International Journal of Biochemistry Research & Review

31(4): 11-16, 2022; Article no.IJBCRR.86918
ISSN: 2231-086X, NLM ID: 101654445

Comparative Analysis of Protein in Selected Infant Formula Using Dye-Binding and Formol Methods

Suleiman Zakari [a*], Moses Martha [b], Inalegwu Bawa [a], Celestine O. Ogbu [a], Hajara Zakari [c], Ocheleka M. Itodo [a] and Ameh Felicia Matilda [d]

[a] Department of Biochemistry, College of Medicine, Federal University of Health Sciences, Otukpo, Benue State, Nigeria.
[b] Department of Chemistry, Federal University of Agriculture, Makurdi, Benue State, Nigeria.
[c] Department of Biological Sciences, Federal University of Health Sciences, Otukpo, Benue State, Nigeria.
[d] Department of Chemistry, Federal University of Health Sciences, Otukpo, Benue State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Specifically, conceptualization and methodology was designed by Authors SZ and MM carried out investigation. Analysis was carried out by Authors HZ, AFM, SZ, COO and OMI wrote the original draft. Authors IB and COO did review and editing. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2022/v31i430313

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/86918

Original Research Article

Received 06 March 2022
Accepted 13 May 2022
Published 21 May 2022

ABSTRACT

Aim: The protein content of some infant milk formula was determined using Dye-binding and Formol methods in comparison to the manufacturer's values as reference.
Study Design: The study was an experimental design.
Place and Duration of Study: This study was carried out at the laboratory of Chemistry department, Federal University of Agriculture, Makurdi, Nigeria between June, 2021 and December, 2021.
Methodology: Dye-binding Method was done by weighing about 5 g of the baby milk samples into beakers and 100 mL distilled water was added and mixed thoroughly. 20 mL of diluted 80% orange G dye solution was added to 2 mL of the milk solution and shaked vigorously. The sample was centrifuged at 820 rpm for 5 minutes and a clear filtrate obtained whose absorbance was then analyzed using spectrophotometer. For Formol Method I g of each of the milk samples was weighed into different beakers then 10 mL of distilled water and 0.7 mL saturated potassium oxalate was added along with 3 drops of phenolphthalein indicator into the mixture. Two drops of

**Corresponding author: E-mail: zakarisuleiman13@gmail.com;*

NaOH solution was titrated into the mixture and a pink colour appeared which diminished immediately. About 2 mL of formaldehyde was added and shaken for few seconds then titrated using sodium hydroxide (NaOH) until the mixture turned light pink.
Results: The mean and standard deviation of protein content using Dye-binding method are; Peak baby milk 2.49 ± 0.13 g, My boy milk 2.54 ± 0.06 g. For formol methods Peak baby milk has 0.13 ± 0.01 g and My boy milk 0.1 ± 0.01 g. The result showed that both infant formula under the Dye-binding method and formol method was significantly different ($p<0.05$) when compared to the reference value (1.7 Peak baby and 0.11 My boy). However, values obtained from Formol method for My boy milk was closest to the reference (factory value) only a slight difference of about 0.01 g.
Conclusion: In conclusion, Formol method is more closely related to the reference values (manufacturer's values). Thus, the Formol titration may be used with confidence as a quick test for approximating the protein content of skim milk solids which is typical of baby milks.

Keywords: Protein; dye-binding; formol methods; indicator; infant; formula; titration; Makurdi Metropolis; Benue State.

