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PROTEIN CONTENT CHANGES IN TWO INFANT FORMULAS OF MILK POWDER CAUSED BY PHYSICOCHEMICAL VARIATIONS IN RELATION TO STORAGE CONDITIONS OF MILK POWDER

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ABSTRACT

Protein is considered one of the essential nutrients in infant's milk powder. it should have of typical structure and fit to human consumption especially when suitable conditions of storage are maintained away from the ambient factors which promote the degradation of milk powder constituents. Physicochemical properties play a crucial role on quality and quantity of protein. It was revealed that physicochemical properties and protein content didn't change when samples of milk powder cans were kept airtight closed whether in the fridge or at lab. Thus these samples were considered safe, nutritional, and fit to consumption. As samples of milk powder cans were kept open, physicochemical properties and protein content changed that is moisture increase in both milk brands A3, B3 ranged from 1.05% to 5.44% and from 2.92% to 7.16% respectively during the thirty days of timeline. In the present study, the influence of milk powder storage was investigated for its physicochemical i.e. acidity, moisture and flow ability which affect by turn on protein content. Lactic acid based acidity increase ranged from 0.055% to0.20% for sample A3 and from 0.059% to 0.283% for sample B3. Due to these changes protein content decreased from 9.63% to 0.283% and from11.57% to 10.88% for samples A3 and B3 respectively. Protein decline can be attributed to its degradation into amino acids. Furthermore, accompanied variation in sensory quality and lumping of milk powder occurred by which samples of milk powder cans A3,B3 were considered unfit to human consumption.

INTRODUCTION

Although mother's milk is the best choice for new born, alternatives to breast milk played a major role in infant nutrition when mother's nursing is not sufficient or doesn't meet their nutritional options.[1]. According to Syrian standards numbered 197/1996 , the modified formula of powdered infant milk has become equivalent to that of mother's milk in terms of composition ratio carbohydrates, proteins, fats, vitamins and minerals that meet the daily nutritional requirements for infants. Protein has an essential role in body infant's growth and development. Protein requirements are also higher in infants and children than for adults [2]. Protein is a major structural component of hormones, enzymes, nails; hair and blood transport molecules that maintain cell *viability* and ensure body health and growth. Besides, proteins are a vital source of energy [3].Edema or swelling disease is commonly associated with infant's low intake of protein hence a remarkable decrease in total serum proteins. Kwashiorkor is a disease noticed in severe cases of infantile malnutrition with manifested with edema, changes in hair and skin and *hepatomegaly* and *fibrosis* [4]

Milk powder consists of lactose, fat, protein…etc.) there is a relationship between milk powder features and its constituents that is any change in powder formula features will affect its constituents and vice versa [5]. Physicochemical properties of milk powder have a significant role in preserving of the product quality. However, upon home usage and storage physicochemical properties of the powder change and milk constituents including protein change [6]. Physicochemical properties of milk powder such as moisture, flowability, particle sizes, caking and solubility were determined using suitable methods; moisture was determined using oven drying [7], total acidity was measured based on acid-base titration[8], flowability(Hausner ratio) was investigated using angle of repose and Carr's compressibility index[9,10] the sizes of particles were measured using sieving[11] caking was tested[5] and solubility was also examined[12]. Protein content was determined in food samples of liquid and powder milk using Kjeldahl's analytical method [13], spectrophotometric methods of biuret, Lowry, Bradford, and ultraviolet [14], a ninhydrin method [15] and electrophoresis [16]. The major object of the present study was to monitor the influence of some physicochemical properties of powdered milk on its protein content upon consumption. In the present study, some Physicochemical properties of milk powder such as moisture was determined using oven drying, total acidity was measured based on Lactic acid as acid-base titration, flowability (Hausner ratio) was investigated using (Hausner ratio) and Carr's compressibility index. Protein content in different brands of milk powder was determined using Kjeldahl analytical method and spectrophotometric method of biuret. Besides, protein hydrolysis into

amino acid was qualitatively detected using ninhydrin method.

Materials and Methods

Milk powder samples

Six cans of infant milk powder produced by two different companies (A, B) were bought from local pharmacies on 2015. The first three can set was for infants aged from 1-6 months and the second three can set was for infants aged from 6months to one year old. The samples were studied at three different conditions of storage as follows: first sample was kept air tight in the fridge, the second was kept sealed at lab temperature, and the third one was left opened at lab temperature as shown in Table 1. The study was done over one month.

Chemicals and Apparatus:

- Egg albumin (BDH)
- Sulforic acid concentrated 95-97% (CHEMICAL LAB)
- Copper Sulfate 98% (TEKKIM).
- Potassium sulfate 99% (TEKKIM)
- Zinc Oxide (QUALIKEMS)
- Sodium Hydroxide (TEKKIM).
- Boric Acid (TECHNO PHARCHEM)
- Bromocresol green indicator (QUALIKEMS)
- Hydrochloric Acid37%(CHEMLAB)
- Phenolphthalein reagent (BDH)
- Trichloroacetic acid (RIEDEL-DE-HAEN AG)
- Ninhydrin reagent (LOBA CHEMIE)
- Methanol (BDH)
- Vitamin C (BDH)
- Glacial acetic acid (BDH)
- Sodium acetate (HIMEDIA)
- Potassium iodide (BDH)
- Sodium and potassium tartrate (QUALIKEMS)
- Distilled water
- sensitive balance (Precisa XB 220 A)
- kjeldahl digestion and distillation apparatus (Buchi)
- automatic burette
- electrical oven (Janat)
- spectrophotometer (UV -530V Jasco)
- water bath (EMKO,ESM-3711-H)
- Centrifuge (R4U-REMI MOTOR)

Table 1 The studied sample of infant milk powder

Infant milk powder	Storage conditions	Protein content	Number of samples
A(for infants aged from 1-6 months)	**A1:kept airtight closed in the fridge**	**9.65g**	**1can(400g)**
	A2:kept airtight closed at lab		**1can(400g)**
	A3:kept open at lab		**1can(400g)**
B(for infants aged from 6months to one year old)	**A1:kept airtight closed in the fridge**	**11.50g**	**1can(400g)**
	A2:kept airtight closed at lab		**1can(400g)**
	A3:kept open at lab		**1can(400g)**

Preparation of solutions and reagents.

- Sodium acetate buffer 4N:0.108 g of Sodium acetate was mixed with 20 ml of glacial acetic acid. Solution pH was adjusted to 5.5 using sodium hydroxide (2M) and the volume was made up to 100 ml with distilled water.
- Ascorbic acid solution: 0.1 g of Ascorbic acid was dissolved in 1ml of distilled waters.
- Ninhydrin reagent solution: 0.2 g of ninhydrin was dissolved in 7.5 ml of methanol, 2.5 ml of sodium acetate 4N.4 and µl of vit C solution were then added. The color developed pale red.
- Biuret reagent solution: 1.5 g of aqueous copper sulfate were dissolved in sodium hydroxide 0.2N, 4.5 g of sodium potassium tartrate was added then 2.5 g of potassium iodide was added until completely dissolved. The volume was made up to 500ml using sodium hydroxide 0.2 N

Methods

Moisture content

Moisture content in sample were determined by oven drying according to AOAC [17]

Acidity titration

Titratable acidity of samples was determined according to AOAC [17]

Flowability

The flowability of the studied samples was evaluated according to [18]

Protein content

Kjeldahl method was used for the determination of protein content in the studied samples according to AOAC [17].

