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Comparative Analysis of Physicochemical Parameters in Raw and Evaporated Milk from Sebha City, Libya

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Abstract The present study was carried out to determine chemical and physical properties and heavy metals in order to evaluate the potential health risks to humans. A total of fourteen randomly milk samples (7 raw, 3 concentrated and 4 whole milk samples) were collected from different outlets in Sebha, Libya in order to study the physicochemical parameters, minerals and heavy metals of milk samples. The chemical composition was determined by protein, fats, moisture, ash, fibre, carbohydrates. The moisture content (82.6%), pH (6.61), acidity (0.27%), density (1.03g/ml), total solids (16.8%), fat (3.22%), protein (3.16%), total sugar (6.15%), sucrose (1.68g/100ml), NaCl (0.54%), urea (10.48%) were reported. The pH values had the following order: whole>concentrated> raw milk samples. The mean concentrations (mg/l) of metals in analyzed milk samples were ranged between K (4.50E-03), Na (7.00E-03), Ca (20.2E-03), Mg (45.0E-03), Cd (5.60E-03), Zn (4.00E-03).
Keywords: Milk, heavy metals, minerals, protein, urea, fats, salts.

تحليل مقارن للخواص الفيزيوكيميائية والحيوية لعينات الحليب الطبيعي والصناعي المختارة من مناطق مختلفة في مدينة سبها

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ملخص أجريت هذه الدراسة لتحديد الخواص الفيزيائية والكيميائية والحيوية لعدد 14 عينة من الحليب المبستر والمركز والطبيعي المباع في مدينة سبها ومقارنتها بمعايير منظمة الصحة العالمية. أظهرت النتائج لعينات الحليب أن كل الخواص تعتمد على نوعية الحليب ولقد أثبت ذلك بواسطة التحليل الإحصائي. حيث لوحظ أن متوسط نسبة الرطوبة 82.6 %، متوسط المواد الصلبة الكلية 16.8%،متوسط الرقم الهيدروجيني 6.6،متوسط قيم الكثافة 1.03 g/ml،متوسط الحموضة 0.27%، متوسط كلوريد الصوديوم 0.54% ، متوسط نسبة السكريات الكلية 6.15%، متوسط نسبة السكروز 1.68g/100ml،متوسط نسبة الدهون 3.22% ،متوسط نسبة البروتين 3.16% ، متوسط نسبة اليوريا 10.48%. نلاحظ ان تراكيز العناصر والمعادن الثقيلة متقارب جداً فمتوسط تركيز الصوديوم والبوتاسيوم والكالسيوم والماغنيسيوم والكادميوم والزنك يكون على التوالي لجميع العينات (mg/L0.007,0.0045,0.0202,0.45,0.056,0.04). و وجد ان بعض الحليب الصناعي مغشوش باضافة ماء والبعض الاخر بنزع الدهن.

الكليمات المفتاحية: الحليب، العناصر الثقيلة، المعادن، البروتين، اليوريا، الدهون، املاح.

Introduction

A major concern worldwide is an environmental pollution from industrial and agriculture processes, which increases the level of toxic materials in ecosystems like plants and animals. However, One of the most popular dairy products is milk as a complex, bioactive substance, which is considered to be the most important components for human and widely consumed by human children due to it is completely food containing proteins, fats, sugars, vitamins and minerals with 38 micro and trace elements reported [1, 2]. Also, milk is an excellent source of macro-elements (Ca, K, and P) and micro-elements (Cu, Fe, Zn, and Se) [3, 4]. Moreover, other contaminated metals might reach a high level, which is harmful to humans, entering milk during production and packing processes [3, 5].In recent years, quality of raw and powdered milk

has been paying a great attention in worldwide concern. The measurements of heavy metals in milk is important for controlling the level of heavy metal toxicity [3, 6, 7]. However, to the best of the knowledge of the researchers, there is no report on the quality of milk such as physicochemical properties and heavy metals in powder and produced milk at Sebha City, Libya. Therefore, the aims of this study are: to determine the physicochemical properties (protein, fats, moisture, ash, fiber, carbohydrates, pH, acidity, total solids, fat, protein, sugar); to determine elements (Na, k, Ca, Mg) and heavy metal levels (Fe, Cd, Mn, Zn, Pb and Ni) in 14 different brands of milk productions, where either legally imported into Libya, made in Libya or from local farms.