1. INTRODUCTION

Milk has being the first food of animals that depends on breast milk at birth; it provides all of the energy and nutrients required for the younger animal's normal growth and development. With the exception of humans, all mammals stop drinking milk after weaning. Milk, on the other hand, is a good source of minerals like calcium, phosphorus, sodium, potassium, chloride, iodine, magnesium, and iron. Calcium and phosphorus, which are required for bone growth and the normal development of neonates, make up a bigger portion of these mineral elements in milk [1]. Dairy food products are regarded to be an essential component of a healthy diet. It also contains a variety of bioactive ingredients, including high-quality proteins, lipids, carbs, lactose, vitamins, minerals, enzymes, hormones, immunoglobulins, and growth factors [2]. These nutrients not only assist meet human nutritional needs, but they also have an important role in avoiding diseases like hypertension and cardiovascular disease, obesity, osteoporosis, dental caries, poor gastrointestinal health, colorectal cancer, and ageing [3]. Milk is a practically complete food since it contains a good amount of protein, fat, and essential minerals. Milk and its products are important parts of the daily diet, particularly for vulnerable groups like infants, school-aged children, and the elderly [2].

Breast milk contains all of the energy and virtually all of the nutrients needed for an infant's growth and development during the first 4 to 6 months of life, as well as a variety of immunological variables and bioactive components [4]. Infant formula is defined by the United States Federal Food, Drug, and Cosmetic Act (FFDCA) as "a food that purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk." Purified cow's milk whey and casein are employed as protein sources, and a combination of vegetable oils is used as a fat source in most infant formulae [5]. Regardless of the fact that it is recommended that babies be breastfed exclusively for the first six months, only about 40% of infants under this age are exclusively breastfed globally. The vast majority of American babies are not exclusively breastfed for the first six months of their lives – in 2005, only 12% of babies were exclusively breastfed for the first six months [6], with over 60% of babies aged two months being fed formula [7], and one in every four breastfed infants receiving infant formula within two days of birth [7].

Proteins are highly, complicated molecules that play important roles in the human body. The construction, function, and control of the body's tissues and organs are all dependent on them [4]. Proteins, together with carbohydrates and lipids, the energy-giving components in the diet, play a crucial part in the growth and maintenance of the human body. Furthermore, proteins have a variety of other roles in the body, including enzymatic activity and the movement of nutrients and other biochemical molecules through cellular membranes [8]. It is critical to give the body with high-quality proteins through nutrition in order to sustain these vital activities. Increased turnover of muscular proteins arises from insufficient intake of dietary proteins containing necessary amino acids, resulting in diminished growth and loss of muscle mass. Immune dysfunction, as well as decreased hormonal and enzymatic activity, may result [9].

Milk is considered to be a complex food that contains essential components (proteins, fats, and vitamins) that have been shown to have health advantages [7]. Milk, being animals' first food, provides all of the energy and nutrients required for the neonate's normal growth and development. Milk consumption stops for all mammals after weaning, with the exception of humans, who continue to drink milk throughout their lives. Milk and dairy products are regarded to be an important part of a healthy diet. It also contains a variety of bioactive components, including high-quality proteins, lipids, carbs, lactose, vitamins, minerals, enzymes, hormones, immunoglobulins, and growth factors. These components not only assist meet human nutritional needs, but they also have an important role in the prevention of diseases like hypertension and cardiovascular disease, obesity, osteoporosis, dental caries, poor gastrointestinal health, colorectal cancer, and ageing [8].

To evaluate the protein content in food, a variety of analytical procedures have been developed over time. However, just a few of these are commonly utilized, and the reason for this may be due to a range of variables, such as tradition (using established analytical processes in laboratories), a lack of analytical infrastructure, or the high economic expenses associated with specific approaches. A first group of methods based primarily on the measurement of chemical or structural characteristics of the proteins, such as nitrogen, free NH3 groups (from the N-terminus and basic amino acids), CO-NH peptide bonds, and aromatic amino acids, can be distinguished among the methods currently used for milk protein analysis. These techniques are best suited to determining total proteins as well as the major nitrogen fractions in milk. They are commonly employed for a variety of objectives, including animal breeding and feeding, quality-based milk payout to producers, and raw material control prior to processing. The goal of this study was to examine baby infant formula using a rapid and simple test for approximating the protein content of skim milk solids, which is typical of baby milks, in order to find the most accurate method that could be useful in the food industry's routine quality control/assurance process.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples of baby milk (Peak baby and Myboy) were obtained within commercial market in Wurukum part of Makurdi metropolis, Benue state.

