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The Contents of Some Essential Major and Trace Elements in Various Types of Processed Milk Products Collected from Benghazi Markets

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ABSTRACT

Milk is an important food; it supplies our body with the essential mineral's requirements. In this study, some major (Calcium, magnesium, phosphorus) and minor (copper, iron, zinc) essential minerals were determined in seventy-eight random processed milk samples. These samples were collected from different markets in Benghazi city, Libya during 2017. The order of major essential elements concentrations in all milk samples was calcium> phosphorous> magnesium. The mean concentrations of calcium were recorded at 4262 ±2771mg/kg, 5006 ±896mg/kg, 1276 ±319mg/kg and 1231 ±154mg/kg, while mean concentrations of magnesium were 560.1 ±82mg/kg, 419.4 ±153mg/kg, 249 ±7.1mg/kg, 127.7 ±21mg/kg, in powdered, infant formula, evaporated and sterilized milk samples, respectively. Phosphorus concentration varied between 3322-7692mg/kg, 2622-5490mg/kg, 1381-4720mg/kg and 1049-2326mg/kg in powdered, infant formula, evaporated and sterilized milk, respectively. The contents of trace essential minerals, included copper, iron and zinc, were detected in all collected samples. The highest copper content was recorded in powder milk, with a mean concentration 2.13 ±0.84mg/kg, followed by infant formula products, with mean concentration 1.15±0.95mg/kg. The mean concentrations of zinc and iron in milk powder products were detected at 33.48 ±17.4mg/kg and 27.60mg/kg, respectively. The concentration of minor element in evaporated and sterilized milk samples were in concentration lower than 1mg/kg, except zinc in evaporated milk, which varied between 2.80-5.77mg/kg. The contents of essential minerals were agreed with some values that recorded in published results, for same products of processed milk.

محتوي بعض العناصر الأساسية الرئيسية والثانوية في أنواع مختلفة من الحليب المصنع التي تم جمعها من أسواق بنغازي

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الكلمات المفتاحية:
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بنغازي

الملخص

يعتبر الحليب غذاء مهم، فهو يمد أجسامنا بمتطلبات المعادن الأساسية. وفي هذه الدراسة، تم تحديد بعض المعادن الأساسية الرئيسية (الكالسيوم، والماغنسيوم والفوسفور) والثانوية (النحاس، والحديد والزنك) في ثمانية وسبعين عينة من الحليب المصنع التي تم جمعها عشوائيًا من أسواق مختلفة في مدينة بنغازي، ليبيا خلال عام 2017.وجد في هذه الدراسة إن ترتيب تراكيز العناصر الأساسية الرئيسية في جميع عينات الحليب هو: الكالسيوم> الفوسفور> الماغنيسيوم. وتم تسجيل متوسط تراكيز للكالسيوم عند 4262 ± 2771ملجم/كجم، 5006 ± 896ملجم /كجم، 1276 ±319ملجم/كجم و1231 ± 154 ملجم/كجم، بينما كان متوسط

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تركيزات الماغنيسيوم 560.1 ± 82ملجم/كجم، 419.4 ± 153ملجم/كجم، 249.7 ± 7.1ملجم/كجم، 127.7 ± 21ملجم/كجم، في لكل من عينات الحليب المجفف والأطفال والمبخر والمعقم، على التوالي. كما تراوحت تراكيز عنصر الفوسفور بين 3322-7692ملجم/كجم، 2622-5490ملجم/كجم، 1381-4720ملجم/كجم و1049-2326ملجم/كجم في الحليب المجفف والأطفال والمبخر والمعقم، على التوالي. كما عُينت تراكيز بعض المعادن الأساسية الثانوية؛ ومنها النحاس والحديد والزنك، في جميع العينات التي تم جمعها. وسجل أعلى محتوى من النحاس في الحليب المجفف بمتوسط تركيز 2.13 ± 0.84ملجم/كجم، تلها منتجات حليب الأطفال بمتوسط تركيز 1.15 ± 0.95ملجم/كجم. وسجل متوسط تراكيز لكل من الزنك والحديد في الحليب المجفف عند 33.48 ±17.4ملجم/كجم و27.60ملجم/كجم على التوالي. وكانت تراكيز العناصر الأساسية الثانوية في عينات الحليب المبخر والمعقم أقل من 1 ملجم/كجم، ما عدا عنصر الزنك في الحليب المبخر، حيث تراوح تركيزه بين 2.80-5.77ملجم/كجم. كانت محتويات المعادن التي تم تحليلها في منتجات الحليب متوافقة مع بعض القيم المنشورة لنفس منتجات الحليب المصنعة في بلدان مختلفة.

