Physico-analytical Studies on Commercial Milk Samples

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Summary: Physical properties such as density, viscosity, pH and conductance of commercial milk samples have been determined. Total solids, water, sugar and protein contents of milk samples are also estimated. A simple and low cost digestion method has been developed for quantitative analysis of Na, K, Ca, Mg and Zn in milk samples using flame atomic absorption spectrometry. The method involves partial digestion of samples using nitric acid at 105 ± 5°C in oil bath for forty-five minutes. Relative standard deviation for Na, K and Ca is better than 1% and for Mg and Zn around 2%. The concentration ranges for Na, K, Ca, Mg and Zn are 240.00 -776.50, 422.50 - 710.00, 831.25 - 1172.50, 51.23 - 108.51 and 2.88 - 7.31 μg ml^{-1} respectively.

Introduction

The accurate measurements of sugar, fat, protein, metal and other contents in the foodstuffs play an important role in determining the food value and suitability of the diet for a variety of healthy and sick personals. The intake demand of contents listed above varies according to the physiological conditions/age of human beings. Milk is considered as a vital food item for the human beings of all ages. Quantity and quality of its intake is variable and depends on many social, economical and personal factors. Analysis of famous commercial milk brands is necessary to develop a criterion for maintenance of food value and to relate the same with dietary requirements of people. Many methods are available in literature to estimate sugars, fats and proteins in milk [1-4].

Elemental analysis of major and trace elements of milk is important for the assessment of the nutrients and environmental factors for human. The environmental term includes the origin (i.e. type of animal, quantity and quality of its food & water); and its processing and preservation etc.

Chemical digestion methods are important in the analysis of metals in any sample including foods. Total and partial digestion techniques have been reported by the others [5-11] and us [12-19] to analyze metals in a large variety of samples from natural and artificial sources. Partial digestion method is recently used to bring the metals in solution from many natural samples, e. g. leaves [20] and animal serum [21]. This work is in continuation to our efforts [12-19] regarding analysis of food items and to help establishing national standards for edibles. In this work, we report physical parameters and food contents such as sugars, proteins, water, total solids and metal contents in famous brands of commercial milk samples. Flame atomic absorption spectrometry (FAAS) is used to analyzes the metal contents.

Results and Discussion

Physical Properties

Physical properties such as density, viscosity, pH and conductance were determined using literature methods. The experimental value ± standard deviation as well as the literature values [1, 4] are given in table 1. Each measurement is an average of at least three independent experiments of each brand of milk for 20 samples.

Table 1: Physical Properties of Commercial Milk Samples at 25°C.

Samples	Density ± 0.0001 (g/ml)	Viscosity ± 0.01 (mpa-s)	pH ± 0.01	Conductance ± 1 μ S (S/cm) x 10^{-4}
1*	1.0334	1.73	6.08	0.5
2*	1.0365	1.52	7.07	0.5
3*	1.0358	1.73	6.95	0.4
4*	1.0353	2.20	6.90	0.4
5**	1.0311	1.55	7.00	0.4
6**	1.0340	1.73	7.33	0.4
Lit.	1.028 – 1.032	1.53 – 3.33	6.00 – 7.50	--

* Commercial tetra-pack
** Fresh milk sample

Density and viscosity data refer to the level of purity and homogeneity of milk samples. pH of milk indicates the acidic, basic or neutral nature of the sample and helps in controlling the microbial growth [3].

Biochemical Analysis

Percentage of water, total solids, sugar and proteins were estimated using literature methods [1-

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4]. The experimental value ± standard deviation, along with the literature values [1-4] are given in table 2.

Table 2: Biochemical Analysis of Commercial Milk Samples.

Samples	% Water ± 0.01	% Solids ± 0.01	% Sugars ± 0.01	% Proteins ± 0.01
1	87.43	12.57	5.50	1.93
2	87.46	12.54	5.24	1.88
3	87.86	12.14	5.51	1.87
4	87.82	12.18	5.80	1.93
5	88.85	11.15	5.18	1.86
6	83.74	16.26	6.01	1.82
Lit.	85.50 – 89.50	14.50 – 10.50	4.30 – 7.00	1.50 – 4.00

Percentage sugar and protein contents were also estimated in the following laboratories in Multan using patented methods: I. Shahab Laboratory, II. Central Laboratory Nishtar Hospital and III. CMH Laboratory. The results of these laboratories also support our data within the limits of standard deviation.