Spectrophotometric determination of protein - Biuret Method

A gradient series of egg albumin standards containing 0,0.2, 0,3, 0.4,0.5,0.8,1,1.2,1.4,1.6,2were prepared.300 g of egg albumin was weighed and dissolved in 1 liter of distilled water at which the concentration was 0.3mg/ml Extracted protein solution was placed in the test tubes to which biuret reagent was then added to. The tubes were thereafter kept at 37˚C for 10 minutes. After cooling absorbance was read at 540nm.

Ninhydrin qualitative protein detection

1g of milk powder sample was weighed and dissolved in 9 ml of distilled water. 1 ml of Trichloroacetic acid TCA 105 was added and the result was centrifuged at 3000rpmfor 10 minutes. At the end of the centrifugation two layers were obtained one of them was a precipitate containing protein and the other supernatant layer contained the rest constituents of the sample. The second layer was separated by filtration and ninhydrin reagent was added until the color developed violet as an indicator for protein hydrolysis into amino acids

Results and Discussion:

Standard curves

A standard or calibration curve was created with a correlation coefficient of 0.966 the resulted curve was a straight line ranged from the origin after zeroing out the spectrophotometer using a blank consisted of biuret reagent and distilled water. While x axis represents protein concentration, Y axis represents absorbency, where concentration was measured in mg/ml and the absorbance was detected 540nmas shown in Fig1. Table 2 reveals the concentrations and the absorbencies of gradient series of egg albumin standard solutions standard solutions. Where the tube1represents a blank which is consisted of biuret reagent and distilled water without the studied sample.

Table 2 Gradient series of egg albumin standard solutions

Tube serial number	1	2	3	4	5	6	7	8	9	10	11
Standard protein solution (ml)	**0**	**0.2**	**0.3**	**0.4**	**0.5**	**0.8**	**1**	**1.2**	**1.4**	**1.6**	**2**
Distilled water(ml)	**2**	**1.8**	**1.7**	**1.6**	**1.5**	**1.4**	**1**	**0.8**	**0.6**	**0.4**	**0**
Biuret reagent(ml)	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Protein concentration(mg/ml)	**0**	**0.030**	**0.045**	**0.060**	**0.075**	**0.12**	**0.15**	**0.18**	**0.21**	**0.24**	**0.30**
Absorbance	**0.00**	**0.1355**	**0.1793**	**0.2477**	**0.2945**	**0.4566**	**0.5833**	**0.6833**	**0.7743**	**0.8812**	**1.1961**

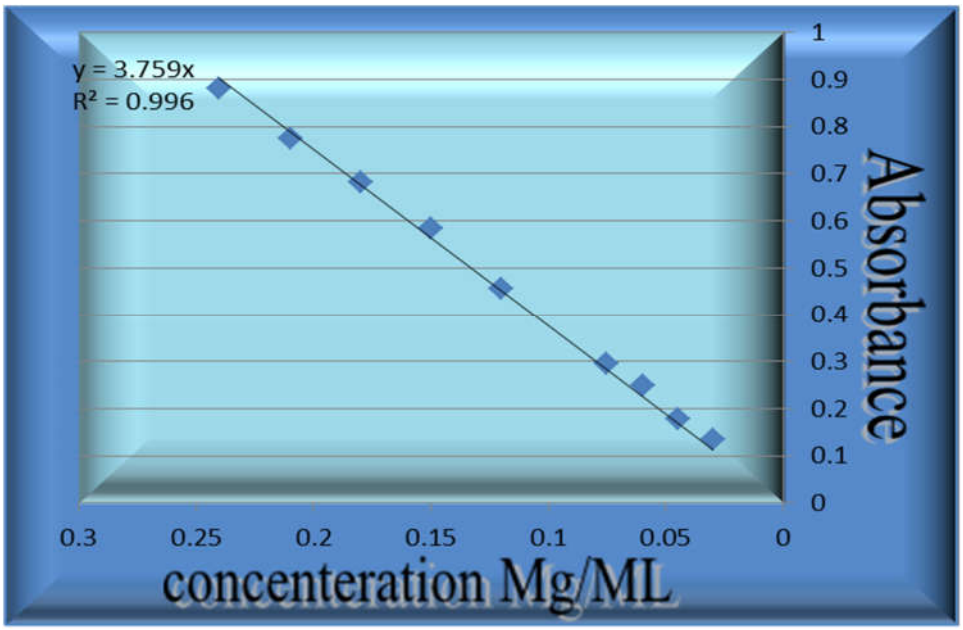

Figure 1 Detected absorbance at 540nm against gradient series of egg albumin standard solutions

Physicochemical properties of milk powder:

Fridge storage of a tightly closed can of milk powder sample A1:

Milk powder can A1 was placed tightly closed in the fridge and monitored throughout 30 days with 5 day intervals. Moisture was measured by oven drying until constant weight and it was found to be 0.99% at the first day. This value is considered acceptable according to Syrian standard specification number 197/1996 which states that maximum moisture should not exceed 4% utmost in infant milk product. Moisture measurement was done each day in triplicate and relative standard deviation RSD was calculated. The all findings were less than 10% indicating that all measurements were accurate. Despite moisture content was noticed to increase in the milk sample A1 during fridge storage, after milk can opening, this rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Lactic acid based acidity was titrated in solid mater and it was found to be 0.053% at the first day of experiment. This value is considered acceptable according to Syrian standard specification number 197/1996 which states that maximum Lactic acid based acidity should not exceed 4% in infant milk product. Acidity titration was done each day in triplicate. and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate

Milk powder flowability was determined based on tapped density and bulk after sifting. Besides,

flowability or Hausner ratio was derived from calculated compressibility Carr's index. It was noticed that the flowability is suitable at the first five days of work timeline after opening the product can as well as after 20-30 days of fridge dwell timeline. Table 3

Table 3 Variations in physicochemical properties of milk powder can sample A1 which that was kept tightly closed in the fridge

A1	The sample kept tightly closed in the fridge								
	moisture		acidity			flowability			
Timeline	Moisture content%	RSD	Total acidity per dry weight basis	Titratable acidity as lactic acid	RSD	Hausner ratio	flowability	Carr's index	flowability
1	0.99	1.62	0.59	0.053	1.88	1.23	suitable	18.34	suitable
5	1.39	0.05	0.62	0.056	1.78	1.25	suitable	19.96	suitable
10	1.56	1.73	0.65	0.059	6.77	1.26	passable	21.14	passable
15	2.35	2.49	0.67	0.06	1.17	1.27	passable	21.09	passable
20	2.54	3.98	0.74	0.067	3.5	1.29	passable	22.85	passable
25	2.69	5.21	0.79	0.071	3.59	1.30	passable	23.08	passable
30	2.96	0.63	0.86	0.077	5.35	1.32	passable	23.97	passable

