Original Research Article

Comparative analysis of protein in selected infant formula using dye-binding and formol methods

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: Experimental study.
Place and Duration of Study: This research was done at the laboratory of Chemistry department of 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 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, Myboy milk 2.54 ± 0.06 g. For formol methods Peak baby milk has 0.13 ± 0.01 g and Myboy milk 0.1 ± 0.01 g. The result showed that for Peak baby, the Dye-binding method was significantly different ($p<0.05$) when compared to the reference value. There was no significant difference ($p>0.05$) between the values obtained from Formol method for myboy milk compared to the reference (factory) 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, as the first food for mammals, supplies all the energy and nutrients needed for the proper growth and development of the neonate. For all mammalians, the consumption of

milk ends at the weaning period with the exception of humans that continue consuming milk throughout their life. However, milk is an important source of mineral substances, especially calcium, phosphorus, sodium, potassium, chloride, iodine, magnesium, and small amounts of iron. Amongst these mineral constituents, calcium and phosphorus constitute a larger fraction in milk which is needed for bone growth and the proper development of newborns [1]. Milk and derived dairy products are considered an important constituent of a balanced diet. Moreover, it is a source of many bioactive components, such as high-quality proteins, lipids, carbohydrates, lactose, vitamins, minerals, enzymes, hormones, immunoglobulins, and growth factors, among others [2]. These components not only help meeting human nutritional requirements, but also play a relevant role in preventing various disorders such as hypertension and cardiovascular diseases, obesity, osteoporosis, dental caries, poor gastrointestinal health, colorectal cancer, ageing, and others [3]. Milk is considered as a nearly complete food since it is a good source of protein, fat and major minerals. Milk and their products are main constituents of the daily diet, especially for vulnerable groups such as infant's, school age children and old age [2].

In humans, breast milk provides all the energy and nearly all nutrients required for infant growth and development during the first 4 to 6 months of life, as well as various immunological factors and bioactive components [4]. According to the U.S. Federal Food, Drug, and Cosmetic Act (FFDCA) which defines infant formula as "a food which 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. The most commonly used infant formulas contain purified cow's milk whey and casein as a protein source, a blend of vegetable oils as a fat source [5]. Despite the recommendation that babies be exclusively breastfed for the first 6 months, less than 40% of infants below this age are exclusively breastfed worldwide. The overwhelming majority of American babies are not exclusively breastfed for this period – in 2005 under 12% of babies were breastfed exclusively for the first 6 months [6], with over 60% of babies of 2 months of age being fed

formula [7], and approximately one in four breastfed infants having infant formula feeding within two days of birth [7].

Proteins are complex molecules that play critical roles in the body. They are required for the structure, function, and regulation of the body's tissues and organs [4]. Proteins have a major role in the growth and maintenance of the human body along with carbohydrates and lipids, the energy giving nutrients in the diet. In addition, proteins also pose a wide range of other functions in the body, such as enzymatic activity and transport of nutrients and other biochemical compounds across cellular membranes [6]. In order to maintain these important functions, it is essential to provide the body with good quality proteins through diet. Inadequate intake of dietary proteins containing essential amino acids results in increased turnover of muscular proteins, leading to reduced growth and loss of muscle mass. Impaired immunity, as well as reduced hormonal and enzymatic activity may subsequently follow [9].

Milk is a complex food containing basic nutrients (e.g. proteins, lipids, vitamins) with positive health benefits [7]. Milk, as the first food for mammals, supplies all the energy and nutrients needed for the proper growth and development of the neonate. For all mammalians, the consumption of milk ends at the weaning period with the exception of humans that continue consuming milk throughout their life. Milk and derived dairy products are considered an important constituent of a balanced diet. Moreover, it is a source of many bioactive components, such as high-quality proteins, lipids, carbohydrates, lactose, vitamins, minerals, enzymes, hormones, immunoglobulins, and growth factors, among others. These components not only help meeting human nutritional requirements, but also play a relevant role in preventing various disorders such as hypertension and cardiovascular diseases, obesity, osteoporosis, dental caries, poor gastrointestinal health, colorectal cancer, ageing, and others [8].