Metal Aanalysis

Digestion Conditions

To find the appropriate amount of nitric acid required for complete extraction of metals, four sets of test tube pairs each containing 10 ml of milk and 2, 5, 8 & 11 ml of nitric acid respectively were taken and digested for 30 minutes at 105 ± 1° C in oil bath. Blanks were similarly prepared. After dilution and making up the volume, samples were analyzed for metal contents by FAAS using instrumental conditions described in the experimental. Results indicated that 5 ml nitric acid is sufficient to bring the maximum of test elements into solution and excess of acid has no effect on the results.

To optimize the digestion time, a set of 10 ml of a milk sample in duplicate was digested with 5 ml nitric acid for 15, 30, 45 & 60 minutes at 105 ± 1° C and analyzed as described above. Blanks were similarly treated. Analytical results showed that 30-45 minutes were sufficient to bring the metal contents into solution. Hence a heating time of 45 minutes at 105 ± 1° C was used in further work for 10 ml of milk sample containing 5 ml nitric acid.

Blank Values

Blank values for the metals to be analyzed in milk samples are determined for assessing the possible contamination level in the procedure adopted. A set of five test tubes, each containing 10 ml water (instead of milk) and 5 ml nitric acid was passed through the sequence of steps. After dilution and making up the volume to 25 ml with water, the blanks were analyzed for sodium, potassium, calcium, magnesium and zinc by FAAS. Concentration ranges in μg ml^{-1} for Na: 2.3 ± 2; K: 0.3 ± 0.2; Ca: 0.3 ± 0.2; Mg: 0.1 ± 0.09; and Zn: 0.03 ± 0.01 were obtained. These blank values are insignificant to cause serious error in the methodology.

Calibration Statistics

Calibration curves were obtained for metals (Na, K, Ca, Mg & Zn) using standard solutions in 5% aqueous nitric acid. They were linear and correlation coefficient of each curve was above 0.9900, which indicated a best fit between concentration of the standard solutions and respective absorbance values. Statistical parameters of calibration curves are given in table 3.

Table 3: Statistical Parameters for Calibration Curves: Y = a + bX.

Element	Conc. Range (ppm)	Slope (b)	Corr. Coeff (r)	Intercept (a)
Na	0.00 – 1.50	0.4166	0.9966	0.0372
K	0.00 – 2.50	0.0713	0.9997	0.0008
Ca	0.00 – 10.00	0.0196	0.9983	0.0160
Mg	0.00 – 2.00	0.6680	0.9981	–0.0026
Zn	0.00 – 1.00	0.1957	0.9980	0.0037

Matrix Components

Calcium, chromium, zinc, iron, aluminum, manganese, copper, sodium, potassium, magnesium and cobalt are reported as matrix components in milk [1, 3]. These elements were analyzed by FAAS. The observed upper limits of elements [1, 3] in ppm are: Ca = 1500; Cr, Co, Cu, Mn & Al = 4 – 5; Zn = 14; Fe = 20; Na = 650; K = 600 & Mg = 150. Interference due to these elements is minimized by their addition at their maximum levels in blanks, standard solutions and during standardization procedure. It gave good matrix matching.

Accuracy, Precision and Recovery

Accuracy of the method was verified by standard addition/recovery method [12, 13]. A milk sample, in duplicate, with and without addition of known amounts of Na, K, Ca, Mg and Zn was digested and analyzed as described in experimental. Sample blanks were similarly prepared. The results are given in table 4.

The results indicate that the %age recovery of the elements is satisfactory and within the tolerable

Table 4: Recovery (%) of Na, K, Ca, Mg and Zn in a Commercial Milk Sample.

Element	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	%age Recovery
Na	--	248.75 ± 0.01	--
	25.00	271.10 ± 0.02	99.00
	50.00	294.45 ± 0.02	98.56
K	--	422.50 ± 0.04	--
	25.00	445.15 ± 0.05	99.46
	50.00	470.20 ± 0.05	99.51
Ca	--	783.75 ± 0.04	--
	25.00	802.00 ± 0.05	99.29
	50.00	830.00 ± 0.05	99.55
Mg	--	61.25 ± 0.01	--
	5.00	64.98 ± 0.02	98.08
	10.00	70.39 ± 0.02	98.79
Zn	--	2.88 ± 0.01	--
	1.00	3.80 ± 0.01	97.94
	2.00	4.77 ± 0.02	97.75

limits. Precision for n determinations (n = 20) is expressed in relative standard deviation (RSD). It is better than 1% for Na, K and Ca at various occasions and 2% for Mg and Zn. Analytical results obtained for milk samples are given in table 5. Each value is an average of at least three independent measurements in duplicate batches for each sample analyzed thrice.