Lab storage of a tightly closed can of milk powder sample A2:

Milk powder can A2 was placed tightly closed at lab and moisture was measured it was found to be 1.07% at the first day. This value is considered acceptable according to Syrian standard specification number 197/1996. Moisture measurement was done each day in triplicate and relative standard deviation RSD was calculated. The all findings were less than 10% indicating that all measurements were accurate. Despite moisture content was noticed to increase in the milk sample A2 during the 30 days of timeline as milk can kept open, the rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Lactic acid based acidity was titrated in solid mater and it was found to be 0.056% at the first day of experiment. This value is considered acceptable according to Syrian standard specification number 197/1996. Acidity titration was done each day in triplicate. and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. Despite lactic acid based acidity was noticed to increase in the milk sample A2 during the 30 day of timeline, this rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Flowability was determined, It was noticed that it was suitable

during the 30 day of timeline then it became acceptable starting the day 15 to the day 30 of timeline. Table 4

Table 4 Variations in physicochemical properties of milk powder sample can A2 which that was kept tightly closed at lab

A2	The sample kept tightly closed at lab								
	moisture		acidity			flowability			
Timeline	Moisture content%	RSD	Total acidity per dry weight basis	Titratable acidity as lactic acid	RSD	Hausner ratio	flowability	Carr's index	flowability
1	1.07	4.94	0.63	0.056	5.21	1.22	suitable	17.78	suitable
5	1.48	1.80	0.64	0.058	3.44	1.26	passable	20.62	passable
10	2.01	2.59	0.66	0.060	2.88	1.29	passable	22.00	passable
15	2.62	3.17	0.69	0.062	4.26	1.30	passable	22.91	passable
20	2.94	4.78	0.74	0.067	1.08	1.31	passable	23.21	passable
25	3.01	1.17	0.81	0.073	1.67	1.32	passable	23.87	passable
30	3.18	0.63	0.86	0.077	5.35	1.32	passable	23.97	passable

Lab Storage of an opened can of milk powder sample A3:

Milk powder can A3 was placed open at lab and moisture content was measured and it was found to be 1.05% at the first day of experiment. According to Syrian standard specification number 197/1996 the result moisture content is passable. Moisture measurement were done each day in triplicate and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. It was noticed that despite moisture content of milk powder sample A3 increased at the first fifteen days after opening the can, the rise was still within the passable limits mentioned in Syrian standard specification number 197/1996. Starting from day20 till day30, moisture content increased exceeding the limit recommended by Syrian standard specification. Lactic acid based acidity was titrated in solid mater and it was found to be 0.056% at the first day of experiment. This value is considered passable according to Syrian standard specification number 197/1996. Acidity titration was done each day in triplicate and relative standard deviation RSD was calculated. The all findings were less than 10% indicating that all measurements were accurate. It was noticed that despite Lactic acid based acidity titrated in solid mater of milk powder sample A3 increased at the first twenty days after opening the can, the rise was still within the passable limits mentioned in Syrian standard specification number 197/1996. Starting

from day 25 till day 30, acidity increased exceeding the limit recommended by Syrian standard specification. As for flowability that was determined, It was noticed that it was suitable at the first day after opening the product can and starting from the day 5 till the day10 of timeline flowability was passable but after the day 15 till day 30 it became to be weak due to the increase of moisture content that led to milk powder clumping. Table 5

Table 5 Variations in physicochemical properties of milk powder can sample A3 which was kept open at lab

A3	The sample kept open at lab								
	moisture		acidity			flowability			
Timeline	Moisture content%	RSD	Total acidity per dry weight basis	Titratable acidity as lactic acid	RSD	Hausner ratio	flowability	Carr's index	flowability
1	1.05	3.08	0.61	0.055	1.82	1.25	suitable	19.54	suitable
5	2.60	2.66	0.65	0.059	1.19	1.26	passable	20,42	passable
10	3.11	3.11	0.98	0.089	5.27	1.33	passable	24.59	passable
15	3.45	2.70	1.00	0.090	5.27	1.36	weak	26.45	weak
20	4.00	1.05	1.57	0.141	1.12	1.38	weak	27.74	weak
25	4.86	5.82	2.02	0.182	0.97	1.41	weak	29.23	weak
30	5.44	4.41	2.53	0.202	6.34	1.45	weak	30.82	weak

Fridge Storage of a tightly closed can of milk powder sample B1:

Milk powder can B1 was placed tightly closed in the fridge and moisture was measured it was found to be 2.57% at the first day. This value is considered acceptable according to Syrian standard specification number 197/1996. Moisture measurement was done each day in triplicate and relative standard deviation RSD was calculated. The all findings were less than 10% indicating that all measurements were accurate. Despite moisture content was noticed to increase in the milk sample B1 after milk can opening, this rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Lactic acid based acidity was titrated in solid mater and it was found to be 0.059% at the first day of experiment. This value is considered acceptable according to Syrian standard specification number 197/1996. Acidity titration was done each day in triplicate. and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. Despite lactic acid based acidity was noticed to increase in the milk sample B1 during the 30 day of timeline, this rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Flowability was determined, It was noticed that it was suitable during the 30 day of timeline Table 6

Table 6 Variations in physicochemical properties of milk powder can sample B1 which that was kept tightly closed in the fridge

B1	The sample kept tightly closed in the fridge								
	moisture		acidity			flowability			
Timeline	Moisture content%	RSD	Total acidity per dry weight basis	Titratable acidity as lactic acid	RSD	Hausner ratio	flowability	Carr's index	flowability
1	2.57	1.80	0.65	0.059	1.18	1.27	acceptable	21.26	acceptable
5	3.21	3.45	0.67	0.060	1.93	1.28	acceptable	21.34	acceptable
10	3.40	0.29	0.73	0.065	5.93	1.30	acceptable	23.84	acceptable
15	3.45	4.15	0.80	0.072	4.50	1.33	acceptable	24.43	acceptable
20	3.67	1.65	0.84	0.078	1.61	1.33	acceptable	24.67	acceptable
25	3.78	2.14	0.90	0.081	3.75	1.34	acceptable	25.37	acceptable
30	3.88	2.97	0.95	0.086	4.72	1.34	acceptable	25.37	acceptable

Lab storage of a tightly closed can of milk powder sample B2:

Milk powder can B2 was placed tightly closed at lab and moisture was measured it was found to be 2.72% at the first day. This value is considered acceptable according to Syrian standard specification number 197/1996. Moisture measurement was done each day in triplicate and relative standard deviation RSD was calculated. The all findings were less than 10% indicating that all measurements were accurate. Despite moisture content was noticed to increase in the milk sample B2 during the 30 days of timeline as milk can kept open, this rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Lactic acid based acidity was titrated in solid mater and it was found to be 0.059% at the first day of experiment. This value is considered acceptable according to Syrian standard specification number 197/1996. Acidity titration was done each day in triplicate and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. Despite lactic acid based acidity was noticed to increase in the milk sample B2 during the 30 day of timeline, this rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Flowability was determined, It was noticed that it was suitable during the 30 day of timeline then it became acceptable starting the day 15 to the day 30 of timeline. Table 7