2. Materials and Methods

2.1. Reagents: All reagents and chemicals were purchased from Merck, Germany and BDH, England and were used of analytical grade. Standard solution of heavy metals for atomic absorption spectroscopy were prepared by diluting the stock solution of 1000 mg/l. Deionized water was used throughout this work

2.2. Apparatus: Atomic absorption spectrometer (AAS) series S from Thermo Company was used. Conductivity and pH values were measured using Orion 4 Star, USA.

2.3. Sample collection: A total of 14 samples of commercially available milk were purchased and collected twice from different 14 sites around Sebha City, Libya during October, 2015 to February, 2016. The samples were collected with size of a liter for each raw milk immediately after milking. All samples were kept in their original packages. Four different animal species namely goat, sheep, cow and Camel were studied. A new bourn milk of cow and goat were obtained from different farms in Sebha City, Libya. Human milk was collected at Sebha Medical Center. Forth whole milk and three concentrated milk were obtained from local supermarket produced from different foreign companies. All samples were transferred in well labeled, sterile, plastic bottles and kept in a cooler with ice pack and finally carried immediately to the laboratory and kept frozen at 4.0°C as shown in Figure 1 and listed in Table 1.

Figure 1. Libyan map of milk sample collection

2.4. Sample preparation: All milk samples were transferred into sterilized dark bottle and kept in laboratory refrigerator at -4.0°C for further analysis. At the experimental part, milk samples were heated at 35°C and the mixture was well mixed and left to cool at room temperature for 3 mins in order to remove air bubbles**.**

2.5. The physicochemical properties: The physicochemical properties of milk samples were determined using different American Society for Testing and Materials, ASTM, such as: ASTM D4959-16 for moisture content, D6128-00 for TSC, D1989-20 for SNF, D2937-00 for density, D974-06 for acidity, D632-12 for NaCl and D5258-92 for heavy metals, and Lane-Eynon method for total sugar, Resr-Gottliebs for fats.

2.6. Determination of heavy metals: In order to determine the level of heavy metals in milk samples, ASTM D5258-92 were modified and used. Approximately, 3.0 g of each samples were weighted and immersed into 10 ml of nitric acid (2:3, 65%) and slightly heated for 10 min before addition of 10 ml H_2O_2. The mixture was boiled until clear solution was obtained. The mixture was left to cool and the volume was adjusted to a suitable volume 100 ml with deionized water. The concentrations of heavy metals, Pb, Ni, Zn, Cd, Mn, in filtrate of digested mixture were determined using AAS with standard curves using metal chloride salts.

2.7. Estimated daily intake of metals from milk The estimated daily intake (EDI) of metals could be determined by using the following equation:

$$EDI\,(mg/kg) = \frac{Ci \times 39.2}{60} \quad \textbf{(1)}$$

Where: 39.2 mg/day=daily milk consumption rate, 60kg= average body weight, Ci=metal concentrations [8].

Table 1. Sampling collection information

Sample Number	Sample Name	Product	Country	Date		Notes
				Product	Expired	
MS1	Judi	Production Par Judi Food Industries	Tripoli-Libya	11\2\2016	10\8\2016	Sterilized
MS2	Nadec	Saudi Arabia	Saudi	21\9\2015	21\6\2016	Sterilized
MS3	Rainbow	Friesland Campina	Belgium	18\11\2015	14\8\2016	Sterilized
MS4	Azahrat	Shamal Al Mutawassat		15\8\2015	11\5\2016	Sterilized
MS5	Rainbow	not Found	Holland	10\2015	10\2016	Concentration
MS6	Judi	not Found	Germany	7\2015	7\2016	Concentration
MS7	Azahrat	Hoch wail Foods GmbH		11\2015	11\2016	Concentration
MS8	Goat	Sebha-Libya	Farm	1\3\2016	4\3\2016	Natural
MS9	Camel	Sebha-Libya	Farm	7\3\2016	11\3\2016	Natural
MS10	Cow	Sebha-Libya	Farm	6\3\2016	9\3\2016	Natural
MS11	Sheep	Sebha-Libya	Farm	1\3\2016	4\3\2016	Natural
MS12	Colostrum sheep	Sebha-Libya	Farm	4\5\2016	7\5\2016	Natural

