel pan. The heating was carried out using liquid petroleum gas. The milk was heat desiccated with continuous steering. Milk, when evaporated with approx. 70% water, a semisolid mass is obtained known as *Khoa*. *Khoa* samples were prepared by the procedure documented by [25].

Paneer preparation

Paneer is indigenous small sized, unripened cheese prepared by acid coagulation or rennet-coagulation. *Paneer* was formulated by following the protocol described by Prajapati et al. [26]. For paneer preparation 4 kg milk was placed in a steel container and heated to 80 °C. Milk was cooled to 60 °C and 10% citric acid was mixed dropwise till milk was coagulated. After 30 minutes coagulated milk was passed through muslin cloth to separate paneer from whey. The *paneer* was washed with cooled water three times to efficiently separate whey. The paneer was then placed under 5 kg weight to expel out the whey.

Sample preparation

Five distinct procedures of organic matter incineration was used for sample preparation. For milk samples, the sample volume was 5 g and for *khoa* and *paneer* sample was 1 g.

Wet digestion on hot plate using tri acid mixture (HNO_3:$HClO_4$:H_2SO_4 in 3:2:1 ratio)

When sample organic matter is incinerated in organic acids, the process is known as wet digestion. Previously single acids, mixtures of acids in different ratios were used. Non spiked and calcium spiked milk and water samples were weighed in a Kjeldahl glass digestion tube and digestion was carried out on a hot plate up to fuming cease. Poured 10 ml tri acid mixture (HNO_3:$HClO_4$:H_2SO_4 in 3:2:1 ratio) in Kjeldahl tubes. And kept it in a Kjeldahl digestion assembly until a clear solution appears. Add 1ml/L (5%) of lanthanum solution and samples were diluted with deionized water 1000 times.

Dry digestion

Weight silica crucible accurately and milk and milk product samples were placed in crucible and note down the weight. Crucibles were placed in an oven at 100 °C for 1 h. Samples were charred on heater and incinerated at 600 °C in muffle furnace for minimum 16 h. The inorganic residue was solvated in 1 ml conc. HNO_3, and 1 ml/L (5%) of lanthanum solution was added and samples were diluted with deionized water 1000 times [17].

TCA coagulation method

Take 5 g of milk in a 100 ml volumetric flask, 50 ml of 24% (w/v) TCA was added and diluted to the final volume with deionized water. At every 5 minutes interval, samples were shaken for 30 minutes and finally filtered. A 5 ml aliquot of the filtrate was transferred to a 50 ml volumetric flask and add 1 ml/L of lanthanum (5% solution), and samples were diluted with deionized water [27].

Wet digestion in Kjeldahl digester

The samples were digested in Kjeldahl digestion assembly. Samples were weighed in Kjeldahl tubes and 25 ml tri acid mixture was poured in it. The temperature of Kjeldahl digester was increased at the rate 50 °C/10 minutes up to 400 °C. After attaining 400 °C temperatures, samples were kept for 4 h. After 4 h digestion, 1 ml/L of lanthanum (5% solution) was added, and samples were diluted with deionized water 1000 times.

Wet digestion on hot plate (HNO_3 and $HClO_4$)

In wet digestion, samples were weighed in 300 ml Kjeldahl flask. Samples were charred on hot plate. After fumes subside, 10 ml HNO_3 was added in Kjeldahl flasks. When organic matter was incinerated, a clear solution was obtained, then 10 ml $HClO_4$ was added and kept on hot plate for 4 h. In between, if acid was evaporated, 5 ml acid was added. 1 ml/L of lanthanum 5% solution was added to it and samples were diluted with deionized water 1000 times.

Reference material

Calcium carbonate with 99.95% minimum assay was used as reference material. The calcium carbonate was dried at 200 °C for 8 h.

AAS analysis

For analysis of calcium, Flame absorption AAS was used. The air-acetylene flame was used with flow rate of 1.5 L/minute for acetylene and 15 L/minute for air. Data collected and analyzed using the SMZ software. Atomizer height was set at 7 mm with slit width of 0.7 nm.

Linearity of calibration curve

Calibration curves were generated by plotting the peak area of standard calcium carbonate versus the theoretical concentration. Linear Regression Analysis was applied to determine linearity of calibration curve. In AAS, standard curve was plotted before every sample analysis. Analysis of samples was preceded only if R^2 is above 0.99.

