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Cow Milk Total Protein Analysis and Daily Intake Estimation in Armenia

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ABSTRACT

Protein plays a critical role in both the nutrition and biological functions of milk, which is an important part of a balanced diet. In this study, we installed and implemented the fully automated Kjeldahl method, enabling the precise determination of the total protein content in cow milk. The investigation involved cow milk samples collected from Yerevan markets, representing 9 producers, over the years 2021 and 2022. The mean total protein content in the analyzed milk samples was found to be 2.93 g per 100 g.To determine the significance and contribution of milk protein in the adult population's diet in Armenia, the daily protein intake through milk consumption was evaluated. Two consumer clusters were identified: one with moderate milk consumption (0.067 kg per day) and another with higher intake (0.208 kg per day). The contribution of milk protein to the overall protein intake in the diet of the adult population ranged from 2.62 % to 8.13 %. Despite varied levels of milk protein consumption, it is essential to acknowledge that Armenian protein sources extend beyond milk.

Introduction

Milk, a remarkably intricate fluid, comprises a diverse array of constituents, with primary components including water, lipids, sugars, and proteins. Additionally, it contains trace levels of vitamins and minerals, among other bioactive compounds (Goulding, et al., 2020). Globally, over 6 billion individuals incorporate milk and its derivatives into their diets, underscoring its high importance in human nutrition (FAO, 2018). Projections for 2022 indicate worldwide milk production reaching nearly 930 million tons, reflecting a 0.6 percent growth from 2021 (FAO, 2022). Milk stands out for its protein content, housing a diverse array of more than 100 distinct protein types, categorized into three nitrogen fractions: abundant caseins, serum or whey proteins (comprising lactalbumin, lactoglobulin, blood serum albumin, and immunoglobulins), and the nonprotein nitrogen fraction, which encompasses milk fat globule membrane proteins alongside a wide spectrum of enzymes and hormones (Dupont, 2011).

Due to its high protein content, milk serves as a vital source of essential amino acids in the human diet (Wolfe, 2015; Goulding, et al., 2020).

In Armenia, milk and dairy products have an important role in the local diet. Over the past decade (2012-2022), milk production has consistently ranged from 618.2 thousand tonnes (the lowest in 2012) to 758.2 thousand tonnes (the highest in 2017). In 2022, there was a decrease in milk production compared to previous years, totaling 623.1 thousand tonnes. The nation exhibits a high degree of self-sufficiency in milk production, ranging from 82 % to 93% (ArmStat, 2023). Considering the importance of milk nutrients, particularly proteins, in the diet of the Armenian adult population, a knowledge gap emerges regarding how these nutrients contribute to the nutrition of the population.

International authorities have established Dietary Reference Values (DRVs) and protein intake recommendations for consumers. These guidelines suggest an average requirement (AR) ranging from 0.66 to 0.8 grams per kilogram of body weight per day, as well as a Population Reference Intake (PRI) of 0.83 grams per kilogram of body weight per day, among other parameters (WHO/FAO/UNU, 2007; EFSA NDA, 2012). Armenia, as a member of the Eurasian Economic Union (EEAU), aligns itself with the Customs Union Technical Regulation for food product labeling (TR CU, 2011). This regulation encompasses diverse stipulations for labeling food products, including the specification of the average daily requirement for essential nutrients, such as protein, which has been established at 75 grams per day (TR CU, 2011).

Numerous methods are available for assessing protein content in food items, including spectrophotometric techniques, the Duran method, the Lowry method, and the Biuret method, among others (Jiang, 2014). One of the most widely adopted methodologies is the Kjeldahl method, initially designed for the brewing industry in 1883 and subsequently adapted, with modifications, for application in various food industries, including dairy production (Evers and Hughes, 2002; Goulding, et al., 2020). Despite significant updates to the Kjeldahl method and its equipment in recent years, the fundamental three-step approach involving digestion, distillation, and titration for determining protein content in dairy products has remained unaltered (Dupont, 2011; Licon, 2022). This method calculates total protein content based on nitrogen levels in milk, employing a nitrogen-to-protein conversion factor of 6.38 (FAO/WHO, 2019). It is noteworthy that the Kjeldahl method does not differentiate between protein-based nitrogen and non-protein nitrogen, encompassing inorganic and organic nitrogen compounds, thereby providing a total protein measurement based on the total unspecified nitrogen content in milk (Evers and Hughes, 2002; Licon, 2022).