Introduction

Minerals are inorganic elements that commonly found in foods and water. Those elements form the major part of dry ash that remains after ignition of organic matter of foodstuffs. These minerals account about 4% of total human body mass. Therefore, they are essential to human life. According to the quantity of human body needs, minerals are classified into two categories which include macro-minerals and trace-minerals. Macro-minerals, are also called major essential minerals, include sodium, calcium, chloride, magnesium, potassium, phosphorus, and sulfur. The major minerals consider as cellular and structural building nutritional materials which taking a part in osmotic and acid/base regulation. From the other point of view, trace minerals (or minor minerals**)** are needed in small quantities. These minerals included iron, copper, zinc, manganese, cobalt, molybdenum, selenium, iodine and fluoride. The trace minerals act as co-factors for many enzymes and play an important role in many physiological functions. The essential minerals are given from a variety of foods, but some foods are especially abundant in these important nutrients [1].

Milk is considered as a completed food, it contains about 250 different nutrients, which include easily digestible proteins, fats, carbohydrates, minerals and vitamins. Milk is an ideal source of both major and minor essential minerals such as; calcium (Ca), potassium (K), phosphorous (P) copper (Cu), iron (Fe), selenium (Se), and zinc (Zn). In milk, minerals occur in several chemical forms, including inorganic ions and salts, or as parts of organic molecules such as proteins, fats, carbohydrates and nucleic acids. The deficiency of essential minerals can cause disturbances in body metabolism and produce some pathological conditions. This fact is particularly true in early childhood, as the milk is the only source of nutrients, especially during the first months of a baby's life [2], [3].

Milk in its natural state is high susceptible to rapid chemical degradation and spoilage by natural enzymes and contaminating microorganisms. Therefore, milk is one of the important foods, that manufactured and packaged in different forms. Many processes have been developed over the years to enhance its utilization, safety and preserving milk for long period. These processes involved pasteurization, sterilization, evaporation, etc. Nowadays, milk processing companies convert and package the raw milk into various final milk products for consumption. Those products included powders, infant formula milk, sterilized, evaporated, drinkable liquid milk, etc. Powdered milk (or dried milk) is one of the most popular dairy manufactured milk due to its long shelf life. Powder milk is produced by evaporating milk to dryness using various mechanical dryers. While in Evaporated milk, only 60% of water content has removed from the fresh homogenized milk. Then the product is cooled, packaged, and sterilized. The raw milk can also be heated at 115-118ºC for a certain time and packed in plastic or glass bottle as sterilized milk products [4]. These milk products are widely consumed food products by Libyan families for all age groups. Also, these milk products are considered as ingredient in other various food products such as bakery, confectionary and other dairy products included ice cream, cheese, yogurt and infant formula. Many brands of infant formula are designed to provide babies with the nutrients they need to grow and develop. Some of infant formula milk are manufactured by adding the essential elements and nutrients to milk powder during processing [4].

The level of minerals in milk must comply with regulatory as well as nutritional and safety requirements. Therefore, reliable analytical methods are required for determining the mineral contents of processed milk products. A review described the main methods and related current official methods for determining minerals in milk products (especially infant formula), was prepared by Eric Pottevin [5]. Even more, the levels of major and minor minerals in various forms of milk were published by huge number of international studies [6-18]. The available literature data show that only one published article regards to the analysis of toxic minerals in processed milk samples collected from Benghazi city markets [19]. Therefore, the purpose of this work is to assess the levels of essential major elements (phosphorus, calcium, magnesium) and essential trace elements (copper, iron, zinc) in different products of processed milk, using spectroscopic methods. The collected milk products include milk powder, infant formula, evaporated, sterilized. These samples are commercially available in markets of Benghazi, the second biggest city in Libya, and extensively consumed by babies and adults in Libya. The obtained results of each element will be compared with the recent reference values of minor and major minerals in same types of processed milk, from different parts of the world.

Experimental

Samples Collection

A total of 78 samples of processed milk products were randomly collected from three different markets in Benghazi city, during 2017. The milk samples include four different types (milk powder, infant formula, evaporated milk and sterilized milk). The same milk products were thoroughly mixed to get homogenous and representative samples. Each sample was preserved in freezing till analyzed. Dry samples were stored in the genuine packaging.