Statistical analysis

The experimental data was noted down in Microsoft 365 (Microsoft Corp., New Maxico, US) and the data was interpreted with the help of Microsoft excel [28]. The value represented in the table are the mean of three concordant readings and the value after ± is the standard error mean. The different

superscript alphabets (a-b) inferred a significant ($P < 0.05$) difference in column.

Results and Discussion

Calibration of Instrument

Liu et al. [29] reported that analysis methods development generally ignored calibration of sampling and pre-condition. Therefore, step by step calibration was carried out. Calibration of analyzing instruments has prime importance. The AAS instrument was calibrated by analyzing the standards *viz.* 1, 2, 4, 6, and 8 ppm. The true values of standard solutions are presented in table 1. The true value ranged between 99.90 to 101.13% for 1 and 4 ppm, respectively (Table 1). The acceptable range of true value is ±5%. All standards true value was below ±5%. The results showed that the AAS instrument used for calcium analysis has high accuracy of analysis. Hall et al. [30] carried out calibration of AAS instrument and ICPOES. They reported that there was no significant difference between the analyzed value of AAS and ICPOES. The deviation between analyzed value and expected value was less than 5%.

Table 1: AAS calibration true value using calcium standards.

Standards conc. (ppm)	True value (%)
1	99.90 ± 1.39
2	100.48 ± 1.07
4	101.13 ± 0.86
6	100.23 ± 0.66
8	100.16 ± 0.45

Table 2: Initial calibration verification for analysis of calcium using AAS instrument.

Standards conc. (ppm)	Initial calibration verification (%)
3	100.52 ± 0.77
5	100.09 ± 0.47
7	100.14 ± 0.49

Initial calibration verification

The verification of calcium analysis method using AAS was carried out using three calcium standard solution concentrations *viz.* 3, 5, and 7 ppm, respectively (Table 2). The calibration curve was plotted using the same five concentrations as mentioned in the above section. The initial calibration verification values ranged between 100.09 to 100.52% for 5 and 3 ppm, respectively. The acceptance criteria for initial calibration verification are ±5% and all samples' values fall within range. Accuracy between instrument types and samples is evaluated based on measurement error and bias. Hall et al. [30] reported 3.3% deviation from reference value for AAS.

Spike/recovery test

The analysis methods are generally validated by Spike/recovery test [29]. The Spike test was performed by adding supplementary amount of calcium *viz.* 500, 600, and 700 ppm, respectively, in milk and water. The all samples showed acceptable percent recovery of calcium from both milk and water samples using five different incineration methods. The dry incineration percentage recovery ranged between 97.44 to 101.93%. Milk spiked samples wet digestion on hot plate with tri acid treatment showed lowest recovery rate, however, water spiked samples showed acceptable recovery rate.

Tri acid digestion on Kjeldahl digestion assembly and di acid digestion on hot plate showed nonacceptable recovery rates. The dry digestion in electric furnace has the most consonant results. Similarly, the positive negative deviation was also lowest for dry digestion and maximum for TCA method (Table 3).

Duplicate sample analysis

The duplicate sample analysis was carried out by digesting one sample in duplicate and their relative percentage difference was determined. The relative percentage difference of duplicate samples was ranged between 3.03 to 8.47% for dry digestion and TCA method, respectively (Table 4). The results showed that dry digestion of sample is the best of all tried digestion methods.

Digestion conditions

Table 3: Spike/recovery test of calcium in milk and water.

Sample preparation	Spiked medium	Calcium content (ppm)	Percentage recovery of calcium		
			Spiked 500 ppm	Spiked 600 ppm	Spiked 700 ppm
Dry incineration	Milk	1294.09 ± 17.04	97.39 ± 2.27	101.87 ± 2.74	98.69 ± 2.76
	Water	1.79 ± 0.37	98.18 ± 1.65	98.90 ± 1.69	98.08 ± 0.87
Wet incineration on hot plate (HNO_3:$HClO_4$:H_2SO_4)	Milk	1320.09 ± 30.45	88.07 ± 1.97	89.83 ± 2.41	89.17 ± 1.02
	Water	1.83 ± 0.39	96.49 ± 1.69	96.59 ± 1.58	95.47 ± 1.25
Wet incineration on Kjeldhl digestion (HNO_3:$HClO_4$:H_2SO_4)	Milk	1247.80 ± 34.32	87.56 ± 1.19	87.33 ± 1.23	86.77 ± 0.87
	Water	1.21 ± 0.37	93.40 ± 2.34	91.37 ± 1.71	90.40 ± 1.86
Wet incineration on hot plate (HNO_3:$HClO_4$)	Milk	1202.80 ± 25.23	85.96 ± 4.07	87.90 ± 3.03	87.77 ± 3.41
	Water	1.18 ± 0.30	92.04 ± 1.86	89.20 ± 2.65	89.15 ± 1.59
TCA method	Milk	1475.8 ± 29.16	104.52 ± 1.33	103.90 ± 0.82	102.54 ± 2.08
	Water	1.02 ± 0.27	89.32 ± 1.09	88.60 ± 2.57	88.83 ± 0.72