The primary objectives of this study encompass the quantification of protein content in locally produced and imported cow milk, the evaluation of cow milk consumption rates within the adult population of Armenia, and the estimation of daily protein intake derived from cow milk among Armenia’s adult population. This research seeks to provide valuable insights into the nutritional dynamics of milk consumption in Armenia, shedding light on the role of milk proteins in the dietary habits of the adult population.

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Materials and methods

Milk sampling

The sampling of cow milk was done in 2021 and 2022 from the various markets in the city of Yerevan. The total number of samples was 10 from 9 cow milk producers and from 1 individual producer (Table 1).

Each milk sample consisted of 3 to 4 sub-samples of each type of the product. To prepare a milk sample with an average weight of 250 mL for laboratory analyses, sub-samples from the same producer were mixed (GOST 26809.1-2014).

Table 1. The cow milk sample list by years*

Year of sampling	Sample Code	Product	Producer
2021	MN1	Prostokvasheno	Danone Russia
	MN2	Laktel	Lactalis Ukraine SC
	MN3	Biokat 3.2 %	Biokat Ltd
	MN4	Bonilat	Bonilat Ltd
	MN5	Bandivan	Bandivan Ltd
2022	MN6	Prostokvasheno	Danone Russia
	MN7	Chanakh	Chanakh Co.Ltd
	MN8	Marianna 3.2 %	Marianna Ltd
	MN9	Ani	Tamara & ANI Ltd
	MN10	Farm milk	Individual producer

*Composed by the authors.

All the milk samples were pasteurized (i.e. heat-treated). Before the analyses, the samples were kept at 4 ^{0}C.

Protein analysis methodology

All analyses were carried out at the Center for Ecological-Noosphere Studies (CENS) of the National Academy of Sciences of Armenia. In this study, the fully automated Kjeldahl method was installed and implemented, enabling precise determination of total protein content in milk. Within the 20TTCG-4A001 research project, a new fully automated Kjeldhal Analyzer K1100F (Hanon Advanced Technology Group Co., Ltd, China) with Graphite Digester system (Hanon Advanced Technology Group Co., Ltd, China) equipped with a waste gas collection hood, was acquired. The analyses methodology was undergoing some modernization. Sample pre-treatment was performed following GOST, the Association of Official Agricultural Chemists (AOAC) and VELP Scientific methodology (GOST 34454-2018; AOAC 991.20; VELP Scientific, 2013), as well as the automatic Kjeldahl Analyzer product manual. Milk samples, before analyses were brought to room temperature and were stirred in the beaker using a magnetic stirrer. After 5 mL of sample was added into 250 mL Kjeldahl digestion tube and in each tube were added catalyst: 3.5 g of potassium sulfate and 0.1 g of copper sulfate pentahydrate, 20 mL of concentrated sulphuric acid (96-98 %) and 5 ml of hydrogen peroxide. The tubes were gently shaked and loaded into the Graphite Digester under the following parameters: 15 min 150 ^{0}C, 15 min 250 ^{0}C, 40 min 420 ^{0}C. The digested samples were cooled down to 50-60 ^{0}C and put into the automatic Kjeldahl Analyzer. Beforehand the system was prepared and loaded with the necessary solvents: sodium hydroxide (32 % solvent), boric acid (4 % with indicators: methylene blue and bromocresol green), distilled water, and as titrant solution sulfuric acid (0.1N). All chemicals used were of analytical grade and were used as received without any further purification and were obtained from Carlo Erba (Italy) and Chem-Lab NV (Belgium).

The second part of analysis, which involved distillation and titration, was conducted using the following parameters: 70 mL of sodium hydroxide, 50 mL of dilution water, and 30 mL of boric acid. For titration, the sulfuric acid solution mentioned above was used.