MS13	Colostrum Cow	Sebha-Libya	Farm	23\5\2016	27\5\2016	Natural
MS14	human milk	Sebha-Libya	Not Reported	30\5\2016	2\6\2016	Natural

2.8. Statistical analysis: The statistical analysis of data was conducted by using SPSS software version 23.0. Significant differences between means were subjected to Anova: Two Factor without Replication and the level of significant was compared at $P<0.05$.

3. Results and Discussion Color: all the milk samples appeared to have a white color due to the presence of casion, while the raw milk samples were having a yellowish color due to the presence of fat, total solids, type of animal and food [9]. The SM12 and SM13 were colored in yellow due to the presence of cartin in green grass food [10].

Taste: the fresh milk has sweet taste due to lower the chlorides and higher sugar milk. This taste is disappeared in few hours due to change the fructose to lactic acid in the presence of bacteria. In this study, all the milk samples were found to be in good taste due to freshly collected products and there was no changing in taste or color during experimental processes.

pH: the pH values of all milk samples were in the range between 6.07 to 7.28 with average of 6.61 with one exception of mother milk SM14 as listed in Table 1. However, the results showed that the pH values had the following order: whole>concentrated> raw milk samples. The lower pH values in few studied milk samples could be due to part of lactose sugar forming lactic acid bacteria [5].

Specific gravity: the specific gravity for all milk studied was listed in Table 2. The average of specific gravity of studied milk samples were 6.61, which above recommended value of 1.04 mg/cm^3. Few samples were below the recommended value, which could be due to high percentage of fats in studied milk samples and low total solids. This is related to water milk added [11, 12].

Moisture content, MC: the moisture content percentages, using to determine microbial growth, of studied milk samples were in the range of 74.0-88.7% with an average of 82.7% as listed in Table 4. This could be true based on the preparation processes or hot weather prevalent at the time of sampling [11, 12]. However, the results showed that the percentage of MC had the following order: whole>raw>concentrated milk samples.

Total solids, TS: the percentage of TS of milk samples were in the range of 11.3-25.8% with an average of 16.8% as listed in Table 2. However, all the studied milk samples had TS percentages near to permissible limits 13% [11]. On the other hand, the lower TS percentages than permissible limits could be due to the high percentage of fats, proteins, sugar and casion [6]. Moreover, the results showed that the percentage of TS had the following order: concentrated> raw>whole milk samples

Total solids none fat, S.N.F: the percentage of S.N.F of milk samples were in the range of 5.70-11.2% with an average of 8.45% as listed in Table 2. The lower S.N.F is related to water add to milk. It was noted that MS1 and MS2 had lower SNF, which were due to water add. Also, the MS6 and MS7 had lower TS and higher SNF than permissible limits (TS>3%), which were due to fat extraction from milk. This was evaluated by using milk added weight equation. Moreover, the results showed that the percentage of NSF had the following order: concentrated> raw>whole milk samples.

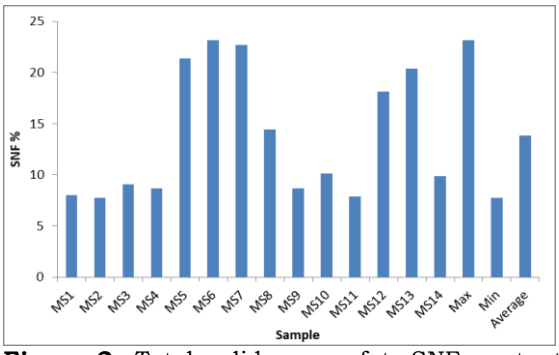

Figure 2. Total solids none fat, SNF content in studied milk samples

Acidity: almost all the percentages of acidity in milk samples were near to the acceptable limit, which between 0.13-0.17% as listed in Table 2. However, the percentages were found to be high in MS12, MS13 and MS14, 0.57, 0.43 and 0.69, respectively, with an average of 0.27%, which could be due to the percentage of SNF or activity of bacteria converting lactic acid from lactose sugar, which is a critical parameter for process and quality control of milk especially in chase [12, 13].