The dry digestion method was shortlisted for sample preparation for calcium analysis in milk. TCA method had the highest interference and background error. All three wet digestion methods have significant interference due to phosphates. Milk contains 899 to 1208 mg/kg phosphorus [31].

Table 4: Relative percent difference of digestion methods in duplicate sample analysis.

Sample preparation	Relative percent difference (%)
Dry incineration	3.02 ± 0.49
Wet incineration on hot plate (HNO_3:$HClO_4$:H_2SO_4)	4.81 ± 0.47
Wet incineration on Kjeldhl digestion (HNO_3:$HClO_4$:H_2SO_4)	6.89 ± 0.66
Wet incineration on hot plate (HNO_3:$HClO_4$)	7.51 ± 0.47
TCA method	8.47 ± 0.35

Phosphates interference was more in case of wet digestion than dry digestion. The condition mentioned in calcium analysis method mentioned in AOAC (2005) for digestion of food is 550 °C. However, 600 °C was chosen as it was higher than sublimation temperature of phosphates. The results showed that the accuracy of calcium analysis was better when dry digestion is carried out at 600 °C temperature than 550 °C temperature. The hypothesis behind the better analysis is that interference is due to reduction in phosphorous because sublimation point of phosphorous is below 600 °C. The boiling point of acids is not more than 400 °C, therefore, digestion of milk during wet digestion does not reduce the phosphates. First organic material is digested, and minerals are in free form. In free form phosphates create interference in calcium analysis. In comparison between wet digestion methods, tri acid mixture (HNO_3:$HClO_4$:H_2SO_4 in ratio 3:2:1) had the lowest interference. Dry digestion at 600 °C was found to be the supreme sample preparation method for calcium analysis of milk and milk products. Thus, further analyses of calcium in milk were carried out by dry digestion method.

Lanthanum concentration used for minimizing interfering compounds

The two salts of lanthanum *viz.* lanthanum chloride and lanthanum oxide were added in three concentrations 10, 20, and 50 µl/10 ml of digested sample of lanthanum 5% solution. It was observed that lanthanum chloride remarkably improves calcium analysis in milk due to minimizing chemical interference more efficiently than lanthanum oxide. The lowest level of lanthanum solution (10 µl/10 ml) was selected because it was observed that concentration increment of lanthanum has no effect on accuracy of calcium detection in milk.

Table 5: Calcium content of milk and milk products prepared in laboratory.

Milk and milk product sample	Mean
Milk	1521.20 ± 49.58
Khoa	7783.00 ± 86.14
Paneer	3903.40 ± 119.31

Linearity

The standard curve was plotted before sample analysis. Analysis of samples was preceded, if R^2 is above 0.99. The standard curve was linear for the concentration used (1 ppm to 8 ppm calcium). Maeaba and Prasad [32] analyzed the food samples and reported a linear range of 2 to 10 ppm with R^2 of 0.9999.

Calcium analysis

Calcium content of products manufactured in lab

The calcium content of milk obtained from National Dairy Research Institute, Karnal was 1521.20 ± 49.58 ppm. The calcium content of *khoa* and *paneer* was 7783.00 ± 86.14 and 3903.40 ± 119.31, respectively. Barreiro et al. [31] reported the calcium content for cow's milk 1062 to 1212 ppm and Walther et al. [33] reported 1090 to 1150 ppm. Mathur et al. [34] analyzed the calcium content of cow milk (1230 ppm) and buffalo milk (1750 ppm). Boghra and Mathur [35] analyzed the calcium content of *khoa* ranged from 6300 ± 250 ppm to 7170 ± 190 6.800 g/kg calcium was reported by Adhikari et al. [36] in *khoa* prepared from buffalo milk. Boghra and Mathur [37] also reported that the calcium content ranged from 4120 ± 161 ppm to 4750 ± 112 ppm in *paneer* samples.