The nitrogen-to-protein conversion factor for milk of 6.38 was used (FAO/WHO, 2019). The data for each sample were automatically calculated by the Automatic Kjeldahl Analyzer as a percentage of nitrogen and a percentage of total proteins. Each sample was analyzed 3 times. The blank sample and standard solution of ammonium sulfate of 1.4 mg/mL nitrogen content for the quality assurance and quality control (QA/QC) of analysis were used. The mean of the obtained standard was 1.33 mg/mL (standard deviation of ± 0.104 mg/mL). Also, the calibration coefficient K-mean value (equal to one) was used for the calibration of the standard.

Milk consumption and protein intake assessment

The data on cow milk consumption among the Armenia's adult population from 18 to 80 years old and above was obtained via 24-hour recall and FFQ (food frequency questionnaire) methods (Pipoyan, et. al, 2023). The data was collected in 2021 by well-trained interviewers, using pre-designed forms of questionnaires. The total number of interviewed residents was 1400 from all 10 marzes of Armenia: Ararat, Armavir, Kotayk, Aragatsotn, Gegharkunik, Lori, Tavush, Shirak, Vayots Dzor, Syunik and from the capital city Yerevan. The response rate of milk consumers was 3.3 %.

The daily intake (*DI*) of total protein via the consumption of cow milk by Armenia's adult population was evaluated and calculated using the following formula:

$$DI=IR \times C, \qquad (1)$$

where *IR*- is the mean daily consumption (ingestion rate) of cow milk among the adult population of Armenia (g/day), *C* is the mean amount of protein in cow milk samples (g/100 g) converted to 1g base.

The statistical one-way analysis of variance (ANOVA) test was used to identify the variance and normality of survey data. Statistical treatment of the survey data was done using IBM SPSS software version 28 (SPSS Inc., Chicago, IL, USA).

Results and discussions

Total protein in cow milk

The data from Kjeldahl analyses is presented in Table 2. The total protein and total nitrogen contents in cow milk samples from 9 different producers over 2 years vary from 0.449 to 0.694 g/100 g, with a total mean of 0.555 g per 100 g of milk, and from 2.81 to 3.14 g/100g, with a mean of 2.93 g in 100g of milk, respectively.

Table 2. Mean of total protein and nitrogen contents in cow milk from local markets of Yerevan (g/100 g)*

Year of sampling	Samples	Total Nitrogen content (mean)	SD**	Total Protein (mean)	SD
2021	MN1	0.694	0.005	3.14	0.024
	MN2	0.678	0.010	3.07	0.044
	MN3	0.661	0.004	2.99	0.019
	MN4	0.620	0.013	2.81	0.059
	MN5	0.627	0.014	2.84	0.065
2022	MN6	0.459	0.003	2.93	0.02
	MN7	0.452	0.002	2.88	0.02
	MN8	0.453	0.012	2.89	0.07
	MN9	0.456	0.015	2.91	0.10
	MN10	0.449	0.008	2.86	0.05
Total mean	0.555	0.109	2.93	0.105	

*Notes: **SD-standard deviation*

*Composed by the authors.

The lowest nitrogen and protein contents in the 2021 and 2022 samples were found in Bonilat (MN4) and the individual milk producer's (MN10) milk samples, measuring 0.620 and 2.81 g/100 g, and 0.449 and 2.86 g/100 g, respectively. The highest levels of nitrogen and protein were detected in the samples of Prostokvasheno (MN1 and MN6), with values of 0.694 and 3.14 g/100 g, and 0.459 and 2.93 g /100 g, respectively (Table 2). In accordance with Technical Regulation 033/2013 of the Customs Union, total protein in milk must be not less than 2.8 % in 100 g (TR CU, 2013). All the analyzed samples met this requirement.

Recent studies have reported varying protein levels in cow milk, ranging from 3.56 to 3.85 g/100 g (Parmar, et al. 2020; Yasmin, et al., 2020). These values are 1.21 and 1.31 times higher than the results obtained in this current research. However, it's important to note that protein levels in cow milk can vary depending on factors such as breed, individuality, stage of lactation, and the health and nutritional status of the animal (Vincent, et al., 2016).