Table 7 Variations in physicochemical properties of milk powder sample can B2 which that was kept tightly closed at lab

B2	The sample kept airtight closed at lab								
	moisture		acidity			flowability			
Timeline	Moisture content%	RSD	Total acidity per dry weight basis	Titratable acidity as lactic acid	RSD	Hausner ratio	flowability	Carr's index	flowability
1	2.72	3.51	0.65	0.059	1.69	1.28	acceptable	21.97	acceptable
5	3.30	4.53	0.71	0.064	1.09	1.29	acceptable	22.66	acceptable
10	3.50	0.77	0.79	0.071	3.72	1.29	acceptable	23.65	acceptable
15	3.53	2.81	0.80	0.072	3.25	1.33	acceptable	24.81	acceptable
20	3.76	1.47	0.84	0.078	1.61	1.33	acceptable	24.81	acceptable
25	3.86	0.37	0.91	0.078	3.59	1.34	acceptable	25.00	acceptable
30	3.95	0.96	0.94	0.085	1.86	1.34	acceptable	25.20	acceptable

Lab Storage of an opened can of milk powder sample B3:

Milk powder can B3 was placed open at lab and moisture was measured it was found to be 2.92% at the first day. This value is considered acceptable according to Syrian standard specification number 197/1996. Moisture measurement was done each day in triplicate and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. Despite moisture content was noticed to increase in the milk sample B2 during the 30 days of timeline as milk can kept open, this rise was still within the acceptable limits according to Syrian standard specification number 197/1996. Lactic acid based acidity was titrated in solid mater and it was found to be 0.059% at the first day of experiment. This value is considered acceptable according to Syrian standard specification number 197/1996. Acidity titration was done each day in triplicate and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. It was noticed that despite Lactic acid based acidity titrated in solid mater of milk powder sample B3 increased at the first fifteen days after opening the can, the rise was still within the passable limits mentioned in Syrian standard specification number 197/1996. Starting from day 20 till day 30, acidity increased exceeding the limit recommended by Syrian standard specification. Flowability was determined, It was noticed that it was passable at the first day after opening the product can and starting from the day 5 till the day10 of timeline flowability was weak but after the day 15 till day 30 it became to be too weak. Table 8

Table 8 Variations in physicochemical properties of milk powder can sample B3 which was kept open at lab

B3	The sample kept open at lab								
	moisture		acidity			flowability			
Timeline	Moisture content%	RSD	Total acidity per dry weight basis	Titratable acidity as lactic acid	RSD	Hausner ratio	flowability	Carr's index	flowability
1	2.92	0.54	0.65	0.059	2.39	1.27	acceptable	21.38	acceptable
5	4.54	2.12	0.74	0.067	1.49	1.40	weak	28.68	weak
10	5.84	1.32	1.04	0.094	2.86	1.43	weak	29.56	weak
15	6.68	1.35	1.83	0.165	5.17	1. 60	very weak	37.39	very weak
20	6.81	1.64	2.12	0.191	1.91	1.61	very weak	38.02	very weak
25	7.00	0.54	2.77	0.250	3.01	1.62	very weak	37.98	very weak
30	7.16	1.96	3.14	0.283	2.28	1.64	very weak	38.96	very weak

Protein content:

Protein content variations in powder milk samples of two brands A and B were determined using kjeldahl and biuret methods taking into consideration different conditions of storage and the accompanied physicochemical variations.

Fridge storage of a tightly closed can of milk powder sample A1:

As for the studied milk powder canA1, it was found that at the first day two comparative values for protein content were 9.67 and 9.26 as obtained with kjeldahl and biuret methods respectively. Both results were close to label value of 9.65.

Kjeldahl and biuret based protein determination was done in triplicate each day and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. It was noticed that kjeldahl determined protein content of can A1 which was kept in the fridge decreased from 9.65% on the first day to 9.2% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline, all result values were less than 4.303 as included a table of ***t*** values (Student's *t*-test**)** of the 95% confidence interval. It's obvious that there wasn't a statistical difference between the first and the last day i.e. there was no significant variation in protein content and the above mentioned decline can be attributed to random analytical errors.

It was noticed that biuret determined protein content of can A1 which was kept in the fridge decreased from 9.26% on the first day to 8.99% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline, all result values were less than 4.303 as included a table of ***t*** values (Student's *t*-test) of the 95%

confidence interval. It's obvious that there wasn't a statistical difference between the first and the last day i.e. there was no significant variation in protein content and the above mentioned decline can be attributed to random analytical errors. Table 9, Fig2

Table 9 Protein content variations in milk powder sample A1 determined by Kjeldahl and Biuret methods during the timeline of 30 days

A1	The sample kept tightly closed in the fridge					
	Protein content determination					
Timeline	Kjeldahl protein per dry weight basis(g/100g)	RSD%	Student's *t*-test	Biuret protein per dry weight basis(g/100g)	RSD%	Student's *t*-test
1	9.67	3.03		9.26	0.50	
5	9.57	2.04	0.88	9.20	0.99	1.16
10	9.46	1.15	3.33	9.18	1.83	0.83
15	9.36	26	2.19	9.13	2.69	0.92
20	9.33	2.13	2.96	9.03	1.46	3.01
25	9.30	2.54	3.39	9.00	1.83	2.73
30	9.24	2.15	3.74	8.99	1.51	3.44

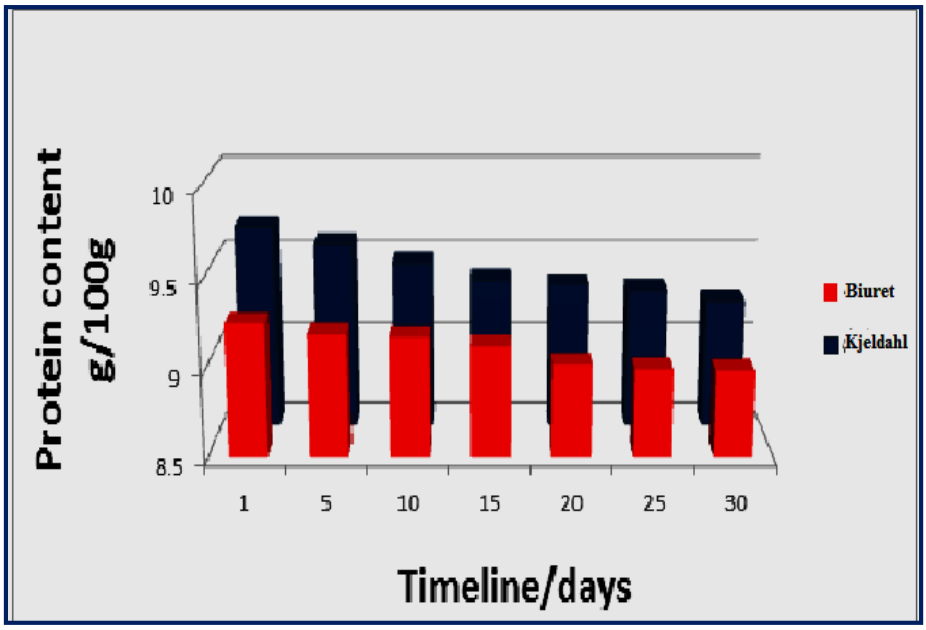

Figure 2 Graphical representation of protein content variations in milk powder sample A1 determined by Kjeldahl and Biuret methods during the timeline of 30 days

Lab storage of a tightly closed can of milk powder sample A2:

As for the studied milk powder canA2, two comparative values for protein content were 9.70 and 9.26 as obtained at the first day with kjeldahl and biuret methods respectively. Both results were close to label value of 9.65.