Varieties of different analytical methods have been developed throughout the years. Only a few of these however, are frequently used, and the reason for the choice of method used in many studies may be due to a variety of factors, for instance tradition (using established

analytical procedures in laboratories), lack of analytical infrastructure or high economic costs associated with certain methods. Among methods currently used for milk protein analysis, a first group of methods based essentially on the measurement of chemical or structural characteristics of the proteins, such as nitrogen, free NH3þ groups (from the N-terminus and basic amino acids), CO-NH peptide bonds and aromatic amino acids can be distinguished. These methods are particularly suitable for the accurate determination of total proteins and the major different nitrogen fractions in milk. They are routinely used for several purposes: animal breeding and feeding, quality based milk payment to the producer and control of the raw material before processing. In this research, we analyzed baby infant formula using rapid and quick test for approximating the protein content of skim milk solids which is typical of baby milks in view of identifying the most accurate method as this would help in routine quality control test of commercial milk products. This study would help to determine the most effective and accurate of the two methods (dye-binding and formol) for determining the protein content of baby milk.

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 Apparatus and Equipments

The apparatus used for this work include spectrophotometer, weigh balance, centrifuge, filter papers, titration unit, Automatic pipette, spatula, beakers, conical flask, funnel, sample bottles and volumetric flask.

2.4 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.

2.5 Analysis of Protein by Dye-binding Method

Dye-binding technique was performed as described by Udy [9]. 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.

2.6 Determination of Protein by Formol Method

Formol method was performed as originally proposed by steineggar [10]. 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

The results are represented in a tabular form. Table 1 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 myboy (with variations in both methods).

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 = $\frac{SD}{Mean} X\ 100$

Table 1 indicates that the highest protein content is present in Myboy (2.54) under Dye-binding method and the lowest amount is present in Myboy milk (0.1) under Formol method. The protein content of each of the milk is given on the packet by the respective company based on the method of production. The percentage of protein of investigated milks differs from the given value. A significant difference is observed in my boy and peak baby between experimental and given value. However, my boy under formol method has the closest 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 [11].

Table 2 shows the statistical comparison between Dye-binding and Formol. Comparing the reference value to the analytical value, the result showed that for Peak baby, the Dye-binding method was significantly different ($p<0.05$) when compared to the reference value. For my boy milk, the result showed no significant difference ($p>0.05$) when comparing with

the reference value, only a slight difference of about 0.01(g) as compared to the reference value.

Table 2: **Statistical Comparison between Dye-binding and Formol methods**

Method	Level of Significance (p value)	
	Peak baby	My boy
Dye-binding	<0.05	>0.05
Formol	<0.05	>0.05

Figure 1 below shows the representation of the protein content for Dye-binding. From the chart in Figure 1, it was observed that My boy had the highest value of protein content, while Peak baby had the lowest value of protein content. Comparing results obtained with that of [12] the protein content in My boy was 0.15, which is higher than the value (0.10) obtained from this work [13]. No significant difference ($p>0.05$) was established statistically between the methods which accounts for the fact that there is close relation between the two methods and each of the methods could produce identical results for routine analysis if other quality assurance parameter were strictly observed.