2.2 Materials

Two sample of powdered baby milk (Peak baby and Myboy) collected from the commercial market in Wurukum, Makurdi.

2.3 Reagents

Analytical and laboratory grade reagents were used in this study. This includes; Sodium hydroxide solution (0.1 M), 80% Orange G dye, distilled water, phenolphthalein indicator, 40% formaldehyde, saturated potassium oxalate.

2.4 Analysis of Protein by Dye-binding Method

Dye-binding technique was performed as described by Udy [9] in triplicate. About 5g of the baby milk samples were weighed into different beakers and 100ml distilled water was added and mixed thoroughly using glass rod. 20ml of diluted 80% orange G dye solution was added to 2ml of the milk solution in a sample bottle and shaked vigorously for 10 minutes for complete mixing. The sample was then kept for 30 minutes before testing. The sample was centrifuged at 820rpm for 5 minutes and a clear filtrate obtained. The absorbance of the filtrate was then analyzed using spectrophotometer at 630nm.

2.5 Determination of Protein by Formol Method

Formol method was performed as originally proposed by Steineggar [10] in triplicate. The burette was filled with 0.1M sodium hydroxide (NaOH) and marked to zero. Ig of each of the milk samples was weighed into different beakers and 10ml of distilled water added to it. After swirling for 30 minutes, 0.7ml saturated potassium oxalate was added along with three drops of phenolphthalein indicator into the mixture. Two drops of NaOH solution was titrated into the mixture and a pink colour appeared which diminished immediately. About 2ml of formaldehyde was added and shaken for few seconds then titrated using sodium hydroxide (NaOH) until the mixture turned light pink.

3. RESULTS AND DISCUSSION

Results presented in table shows the mean and standard deviation of protein content for Dye-

binding and Formol methods. Protein content in peak baby and myboy milk ranges from 0.13 to 2.49 and 0.1 to 2.54 for Formol and Dye-binding methods respectively. Total protein values by the Dye-binding procedure differ with values by Formol method. Standard deviation of the difference between total protein by Dye-binding and by Formol titration was ± 0.12 in Peak baby and ±0.05 in My boy (with variations in both methods). The reason of getting excess protein in all these milks when compared to reference value may be due to the presence of non- protein nitrogenous substances such as urea nitrogen, amino nitrogen, creatinine, uric acid, adenine and guanine [11].

From Table 1, the highest protein content is present in Myboy (2.54 ± 0.06) under Dye-binding method and the lowest amount is present in Myboy milk (0.1 ± 0.01) under Formol method. The protein content of each of the milk according to the manufacturer's reference value is given as 1.7g and 0.11g for peak baby milk and My boy milk respectively. This shows that the protein content of investigated milks differs from the given value. The reason of getting excess protein in all these milks may be due to the presence of non- protein nitrogenous substances such as urea nitrogen, amino nitrogen, creatinine, uric acid, adenine and guanine [12].

Comparing the reference value to the experimental value, A significant difference is observed in my boy and peak baby between experimental and given value. The result showed that both infant milk formula under the Dye-binding method and formol method was significantly different ($p<0.05$) when compared to the reference value. However, my boy under formol method has the closest value [0.1g] when comparing with the reference value of 0.11g, only a slight difference of about 0.01(g) was observed.

Fig. 1 below shows the representation of the protein content for Dye-binding. It was observed that My boy milk had the highest value of protein content, while Peak baby had the lowest value of protein content. Comparing results obtained with that of [13] the protein content in My boy was 0.15g, which is higher than the value (0.10g) obtained from this work [14].

From the shows chart, my boy under formol method has the closest value to manufacturer's value as shown in Fig. 2 [0.1g compared to manufacturer's value of 0.11g].