Experimental

Reagents

Deionized water was used throughout the work and it was obtained from a local fertilizer plant (Pak Arab Fertilizer Ltd. Multan). All the chemicals used were of Merck Anal. R. grade. Nitric acid, used in the work, was 65% with sp. g. 1.40. Standard stock solutions (1000 ppm) were prepared by dissolving appropriate amounts of NaCl, KCl, $Ca(CH_3COO)_2 .2H_2O$, $MgCl_2 .6H_2O$ and $Zn(CH_3COO)_2 .2H_2O$ in 5% aqueous HNO_3. These solutions were diluted immediately before use with 5% HNO_3 to obtain working standard solutions with concentration appropriate to flame atomic absorption (FAAS) spectrometric measurements.

Sample Collection and Storage

Commercial milk samples of famous brands (tetra-packs) were purchased from local city markets. Fresh samples were purchased from a local dairy and a milkman who was a supplier of milk to a local colony. For each brand of milk at least 20 samples were purchased from different places. All samples were stored in their original packing in a fridge at 3° C. Samples once opened were immediately used for studies.

Contamination Control

All glassware were soaked in chromic acid for 24 hours and washed several times with water, rinsed with deionized water, oven dried and stored in dust and fume-free atmosphere without touching their insides. Milk samples digested with nitric acid were filtered using pre-treated filter papers [12–15]. For this purpose Whatman No. 40 filter papers were soaked in 0.1 M (approximately) disodium salt of ethylenediamine tetra acetate and washed several times with water to remove suspected metal impurities. The process was continued until the washings were free from traces of sodium.

Physical Properties

Density, viscosity, pH and conductance of milk samples were measured using established literature methods [3–5, 12].

Biochemical analysis

Total solid, water, sugar and protein contents in milk samples were estimated using standard reported techniques [1–11].

Metal Analysis

Metals were analyzed using flame atomic absorption spectrometer (FAAS). For this purpose, a Hitachi model A–1800 atomic absorption spectrometer equipped with standard burner and air-acetylene flame was used. Standard hollow cathode lamps were used as radiation source for sodium, potassium, calcium, magnesium and zinc. To calculate the absorbance data, atomic absorption (concentration) measurement mode with integration of absorbance signals was used. The optimum

Table 5: Analytical Results ($\mu g\ ml^{-1}$)) of Commercial Milk Samples (n = 20).

Sample No	Na	K	Ca	Mg	Zn
1	776.50 ± 0.20	710.00 ± 0.20	1172.50 ± 0.50	95.00 ± 0.10	5.10 ± 0.01
2	308.75 ± 0.20	452.50 ± 0.20	831.25 ± 0.20	67.50 ± 0.10	3.52 ± 0.10
3	248.75 ± 0.20	422.50 ± 0.20	783.75 ± 0.20	61.25 ± 0.10	2.88 ± 0.10
4	346.25 ± 0.20	671.25 ± 0.20	1171.25 ± 0.20	91.21 ± 0.10	4.48 ± 0.02
5	380.00 ± 0.20	458.75 ± 0.20	777.50 ± 0.20	51.23 ± 0.10	7.31 ± 0.02
6	240.00 ± 0.20	516.00 ± 0.20	1078.76 ± 0.20	108.51 ± 0.10	2.96 ± 0.01

Table 6: Instrumental Parameters for the Determination of Elements by FAAS.

Elements	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Air flow rate (l/min)	Acetylene flow rate (l/min)	Integration time (sec)	Burner height (mm)
Na	589.0	0.4	10.0	9.5	2.2	2.0	7.5
K	766.5	2.6	10.0	9.5	2.3	2.0	7.5
Ca	422.7	0.4	7.5	9.5	2.5	2.0	10.0
Mg	285.8	2.6	7.5	9.5	2.0	2.0	7.5
Zn	213.8	1.3	10.0	9.5	2.0	2.0	7.5

instrumental conditions for each element are listed in table 6.

A 600 ml glass beaker containing paraffin oil was used as an oil bath and a gas burner as heating source. Digestion of milk samples with nitric acid was done in acid washed Pyrex glass tubes of 1.7 x 14.5 cm dimensions.