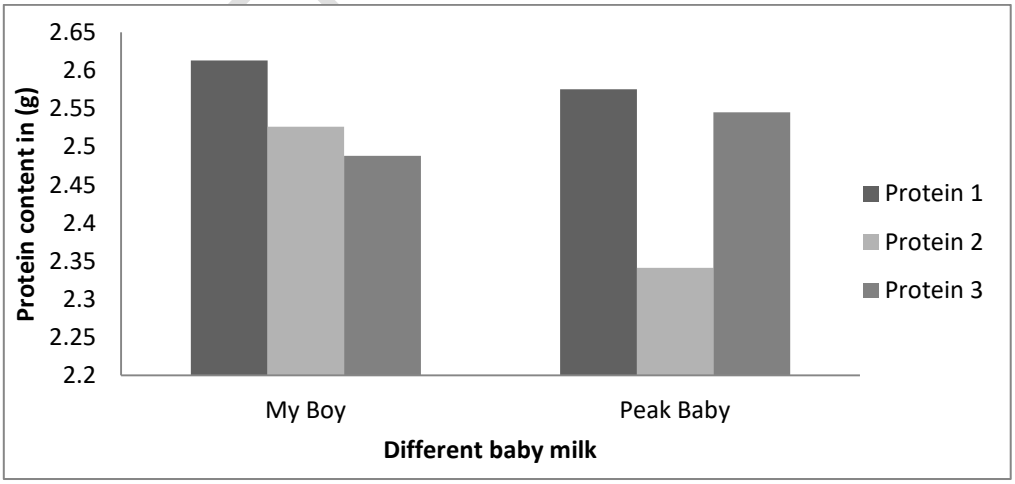

Figure 1: Protein Content for Dye-binding Method

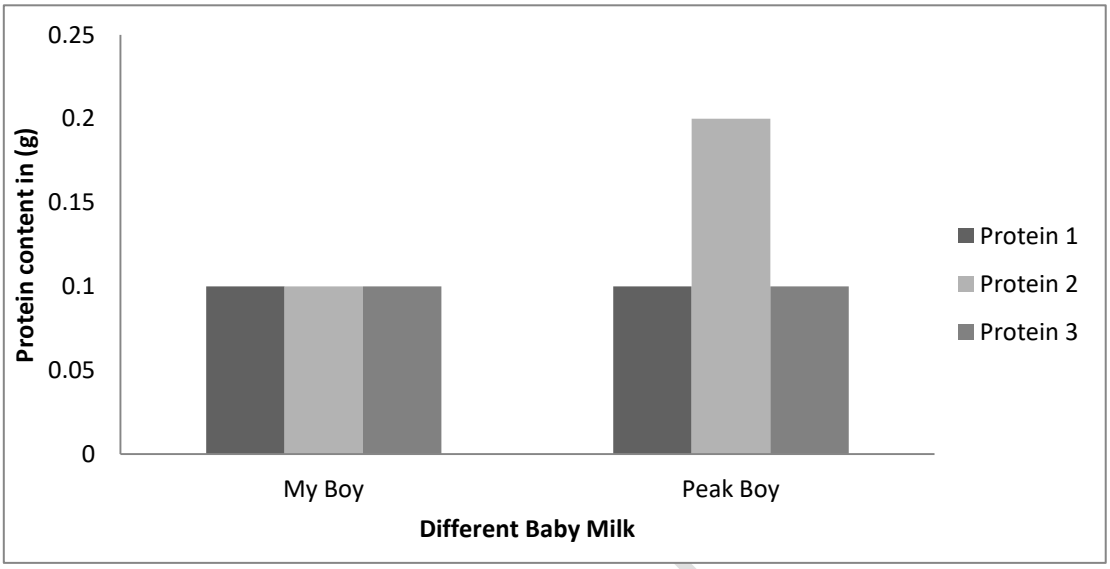

Figure 2: Protein content for formol method

From the above chart, my boy under formol method has the closest value as shown in figure 2. 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 [14].

4. CONCLUSION

In conclusion, Based on findings in this study, it was concluded that analysis of protein content in the two selected baby milk (Peak baby and My boy) using Dye-binding and Formol method carried out, there was no significant difference between the values obtained from Formol method for my boy milk compared to the reference (factory) showed no significant difference at p=0.05. Dye-binding method showed significant difference ($p<0.05$) when comparing the value obtained in My boy (2.54±0.06) against the factory 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.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. 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.

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