Table 1. Mean Protein Content (g) using dye-binding and formol methods

Method	Dye-binding	CV	Formol	CV	Reference
Peak Milk	2.49 ± 0.13	5	0.13 ± 0.01	8	1.7
My boy	2.54 ± 0.06	2	0.1 ± 0.01	10	0.11

Note: CV=Coefficient of Variation = $\frac{SD}{Mean} X\ 100$

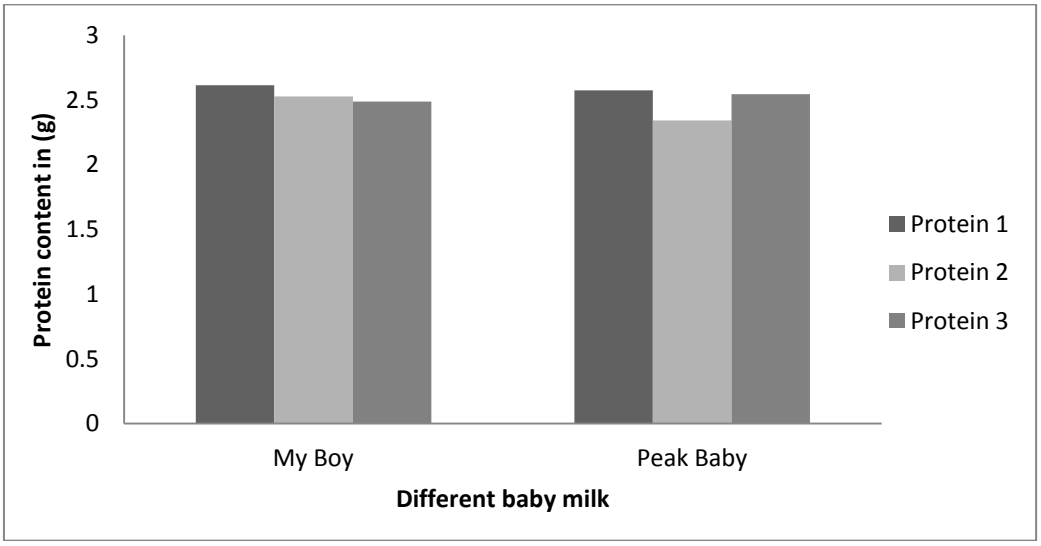

Fig. 1. Protein content for dye-binding method

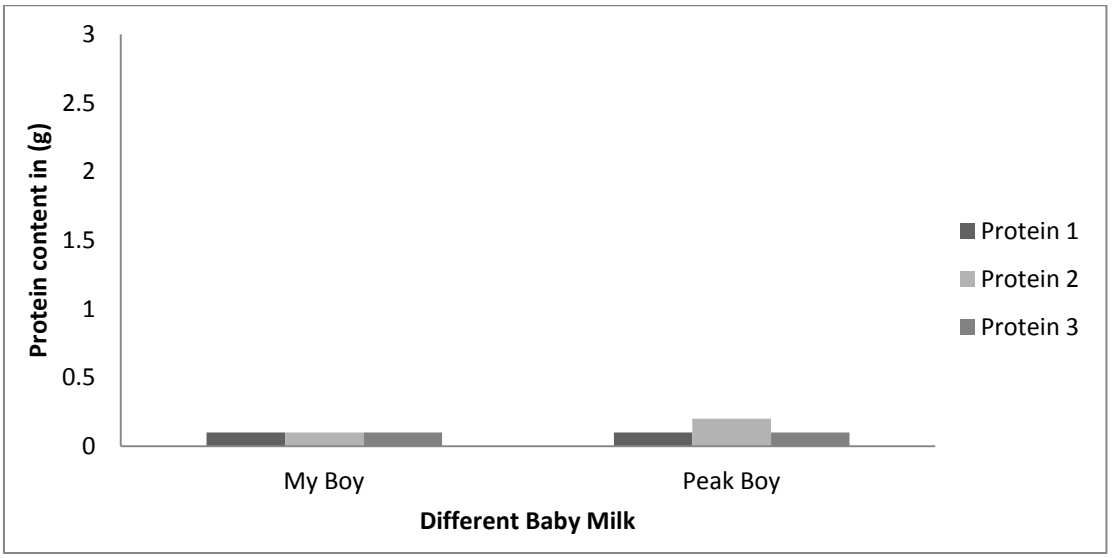

Fig. 2. Protein content for formol method

4. CONCLUSION

In conclusion, Based on findings in this study, it was concluded that there was significant difference (*p<0.05*) between the concentration of protein in Peak baby milk (0.13 ± 0.01, 2.49 ± 0.13) and My boy milk (0.1 ± 0.01, 2.54 ± 0.06) for both formol method and dye-binding methods. However, Formol method for My boy milk (0.1) has closest value to the manufacturer's reference value (0.11). This indicated that the Formol method is more closely related to the reference values (manufacturer's values). Thus, the Formol titration may be used with confidence as a quick test for approximating the protein content of skim milk solids which is typical of baby milks.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al-Wabel NA. Mineral contents of milk of cattle, camels, Goats and Sheep in the central region of Saudi Arabia. Asian Journal of Biochemistry. 3:373-375. DOI: 10.3923/ajb.2008.373.375
2. Gasmalla M, Khadir K, Musa A, Aboshora W, Zhao W. Evaluation of some physicochemical parameters of three commercial milk products. Pakistan Journal of Food Science. 2013;23(2):62–65.
3. Nagpal R, Behare PV, Kumar M. Milk, milk products, and disease free health: an updated overview, Critical Reviews in Food Science and Nutrition. 2012;52(4):321–333.