Digestion, Sample Preparation and Metal Analysis

10 ml aliquot of each milk sample was taken in three separate acid washed test tubes and 5 ml of nitric acid (65%, sp. g. 1.4) was added into each. The test tubes were then placed in a pre-heated oil bath maintained at 105 ± 5° C for forty-five minutes. The tubes were occasionally shaken manually to subside vigorous reactions and to ensure proper mixing. The digested samples were filtered through pre treated filter papers. Filtrate and washings were collected in 25 ml volumetric flasks and made up the volume up to the mark using distilled water. These solutions were appropriately diluted, if required, prior to direct measurements by FAAS. Blanks, standard solutions, sample blanks and samples were directly aspirated and absorbance values measured.

Conclusions

The amount of Na found in milk samples ranges from 240.00 to 776.50 μg ml^{-1} and its recommended level is 300 – 400 ppm [22, 23]. Concentration of K in milk samples varies from 422.50 to 710.50 μg ml^{-1} and permissible level is 500 – 550 ppm [22, 23]. The contents of Ca in milk samples are in the range of 777.50 to 1178.76 μg ml^{-1} and the recommended level is 1100 – 1200 ppm [22, 23]. The amount of Mg analyzed in milk samples lies in the range of 51.23 to 108.51 μg ml^{-1} and recommended level is 70 – 100 ppm [22, 23]. Concentration of Zn in milk samples varies between 2.88 to 7.31 μg ml^{-1} and the permissible level is 10 – 12 ppm [22, 23].

Analytical results indicate that the metal contents in milk samples are within the permitted limits at various occasions. Variations in the metal contents among milk samples may be related to their origin and processing etc. A simple digestion method optimized for milk samples has been reported by us for metal analysis of fruit juices [12]. The present study reveals that it can satisfactorily be applied to milk samples in addition to fruit juices.

References

1. S. A. Lavonson and R. P. McFate, "Clinical Laboratory Diagnosis", 7th Ed., Lae Fabiger, Philadelphia, (1969).
2. G. H. Bell, P. J. Norman and D. E. Smith, "Text Book of Physiology and Biochemistry", 8th Ed. The English Language Book Society, London, (1972).
3. C. H. Wintah, W. D. Wurtz and H. C. Fryer, *J. Dairy Sci.* **39**, 1500, (1954).
4. Milk Ordinance and Code", Recommendations of Public Health Services, Pub. No. 229, (1956).
5. E. W. Williams, *J. Fd. Technol.*, **13**, 367, (1978).
6. H. M. Kingston and L. B. Jassie, *Anal. Chem.*, **58**, 2534, (1986).
7. A. F. Ward, L. F. Marciello, C. Carrara and V. J. Luciano, *Spectrosc. Letters*, **13**, 803, (1980).
8. A. Abu-Samara, J. R. Morris and S. R. Koirtyohann, *Anal. Chem.*, **47**(8), 1475, (1975).
9. N. R. McQuakar, D. F. Brown and P. D. Kluckner, *ibid*, **51**, 1082, (1979).
10. Analytical Methods Committee, *Analyst*, **85**, 643, (1960).
11. *Ibid*, **101**, 62, (1967).
12. S. B. Niazi, F. Mahmood and M. Z. Asghar, *J. Chem. Soc. Pak.*, **19**(2), 122, (1997).
13. S. B. Niazi, A. A. Gill and M. Y. Khokhar, *ibid*, **21**(4), 386, (1999).
14. S. B. Niazi, D. Littlejohn and D. J. Halls, *Analyst*, **118**, 821, (1993).
15. S. B. Niazi, R. L. Ali, M. Y. Khokhar and F.

Mahmood, *J. Chem. Soc. Pak.*, **23**(4), 220, (2001).
16. A. Khan, S. Ahmed, F. Mahmood and M. Y. Khokhar, *Ad. Sci. & Tech.*, **21**(3), 205, (2003).
17. A. Khan, S. Ahmed, F. Mahmood, M. Y. Khokhar and M. Riaz, *Radiochim. Acta*, **91**, 413, (2003).
18. A. Khan, S. Ahmed, S. A. R. Zaidi, F. Mahmood and M. Y. Khokhar, *Sep. Sci. & Tech.*, **37**(13), 3099, (2002).
19. A. Khan, F. Mahmood, S. Ahmed and M. Y. Khokhar, *J. Sol.-Gel.& Tech.*, **27**, 221, (2003).
20. Y. H. Kim, *Sep. Sci. & Tech.*, **35**(11), 2327, (2002).
21. J. Senevirantne and J. A. Cox, *Talanta*, **52**, 801, (2000).
22. European Foods Standards Bulletin, (2000).
23. UNO Edibles Standards, (2001).