The mean total protein value of 2.92 grams per 100 g of milk serves as an important reference point for assessing the nutritional characteristics of the milk samples. Furthermore, the standard deviation (SD), calculated to be approximately 0.105 grams per 100g of milk, indicates the degree of variability in protein content among the samples. A higher SD signifies greater variability, suggesting that some milk samples deviate notably from the mean protein content, while a lower SD indicates more consistency in protein content among the samples. These findings provide essential insights into the protein content of the examined milk samples, enabling a better understanding of the variability and quality of milk products. This information is invaluable for both consumers and producers in ensuring consistent nutritional value in dairy products and making informed dietary choices.

Milk consumption and protein intake

The average daily consumption of cow milk was 263 g per day. Notable, no significant differences in consumption rates were reported between males and females, as determined by the analysis of variance (ANOVA). Besides, the consumption rates showed no statistical significance when comparing consumers and non-consumers of cow milk among the adult population of Armenia. This was addressed by the K-mean cluster test, revealing two groups of consumers with varying weekly consumption rates (Table 3).

In cluster 1, individuals consumed approximately 234.5 g (0.2345 kg) of milk twice a week, resulting in a daily average consumption of 0.067 kg/day. This cluster represents a moderate milk consumption pattern. In cluster 2, the consumption rate was notably higher, with individuals consuming approximately 487.2 g (0.4872 kg) of milk three times a week, leading to a daily average consumption of 0.208 kg/day. Cluster 2 reflects a more frequent and higher milk consumption pattern compared to cluster 1.

Table 3. Daily average milk consumption in clusters*

Clusters	Daily Consumption, kg/day	Consumption Frequency, per week	Daily Average Consumption, kg/day
Cluster 1	0.2345	2 times	0.067
Cluster 2	0.4872	3 times	0.208

Table 4. Daily total protein via cow milk (g/day) intake in clusters*

Clusters	Daily average milk consumption	Daily total protein intake
Cluster 1	67	1.9631
Cluster 2	208	6.094

*Composed by the authors.

These findings provide valuable insights into the diversity of milk consumption habits among different clusters in Armenia. Understanding such consumption patterns is essential for dietary planning, nutritional recommendations, and addressing specific dietary needs within distinct population groups. The daily average consumption values presented in the Table 3 can serve as reference points for assessing milk intake and its contribution to the overall diet in these clusters.

The calculation of daily total protein intake in the two identified clusters, based on the mean protein content of 2.92 grams per 100g of milk and daily average consumption values, provides insights into the nutritional aspects of milk consumption.

In the cluster 1, the average milk consumption is around 67 g/day per individual, resulting in a daily protein intake of about 1.9631 grams from milk. This cluster exhibits a moderate daily protein intake from milk consumption. In contrast, cluster 2 stands out with a higher daily average milk consumption of approximately 208 g/day, leading to a daily protein intake of about 6.094 grams from milk. This cluster demonstrates a notably higher daily protein intake from milk consumption compared to cluster 1. These findings highlight significant variations in daily protein intake patterns within different clusters of milk consumers.

To understand the role of milk in meeting this nutritional requirement, we calculated the percentage of protein contribution from milk based on daily consumption patterns in Armenia. Our analysis revealed that milk, as a dietary source, contributes approximately 2.62 % - 8.13 % of the daily protein intake.

Further studies and international comparisons can provide valuable insights into regional dietary trends and the extent to which milk and other dietary sources contribute to meeting nutritional requirements.

The multidiscipline nature of our study allowed for an in-depth exploration of the interrelationships between cow milk protein content, consumption patterns, and daily protein intake within the Armenian adult population. Understanding these interdependencies is crucial for gaining insights into the nutritional dynamics of milk consumption.

Conclusion

The assessment of daily protein intake through milk consumption in Armenia has provided valuable insights, playing a pivotal role in tailoring dietary recommendations to ensure dietary patterns align with regulatory standards and the health needs of populations. While varying levels of milk protein contribution to daily intake have been observed, it is essential to acknowledge that a comprehensive understanding of protein sources in the Armenian diet extends beyond milk alone. Therefore, to provide a more comprehensive and detailed insight into dietary protein sources, future research should include wider variety of food products. This will enable a more thorough understanding of the contributions of various foods to the overall diet and establish a strong basis for science-based recommendations on nutrition and balanced diets in Armenia. Furthermore, conducting additional studies and international comparisons can provide valuable context for optimizing dietary choices and enhancing nutritional standards.

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