4. Titi Y, Yumei Z, Yibing N, Lili Y, Defu M, Yingdong Z, et al. Chinese Medical Journal, 2014;127(9):1721-1725.
5. U.S. Food and Drug Administration. What is an infant formula. Assessed 10 August, 2021. Available:https://www.fda.gov/food/people-risk-foodborne-illness/questions-answers-consumers-concerning-infant-formula#1. Date
6. World Health Organization. World Breastfeeding Week; August 1–7, 2011. Accessed on August 8, 2021. Available:https://www.who.int/teams/maternal-newborn-child-adolescent-health-and-ageing
7. Center for disease control. Results: Breastfeeding and Infant Feeding Practices - Breastfeeding - CDC. Accessed March 10, 2022. Available:www.cdc.gov
8. Center for disease control. National Immunization Survey (NIS).

Accessed March 10, 2022.
Available:cdc.gov
9. Udy DC. Improved dye method of estimating protein. J. Am. Oil. Chem Soc. 1971;48:29A
10. Steineggar Z. Unters. Nahr. Genu8sm. 1905;10:659 (Abstr.in Analyst, 31, 45).
11. Nagpal R, Behare PV, Kumar M. Milk, milk products, and disease free health: An updated overview, Critical Reviews in Food Science and Nutrition. 2012;52(4):321–333.
12. Haug A, Høstmark AT, Harstad OM. Bovine milk in human nutrition--a review. Lipids Health Dis. 2007 Sep 25;6:25.
DOI: 10.1186/1476-511X-6-25
PMID: 17894873;
PMCID: PMC2039733.
13. Mohammed AA, Gasmalla, Khadir E. Khadir, Abubakar Musa, Waleed Aboshora, Wei Zhao. Evaluation of some physiochemical parameters of three commercial milk products: Pakistan Journal of food science. 2013;23(2):62-65.
14. Eckles CH, Combs WB, Macy H. Milk and Milk Products, 4th ed.; Tata McGraw-Hill Publishing Company Limited, New Delhi; 1973.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/86918