Sodium Chloride, **NaCl**: the percentages of sodium chloride were in recommend range from 0.23% to 0.77% with an average of 0.54% as listed in Table 4. It was noted that mother's milk had the lowest NaCl, which is making mother's milk taste too sweet.

Total Sugar: the concentrations of total sugar in whole, goat and new born milk were ranged from 3g/100ml to 5g/100ml, which near to limit 4.9g/100ml, while the rest of milk samples had higher total sugar than permissible limits with an average of 6.15g/100ml as listed in Table 2.

Sucrose: the concentrations of sucrose in all sample milk were between 2.04-0.79g/100ml with an average of 1.68g/100ml, as listed in Table 2.

Fats: the content of fat in milk samples was in range 2-5% as listed in Table 4. However, almost all of milk samples were close to acceptable data (3.7%) with an average of 3.22%. this could be probably due to type of feeds [12]. However, SM5 had highest density due to natural milk has usually high total dissolved solids with lowest percentage of fat.

Protein: the percentage of protein in milk samples was in range of 1-8%, which is near to acceptable value of 3.7% as listed in Table 2 and shown in Figure 3. However, it was noted that MS2, MS3

and MS4 samples have a higher percentage of protein with an average of 3.6%.

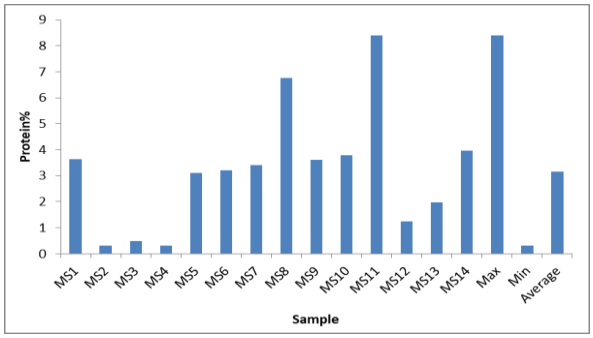

Figure 3. Protein content in studied milk samples

Urea: the percentage of urea in milk samples were between 3-32mg/100ml, which is near to acceptable limit, 6-20mg/100ml with average of 10.47mg/100ml as listed in Table 2 and shown in Figure 4. However, MS12 has a high urea content, which could be due to deficiency of responsible enzymes for converting the ammonia to urea.

Also, MS1 and MS2 have the lowest urea contents due to they have the lowest protein contents.