Kjeldahl and biuret based protein determination was done in triplicate each day and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. It was noticed that kjeldahl determined protein content of can A2 which was kept tightly closed in the lab decreased from 9.70% on the first day to 9.25% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline, all result values were less than 4.303 as included in the table of ***t*** values (Student's *t*-test**)**. It's obvious that there wasn't a statistical difference between the first and the last day i.e. there was no significant variation in protein content and the above mentioned decline can be attributed to random analytical errors. It was noticed that biuret determined protein content of can A2 which was kept at lab decreased from 9.26% on the first day to 8.88% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline, all result values were less than 4.303 as included a table of ***t*** values (Student's *t*-test). It's obvious that there wasn't a statistical difference between the first and the last day i.e. there was no significant variation in protein content and the above mentioned decline can be attributed to random analytical errors. Table 10, Fig3

Table 10 Protein content variations in milk powder sample A2 determined by Kjeldahl and Biuret methods during the timeline of 30 days

A2	The sample kept tightly closed at lab					
	Protein content determination					
Timeline	Kjeldahl protein per dry weight basis(g/100g)	RSD%	Student's *t*-test	Biuret protein per dry weight basis(g/100g)	RSD%	Student's *t*-test
1	9.70	4.09		9.26	0.50	
5	9.57	1.46	1.36	9.17	0.92	1.83
10	9.49	1.23	3.11	9.09	1.77	1.83
15	9.36	2.24	2.80	9.01	1.98	2.43
20	9.29	2.19	3.50	8.99	2.02	2.57
25	9.25	2.25	3.74	8.96	1.57	3.69
30	9.25	2.29	3.68	8.88	1.86	3.99

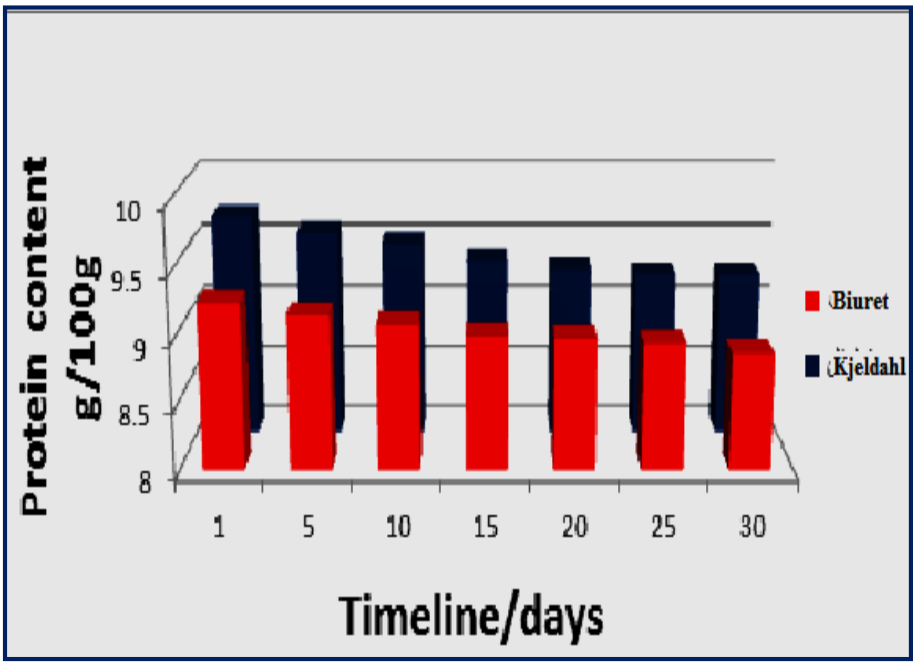

Figure 3 Graphical representation of protein content variations in milk powder sample A2 determined by Kjeldahl and Biuret methods during the timeline of 30 days

Lab storage of an opened can of milk powder sample A3:

As for the studied milk powder canA3, two comparative values for protein content were 9.63 and 9.25 as obtained at the first day with kjeldahl and biuret methods respectively. Both results were close to label value of 9.65.

Kjeldahl and biuret based protein determination was done in triplicate each day and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. It was noticed that kjeldahl determined protein content of can A3 which was kept open in the lab decreased from 9.63% on the first day to 9.80% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline it was noticed that while some result values were less than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the first day till the day 15, other result values were higher than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the twentieth day till the day 30. It's obvious that there wasn't a statistical difference between the first day and the day15. i.e. there was no significant variation in protein content, but there was a statistical difference between the twentieth day and the day30. i.e. there was a significant variation in protein content.

It was noticed that biuret determined protein content of can A3 which was kept at lab decreased from 9.25% on the first day to 8.45% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline it was noticed that while some result values were less than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the first day till the day 15, other result values were higher than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the twentieth day till the day 30. It's obvious that there wasn't a statistical difference between the first day and the day15. i.e. there was no significant variation in protein content, but there was a statistical difference between the twentieth day and the day30. i.e. there was a significant variation in protein content. Table 11, Fig4

Table 11 Protein content variations in milk powder sample A3 determined by Kjeldahl and Biuret methods during the timeline of 30 days

A3	The sample kept open at lab					
	Protein content determination					
Timeline	Kjeldahl protein per dry weight basis(g/100g)	RSD%	Student's *t*-test	Biuret protein per dry weight basis(g/100g)	RSD%	Student's *t*-test
1	9.63	0.78		9.25	0.92	
5	9.61	1.67	0.22	9.19	1.53	0.73
10	9.56	3.14	1.33	9.10	1.63	1.74
15	9.53	1.21	1.51	9.07	1.47	2.34
20	9.12	1.82	5.32	8.72	1.97	5.14
25	8.88	1.60	9.14	8.58	1.42	9.51
30	8.80	1.22	13.4	8.45	2.69	6.10