Market paneer calcium analysis

Eleven *paneer* samples were collected from the local market of Karnal. The *paneer* samples were analyzed in triplicates and a significant difference was observed between all the eleven samples. Calcium content of *paneer* ranged from 4506.67 ± 40.41 ppm to 5823.33 ± 153.73 ppm (Table 6). All market *paneer* samples had higher calcium content in comparison to *paneer* prepared in lab (3903.40 ± 119.31 ppm). This might be because *paneer* available in the market was prepared from skim milk and even skim milk powder was added during manufacture. Due to this solid not fat of *paneer* increased, and this led to an increased mineral content especially, calcium. Sowmya et al. [9] analyzed *paneer* samples for calcium and reported similar calcium content in *paneer*.

Market khoa calcium analysis

In 2018 - 19, 7.58×10^6 MT *khoa* was prepared in India. The *khoa* is consumed as it is and converted into sweet des-

Table 6: Calcium content of market *paneer* samples.

Market *paneer* sample	Mean (ppm)
***Paneer* A**	4506.67 ± 40.41[a]
***Paneer* B**	4766.33 ± 46.01[b]
***Paneer* C**	4899.33 ± 21.22[c]
***Paneer* D**	5208.33 ± 27.32[c]
***Paneer* E**	5622.67 ± 18.44[e]
***Paneer* F**	5885.33 ± 213.41[f]
***Paneer* G**	5970.00 ± 10.82[f]
***Paneer* H**	5170.00 ± 157.16[g]
***Paneer* I**	5185.67 ± 14.84[g]
***Paneer* J**	5318.00 ± 29.51[h]
***Paneer* K**	5823.33 ± 153.73[i]

serts [38]. Market 15 *khoa* specimens were collected from different sweet shops of Karnal. The *khoa* specimens were analyzed in triplicates and a significant difference was observed between all the 15 samples (Table 7). Calcium content of *khoa* ranged from 5735.00 ± 243.77 ppm to 7773.00 ± 162.87 ppm. All market *khoa* samples had lower calcium content in comparison to *khoa* prepared in lab (7783.00 ± 86.14 ppm). Sowmya et al. [9] analyzed *khoa* samples for calcium and reported similar calcium content in *khoa*.

Limit of detection

The limit of detection for calcium analysis was performed by injecting the 3 blanks corresponding to 25 ml of distilled water subjected to the same treatment as the sample. The limit of detection was 0.003 ppm.

Table 7: Calcium content of market *khoa* samples.

Market *khoa* samples	Mean (ppm)
***Khoa* A**	7581.0 ± 124.36^{ab}
***Khoa* B**	7705.33 ± 95.87^{a}
***Khoa* C**	7439.33 ± 289.06^{ab}
***Khoa* D**	7773.00 ± 162.87^{a}
***Khoa* E**	5735.00 ± 243.77^{d}
***Khoa* F**	7559.33 ± 74.47^{ab}
***Khoa* G**	7180.33 ± 145.66^{b}
***Khoa* H**	7027.67 ± 267.93^{b}
***Khoa* I**	7263.67 ± 236.21^{b}
***Khoa* J**	6302.67 ± 182.03^{c}
***Khoa* K**	7595.67 ± 183.93^{ab}
***Khoa* L**	7522.00 ± 179.41^{ab}
***Khoa* M**	7568.67 ± 109.61^{ab}
***Khoa* N**	7606.00 ± 233.09^{ab}
***Khoa* O**	6573.33 ± 241.13^{c}

Limit of quantification

The limit of quantification was 0.020 ppm. Milk is a complex food having protein and calcium interaction that requires additional efforts in sample preparation. The standardized calcium analysis method is dependable, and more accurate results are assured. This method will be used for calcium analysis of milk and milk products. No additional chemical or equipment is required to perform this method. Maeaba and Prasad [32] determined the limit of detection and limit of quantification were 0.0082 and 0.0272 ppm, respectively.

Conclusion

For a long time, no new development in calcium analysis was reported. A manageable and quick analytical method was standardized for the assessment of calcium in milk and milk products. The pretreatment conditions of milk were optimized, and interferences were minimized. Different sample preparation methods were compared, with standardization of lanthanum chloride concentration for correct results. The temperature of incineration was also increased to 600 °C to minimize the phosphate interference. This calcium analytical method will be used for detection of calcium-based neutralizers added in milk, determine the distribution of added calcium in different sections of milk, and for accurate calcium content in milk for labeling. Such standardization of other nutrients analysis in milk should be carried out in future. In future, the applicability of developed calcium analysis method will be checked for other food products also.

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Conflict of Interest

Author declares no conflict of interest.

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