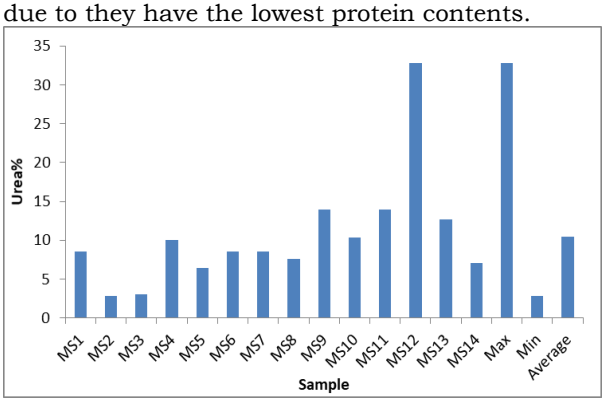

Figure 4. Urea content in studied milk samples

Table 2. The physicochemical properties of milk samples

Sample	PH	MC	TSC	SNF	SP	Acidity	NaCl	TS	Sucros	Fat	Protein	Urea
MS1	6.63	88.73	11.27	8.01	1.02	0.16	0.33	4.69	2.01	3.26	3.64	8.60
MS2	6.98	87.95	12.05	7.74	1.02	0.21	0.50	5.58	1.87	4.51	0.32	2.80
MS3	6.62	87.46	12.54	9.06	1.02	0.18	0.39	5.07	1.85	3.48	0.48	3.10
MS4	6.81	87.76	12.24	8.65	1.02	0.18	0.44	5.13	1.93	3.59	0.30	10.00
MS5	6.41	74.00	25.27	21.35	1.06	0.23	0.66	9.05	1.37	4.40	3.10	6.40
MS6	6.42	74.25	25.75	23.18	1.05	0.24	0.77	6.56	0.79	2.57	3.21	8.60
MS7	6.54	74.99	25.01	22.71	1.05	0.22	0.74	6.63	1.75	2.30	3.42	8.60
MS8	6.61	81.97	18.03	14.42	1.02	0.20	0.62	5.25	2.03	3.61	6.77	7.60
MS9	6.13	87.81	12.19	8.67	1.02	0.14	0.45	6.77	1.99	3.52	3.60	14.00
MS10	6.27	86.37	13.63	10.16	1.02	0.17	0.44	7.37	2.02	3.47	3.78	10.30
MS11	6.86	81.97	11.44	7.90	1.02	0.19	0.61	4.51	2.04	3.54	8.40	14.00
MS12	6.05	79.37	20.63	18.13	1.04	0.57	0.66	4.41	1.21	2.50	1.25	32.80
MS13	6.88	77.53	22.47	20.39	1.06	0.43	0.77	3.54	0.88	2.08	1.97	12.70
MS14	7.28	86.87	13.13	9.87	1.01	0.69	0.23	10.15	1.81	2.26	3.96	7.10
min	6.05	74.00	11.27	7.74	1.01	0.14	0.23	3.54	0.79	2.08	0.30	2.80
max	7.28	88.73	25.75	23.18	1.06	0.69	0.77	10.15	2.04	4.51	8.40	32.80
Average	6.61	82.65	16.83	13.59	1.03	0.27	0.54	6.05	1.68	3.22	3.16	10.47

Calcium and magnesium contents: the concentrations of Ca and Mg in studied milk samples were also determined in studied milk samples as listed in Table 3. The concentrations of Ca, in studied milk samples were ranged between 0.05-1.64 mg/100ml with an average of 0.45mg/l. This is below the recommended daily intake 1200mg/kg per day [12]. The concentrations of Mg, in studied milk samples were ranged between 0.192-0.228 mg/100ml with an average of 0.200 mg/l. This is below the recommended daily intake 350 mg/kg per [12].

Sodium, Na: the concentrations of Na in studied milk sample were determined in studied milk samples as listed in Table 3. The concentrations of Na in studied milk samples were near to each other with an average of 4.03E-02 mg/100ml. This is below the recommended daily intake 500 mg/kg per day [12].

Potassium, K: the concentrations of K in studied milk sample were determined in studied milk samples as listed in Table 3. The concentrations of K in studied milk samples were near to each other with an average of 0.058 mg/100ml. This is below the recommended daily intake limit 2000 mg/kg per day [12].

Cadmium, Cd: the concentrations of Cd in studied milk sample were determined in studied milk samples as listed in Table 3. The concentrations of cadmium, Cd, in studied milk samples were almost none with an average of 4.50E-03 mg/100ml. This is below the recommended daily intake value of 0.50 mg/kg [12].

Zinc, Zn: the concentrations of Zn in studied milk sample were determined in studied milk samples as listed in Table 3. The concentrations of zinc, Zn, in studied milk samples were near to each other with an average of 6.80E-03 mg/100ml. This is below the recommended daily intake value of between 12-15 mg/kg [12].

Nickle and Lead: the concentrations of Ni and Pb in studied milk sample were determined in studied milk samples as listed in Table 3. These heavy metals were not detected in studied milk samples, which could be due to low pollution area.