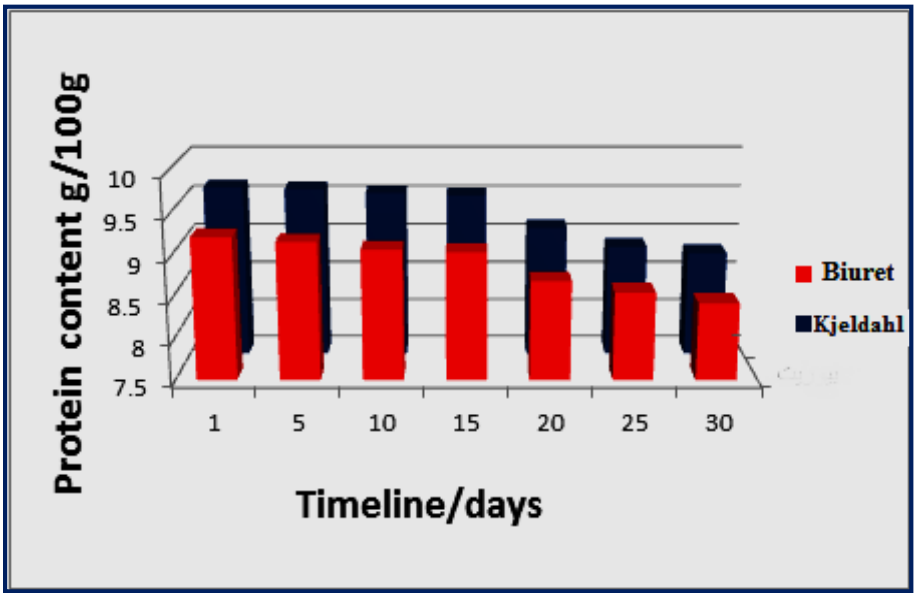

Figure 4 Graphical representation of protein content variations in milk powder sample A3 determined by Kjeldahl and Biuret methods during the timeline of 30 days

Fridge storage of a tightly closed can of milk powder sample B1:

As for the studied milk powder canB1, it was found that at the first day two comparative values for protein content were 11.59and 11.18 as obtained with kjeldahl and biuret methods respectively. Both results were close to label value of 11.50.

Kjeldahl and biuret based protein determination was done in triplicate each day and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. It was noticed that kjeldahl determined protein content of can B1 which was kept tightly closed in the fridge decreased from 11.59% on the first day to 11.21% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline, all result values were less than 4.303 as included a table of ***t*** values (Student's *t*-test**)**. It's obvious that there wasn't a statistical difference between the first and the last day i.e. there was no significant variation in protein content and the above mentioned decline can be attributed to random analytical errors.

It was noticed that biuret determined protein content of can B1 which was kept tightly in the fridge decreased from 11.18% on the first day to 10.93% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline, all result values were less than 4.303 as included a table of ***t*** values (Student's *t*-test). It's obvious that there wasn't a statistical difference between the first and the last day i.e. there was no significant variation in protein content and the above mentioned decline can be attributed to random analytical errors. Table 12, Fig5

Table 12 Protein content variations in milk powder sample B1determined by Kjeldahl and Biuret methods during the timeline of 30 days

B1	The sample kept airtight closed in the fridge					
	Protein content determination					
Timeline	Kjeldahl protein per dry weight basis(g/100g)	RSD%	Student's *t*-test	Biuret protein per dry weight basis(g/100g)	RSD%	Student's *t*-test
1	11.59	1.08		11.18	1.90	
5	11.53	0.95	0.95	11.17	1.57	0.10
10	11.51	0.70	1.71	11.11	1.27	0.85
15	11.47	1.74	1.04	11.05	1.40	1.45
20	11.26	1.73	2.93	11.05	1.53	1.32
25	11.27	1.51	3.26	10.97	1.33	2.52
30	11.21	1.59	3.70	10.93	1.18	3.37

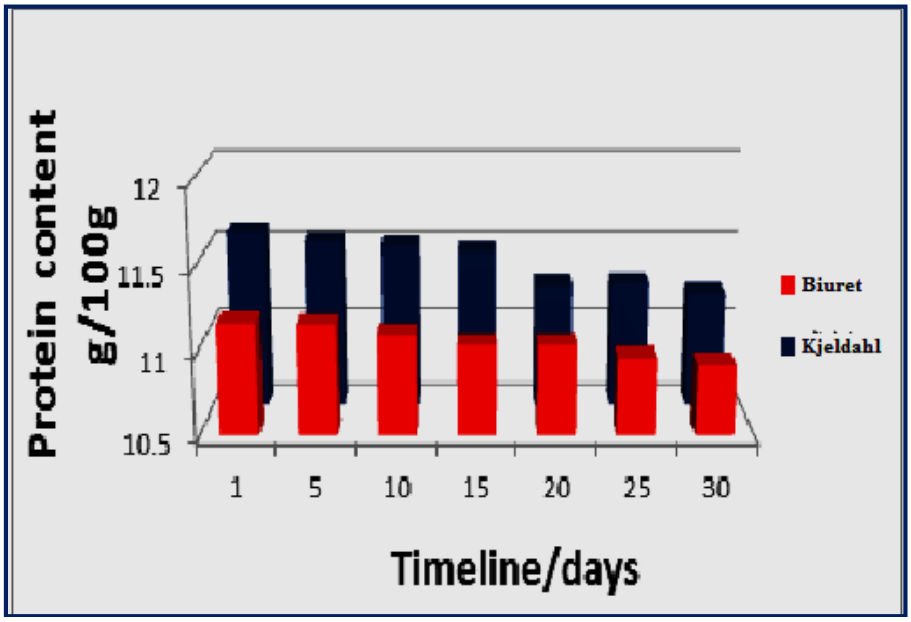

Figure 5 Graphical representation of protein content variations in milk powder sample B1determined by Kjeldahl and Biuret methods during the timeline of 30 days

Lab storage of a tightly closed can of milk powder sample B2:

As for the studied milk powder canB2, two comparative values for protein content were 11.51 and 11.26 as obtained at the first day with kjeldahl and biuret methods respectively. Both results were close to label value of 11.50.

Kjeldahl and biuret based protein determination was done in triplicate each day and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were accurate. It was noticed that kjeldahl determined protein content of can B2 which was kept tightly closed at lab decreased from 11.51% on the first day to 11.18% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline, all result values were less than 4.303 as included in the table of ***t*** values (Student's *t*-test**)**. It's obvious that there wasn't a statistical difference between the first and the last day i.e. there was no significant variation in protein content and the above mentioned decline can be attributed to random analytical errors. Table 13, Fig6

Table 13 Protein content variations in milk powder sample B2 determined by Kjeldahl and Biuret methods during the timeline of 30 days

B2	The sample kept tightly closed at lab					
	Protein content determination					
Timeline	Kjeldahl protein per dry weight basis(g/100g)	RSD%	Student's *t*-test	Biuret protein per dry weight basis(g/100g)	RSD%	Student's *t*-test
1	11.51	1.49		11.26	1.56	
5	11.47	1.57	0.38	11.19	1.06	1.03
10	11.43	1.18	1.03	11.15	1.57	1.09
15	11.38	1.76	1.13	11.05	1.10	2.98
20	11.29	1.47	2.29	10.98	1.34	3.30
25	11.22	1.62	2.75	10.91	1.63	3.40
30	11.18	1.49	3.44	10.87	1.71	3.63

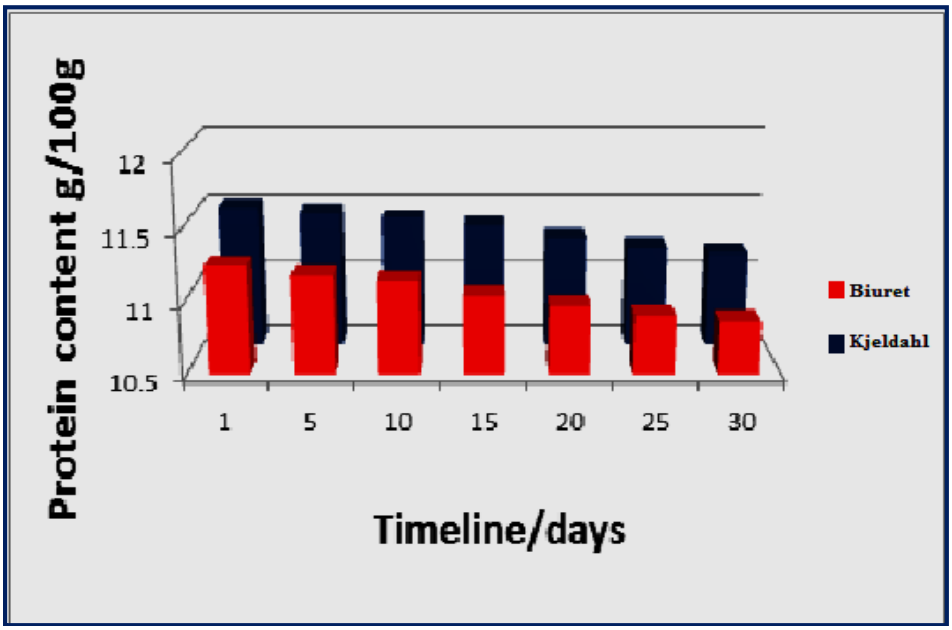

Figure 6 Graphical representation of protein content variations in milk powder sample B2 determined by Kjeldahl and Biuret methods during the timeline of 30 days

Fridge storage of an opened can of milk powder sample B3:

As for the studied milk powder can B3, two comparative values for protein content were 11.51 and 11.26 as obtained at the first day with kjeldahl and biuret methods respectively. Both results were close to label value of 11.50.

Kjeldahl and biuret based protein determination was done in triplicate each day and relative standard deviation RSD was calculated. All findings were less than 10% indicating that all measurements were

accurate. It was noticed that kjeldahl determined protein content of can B3 which was kept open in the lab decreased from 11.57% on the first day to 10.88% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline it was noticed that while some result values were less than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the first day till the day 15, other result values were higher than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the twentieth day till the day 30. It's obvious that there wasn't a statistical difference between the first day and the day15. i.e. there was no significant variation in protein content, but there was a statistical difference between the twentieth day and the day30. i.e. there was a significant variation in protein content.

It was noticed that biuret determined protein content of can B3 which was kept at lab decreased from 11.23% on the first day to 10.59% on the day thirty of timeline. Applying student's T test starting from the first day till the day 30 of timeline it was noticed that while some result values were less than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the first day till the day 15, other result values were higher than 4.303 as included in the table of ***t*** values (Student's *t*-test**)** starting from the twentieth day till the day 30. It's obvious that there wasn't a statistical difference between the first day and the day15. i.e. there was no significant variation in protein content, but there was a statistical difference between the twentieth day and the day30. i.e. there was a significant variation in protein content. Table 14, Fig7.

Table 14 Protein content variations in milk powder sample B3 determined by Kjeldahl and Biuret methods during the timeline of 30 days

B3	The sample kept open at lab					
	Protein content determination					
Timeline	**Kjeldahl protein per dry weight basis(g/100g)**	**RSD%**	**Student's *t*-test**	**Biuret protein per dry weight basis(g/100g)**	**RSD%**	**Student's *t*-test**
1	**11.57**	**2.60**		**11.23**	**1.18**	
5	**11.52**	**1.21**	**0.62**	**11.22**	**1.60**	**0.096**
10	**11.34**	**1.83**	**1.91**	**11.20**	**1.65**	**0.28**
15	**11.30**	**1.56**	**2.67**	**11.08**	**1.38**	**1.70**
20	**11.02**	**1.27**	**6.80**	**10.81**	**1.27**	**5.72**
25	**10.95**	**1.61**	**6.10**	**10.67**	**2.06**	**4.41**
30	**10.88**	**1.57**	**6.99**	**10.59**	**1.29**	**8.09**

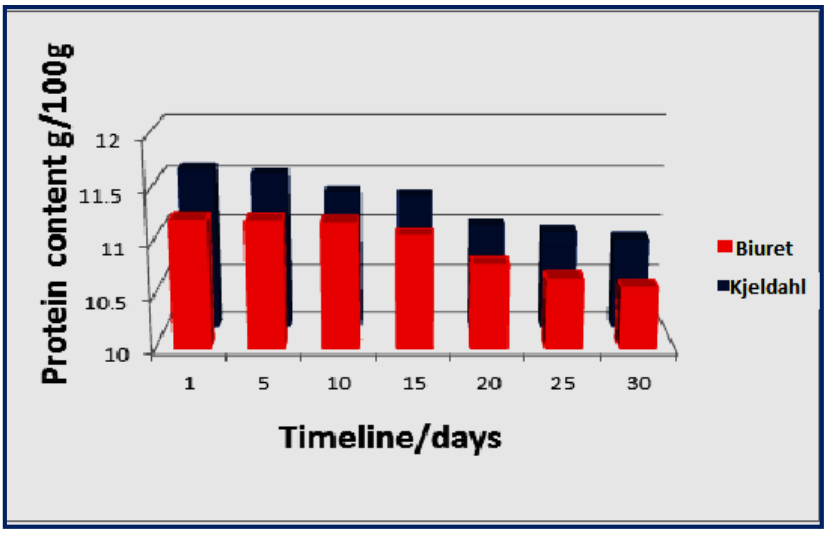

Figure 7 Graphical representation of protein content variations in milk powder sample B3 determined by Kjeldahl and Biuret methods during the timeline of 30 days

Ninhydrin qualitative protein detection:

Samples taken from milk powder cans A1, B1 which kept tightly closed in the fridge and from milk powder cans A2, B2 which were kept tightly closed in the lab were qualitatively detected for protein hydrolysis into amino acids using ninhydrin reagent. It was found that protein in the detected samples didn't hydrolyze into amino acid starting from the first day till the day 30 of timeline. Also, samples taken from milk powder cans A3, B3 which kept open in the lab were qualitatively detected for protein hydrolysis into amino acids using ninhydrin reagent. It was found that protein in the detected samples didn't hydrolyze into amino acid starting from the first day till the day 15 of timeline while it started to hydrolyze into amino acid on the twentieth day of the time.