Table 3. Metal Concentrations in milk samples

Sample	Ca	Mg	Na	K	Cd	Zn	Ni	Pb
MS1	0.70	0.24	0.04	0.05	0.00	0.01	ND	ND
MS2	0.35	0.24	0.04	0.06	0.00	0.00	ND	ND
MS3	0.39	0.38	0.04	0.06	0.00	0.00	ND	ND
MS4	0.43	0.26	0.04	0.06	0.00	0.00	ND	ND
MS5	0.18	0.2	0.04	0.07	0.00	0.00	ND	ND
MS6	0.3	0.06	0.04	0.07	0.01	0.00	ND	ND
MS7	0.16	0.23	0.04	0.07	0.01	0.04	ND	ND
MS8	0.19	0.18	0.04	0.05	0.00	0.01	ND	ND
MS9	0.13	0.12	0.04	0.06	0.00	0.00	ND	ND
MS10	0.12	0.13	0.04	0.06	0.00	0.01	ND	ND
MS11	0.13	0.14	0.04	0.06	0.00	0.00	ND	ND
MS12	1.53	0.23	0.04	0.06	0.01	0.00	ND	ND
MS13	1.64	0.23	0.04	0.06	0.01	0.00	ND	ND
MS14	0.05	0.19	0.04	0.06	0.00	0.01	ND	ND
DEI	--	--	--	--	0.002	--	--	--
RDA	1200	350	500	2000	0.046	15	--	0.02

ND: not detected, RDA: recommended dietary allowance adults (mg/day)

Health risk assessment: the estimated daily intake (EDI) of metals in studied milk samples were investigated. The EDI and RDA values for metals in studied milk samples were listed in Table 3. In order to determine EDI, the average of Cd concentrations were selected. The EDI of Cd in studied milk samples was 0.02 mg/day below the permissible limit [8]. Other EDIs were not able to determine due to the concentration of these metals below detected limit. From these results, it can be concluded that the potential health risk via heavy metals studied is too low.

Statistical studies: The all the statistical values of studied data are given in Table 4. The results from Table 3 indicate that sum squares, SS, and mean of squares, MS, for samples are 73.65 and 5.67, respectively, which lower than those along to properties, 8.84E04 and 5.20E03, respectively. These results suggest that the data can be compared along to properties than samples. The value of Fcal is 5.99E-01 while that of Fcrit is 1.77 along to samples. Fcal is lower than Fcrit and the p-value in rows (p-value>0.05, ANOVA: Tow-Factor Without Replication) indicate that the null hypothesis is accepted along to type of samples and no significant differences along to type of samples. On the other hand, the value of Fcal is 5.50E02 while that of Fcrit is 1.67 along to properties. Fcal is greater than Fcrit and the p-value in rows (p-value<0.05, ANOVA: Tow-Factor Without Replication) indicate that the null hypothesis is rejected and significant differences along to properties. Statistically, the results indicate significant differences in milk properties.

Table 4. Two way analysis of variance of physic-chemical properties and metal concentration of studied milk samples using ANOVA analysis.

ANOVA Source of Variation	SS	Df	MS	F	P-value	F crit
Samples	73.6491	13	5.665316	0.599381	0.853441	1.764653
Properties	88405.44	17	5200.32	550.1857	2.3E-170	1.669171
Error	2088.878	221	9.451937			
Total	90567.97	251				

Df: degree of freedom, SS: sum squares, MS: mean of squares

Correlation Coefficient: The correlation coefficient between two variance was also tested. It was found that there is a great forwarded relationship between SNF and TSC. There is a great relationship between two samples MS6 and MS7. Also, there is no relationship between other parameters.

4. Conclusion: The present study gives important information on the quality of milk in south of Libya. It can be concluded from this research that the quality of sold and produced milk in the area of studied need to be frequently tested and reported using the most validated methods. The values of pH were in the recommended limit with one exception of mother milk. A special attention should be paid to the specific gravity values of studied milk samples due to they were below recommended level. The total solids were also low. The percentage of proteins was too low as well. The content of urea was close to permissible limits. Based on EDI results, it can be concluded that the potential risk via heavy metals is not possible. Based on the results found in this research, the following recommendations are made for human consumers or future work: Frequently routine tests for quality of milk, study the effect of animal age on milk properties and study another point source of raw milk collection and compared with this work.

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