Comparative statistical analysis of kjeldahl and biuret methods:

Protein content in powder milk sample was determined using kjeldahl and biuret methods. It was found that protein content in milk powder product declined during domestic usage. Both kjeldahl and biuret methods revealed decline in protein content but with different ratios. Comparison between two methods was assessed using Student's t-test. Comparison aimed to evaluate an alternative method to a standard method with established reliability and precision (a reference method) that is, biuret method as an alternative was evaluated in terms of reliability and precision to the reference and documented kjeldahl method [19]. In purpose of statistical comparison of the mentioned methods, the following relationship was applied

$t = \frac{|\Delta X|}{S} \sqrt{n}$ **where:** the mean values resulted by both given methods. S : The standard deviation variations of both given methods n : number of measurements. Calculated t values were compared

with tabulated student's t values at significance level of 95%. Differences between values of protein content obtained by both kjeldahl and biuret methods were statistically analyzed starting from the first day till the day 30. All values were higher than 4.303 as included in the table of ***t*** values (Student's *t*-test**)**, thus there is a significant difference between both mentioned methods. It's concluded that findings given by biuret method are inaccurate and it's considered unreliable for protein determination.

Influence of physicochemical properties of milk powder on the sensory qualities and protein content

Physicochemical properties of milk powder cans (A1, A2, A3, B1, B2, B3) were investigated during domestic use. It was noticed that increase in moisture and acidity of milk powder caused variation of its sensory qualities such as odor, taste and appearance. Also, increase in moisture caused milk powder caking and lumping with weaker flowability and lower solubility. Further, higher acidity titrated based on lactic acid in milk caused its degradation that promote bacterial growth and putrid smell. In addition, lipolysis of milk fat led to liberation of fatty acids. Furthermore, it was noticed that in samples of moisture and acidity levels greater than the recommended, a decline in the content of milk powder protein which was hydrolyzed into amino acids during domestic consumption and due to storage conditions. Milk powder cans A1,B1 kept tightly closed in the fridge as well as milk powder cans A2,B2 kept tightly closed in lab during domestic consumption had increase in moisture and acidity within the recommended levels without affecting on flowability, solubility and protein content of the above mentioned samples of milk powder.

Both milk powder cans A3, B3 kept open in the lab temperature had moisture and acidity levels greater than the recommended. These variables were also accompanied by milk powder lumping, weaker flowability, lower solubility and putrid smell. In addition, protein content noticeably declined due to its degradation into amino acids. Thus the studied samples A3, B3 became unfit for human consumption.

CONCLUSIONS

Based on the suggested reference method for the determination of protein content in two brands of milk powder, the influence of variations in physicochemical properties of the studied milk powder products were detected at different conditions.

- Detected physicochemical properties (moisture , acidity) values of protein content at the first day after opening the milk powder cans were within the recommended according to Syrian standard specification number 197/1996 and close to close to label value.
- Protein content didn't influenced when samples of milk powder cans were kept airtight closed in the fridge and at lab temperature due to steady

physicochemical properties within the recommended limits.

- When samples of milk powder cans kept open in the lab temperature moisture, acidity and flowability levels were greater than the recommended limits according to Syrian standard specification. In addition, protein content noticeably declined due to its degradation into amino acids.
- Suitable storage conditions didn't influence on the sensory quality of the studied samples of milk powder on contrary to bad storage conditions which led to lumping, lower solubility of milk powder samples and accompanied by putrid smell. Thus the product would become unfit for human consumption.

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REFERENCES

1. Goedhart,A., Bendels, J., *The Composition Of Human Milk As A Model For The Design Of Infant Formulas: Recent Findings And Possible Applications*, 1994, Nutrition Research Reviews, 7, 1-23
2. Turini,M *Protein Requirements In Early Childhood,*2013*,* Nestlé Nutrition Institute South East Asia And Pacific Rim V No.7 / May.
3. Michaelsen, K., Weaver, L., Branca, F., Robertson, A., *Feeding And Nutrition Of Infants And Young Chlildren* World Health Orgnization 2000 Update Reprint2003 ISSN 0378-225.
4. Manary, M. J., Heikens, G. T., Kwashiorkor: More Hypothesis Testing Is Needed to Understand the Aetiology of Oedema. 2009,Malawi Med J, 21 (3), 106-107
5. Ozkan, N., Withy, B., Chen, X., *Effects Of Time, Temperature, And Pressure On The Cake Formation Of Milk Powders,* 2003, Journal Of Food Engineering 58 - 355–361.
6. Chudy, S., Pikul, J., Rudzinska, M., Macowska, A., *The Effect Of Storage On Physicochemical Properties Of Spray-Dried Milk, Egg And Milk-Egg Mixture*, 2015, Acta Agrophysica, 22(1), 17-26.
7. Elkier, M., Yagoup,. A., *Quality Assessment Of Milk Powders Packed In Sudan*, 2009, Pakistan Journal Of Nutrition 8 (4): 388-391.
8. Gemechu,T., Beyene, F., Eshetu, M *Phsyical And Chemical Quality Of Raw Cows Milks Produced And Marketed In Shashemene Twon Southern Ethiopia,* 2015, Journal Of Food And Agricultural Science Vol.5(2) Pp 7-13, March.
9. Ganesan, V., Rosentrater, A. K., Muthukumarappan, K., *Flowability And Handling Characteristics Of Bulk Solids And Powders – A Review With Implications For DDG,* 2008,. Bio Systems Engineering, 2008 101 - 425 – 435.
10. Momein, A., Sulieman, E., Elamin, M. O., Elkhalifa, A. E., Laleye, L, *Comparison Of Physicochemical Properties Of Spray-Dried*

Camel's Milk And Cow's Milk Powder, 2014, International Journal Of Food Science And Nutrition Engineering, 4(1): 15-19.

11. Benkovic, M., Bauman,I., *Flow Properties Of Commercial Infant Formula Powders*. World Academy Of Science, Engineering And Technology, 2009, Vol:30 -06-24.

12. Thomas, M., Scher, J., Banon, S., *Milk Powders Ageing: Effect On Physical And Functional Properties.* Critical Reviews, 2004, In Food Science And Nutrition, 44:297–322.

13. Amin, M., Flowers,T., *Evaluation Of Kjeldahl Digestion Method.* Journal Of Research (Science), Bahauddin Zakariya University, 2004, Multan, Pakistan.Vol.15, No.2, June, pp. 159-179.

14. *Kamizake, N., Gonclaves, M., Zaia, B.., Zaia, D., Determination Of Total Proteins In Cow Milk Powder Samples: A Comparative Study Between The Kjeldahl Method And Spectrophotometric* Methods, 2003, Journal Of Food Composition And Analysis 16 - 507–516.

15. Starcher, B., *A Ninhydrin-Based Assay To Quantitate The Total Protein Content Of Tissue Samples,* 2001, Analytical Biochemistry 292**,** 125–129.

16. Adedeji, A., Adenikinju, R., Ajele, J., *Serum Protein Electrophoresis Under Effective Control Of Hiv-1 Disease Progression*,2014, *Excli Journal 13:761-771 – ISSN 1611-2156*.

17. AOAC International, *Official Methods of Analysis*, 17th edition, AOAC International, Arlington,VA, 2000

18. Schussele, A., Brandel, A., Note on the measurement of flowability according to the European Pharmacopoeia.2003, International Journal pharmaceutics, volume 257 Issuues1-2, page301-304

19. Lynch, J., Barbano, D. *Kjeldahl nitrogen analysis as a reference method for pro-tein determination in dairy products.* Journal of AOAC international*,* 1999, vol-ume 82 (6), 1389-1398