The samples were given different code numbers such as 1-P to 7-P for seven samples of milk powder, 8-F to 13-F for six samples of infant formula, 14-E to 21-E for eight samples of evaporated milk and 22-S to 26-S for five samples of sterilized milk. Information about the selected samples, has recorded in Table (1).

Table 1: List of milk samples, their codes and countries of production

Milk Type	Code number*	Production Country
powdered	1-P	Egypt
	2-P	Denmark
	3-P	U.A.E
	4-P	Denmark
	5-P	Holland
	6-P	U.A.E
	7-P	France
Infant Formula	8-F	Germany
	9-F	Holland
	10-F	Holland

Milk Type	Code number*	Production Country
	11-F	Spain
	12-F	France
	13-F	France
Evaporated	14-E	Holland
	15-E	Germany
	16-E	Holland
	17-E	Germany
	18-E	Germany
	19-E	Holland
	20-E	Holland
	21-E	Germany
Sterilized	22-S	Libya
	23-S	Libya
	24-S	Egypt
	25-S	Saudi Arabia
	26-S	Germany

*each sample is a homogeneous mixture of 3 same products from 3 markets

Materials

The chemicals used in our work were purchased from qualified companies and were of analytical grade. These chemicals include; nitric acid (65%), was purchased from Dehaenage-Hannover, hydrogen peroxide (30%) was purchased from Eurostar Scientific LTD. Sodium hydroxide, potassium dihydrogen phosphate, sodium vandate and ammonium molybedate were obtained from Riedel-DeHane. The stock standards solutions (1000ppm) of the analysed metals and perchloric acid were supplied from BDH. These standards were used to prepare calibration solutions of each metal, for spectrophotometric measurements. In this work, the reagents and solutions were prepared using de-ionized water (18 MΩ/cm).

Procedure

Analysis of Phosphorous in Milk

For phosphorous determination in milk products, 2g of sterilized milk, 1g of evaporated milk, or 0.5g dry milk, were transferred to a clean pyrex flask and 10 ml nitric acid was added. The flask is heated for 20 min. After cooling the sample to room temperature, 5mL of perchloric acid is added, and the flask is heated vigorously till the white fumes appeared and the sample volume reduced to 2-3 ml. The content of flask was dissolved in 50mL water. Then, the solution is filtered with Whathman No.42 filter paper, 5ml of this solution is mixed with 5ml of 0.04048M ammonium molybedate tetrahydrate solution and 5ml of 0.01414M sodium vandate solution. The volume is completed to 25ml using de-ionized water. Then the absorbance of yellow colored complex is measured at 470nm using double-beam spectrophotometer (CECIL, Cambridge, England) [20].

Analysis of Minerals in Milk

The digestion process of milk products for minerals analysis is performed by weighting 10g of sterilized milk, 5g evaporated milk, or 0.5g of dried milk, into clean dry pyrex flask. 10mL of nitric acid and 3mL of hydrogen peroxide are added. The digestion flask is heated gently until frothing subsided. The heating of digestion flask is continuous till dryness. After cooling the flask to room temperature, the residue is dissolved in 50mL of deionized water and digested. Then, the solution is filtered using Whathman No.42 filter paper [21], [22]. The content of each mineral is determined by measuring the absorbance using Flame Atomic Absorption Spectrophotometer (FAAS) (Analytikjena, model novAA350, Jena, Germany), at the instrumental operating conditions for each element. The instrument is calibrated using standard solution of each element.

The contents of minerals in the digested sample solutions are determined from the calibration curves of each mineral, with considering the dilution factor and sample weight. All samples are analysed in triplicate. The average values are used for representing the contents of minerals in various milk samples. Blank solutions are prepared under identical conditions [22].

Method Validation

The reliability of FAAS method for analysis of calcium, magnesium, copper, iron and zinc were assessed by analyzing Standard Reference Milk Powder(A-11) obtained from "International Atomic Energy Agency". Limit of detection (LOD) and precision of the elements were calculated. The recovery assays were satisfactory, ranging from 98 to 100%.

Statistical analysis

The calculation of statistical values of basic variation (arithmetic mean, standard deviation, maximum and minimum values) were carried out using Statistical Package for Social Analysis (SPSS version 19.0, IBM, Chicago, III., USA) Program. The analysis of data was performed using one-way analysis of variance (ANOVA) to examine the statistical significance at 95% (P<0.05) confidence level. Least Significant Difference (LSD) was used to estimate the significance of difference between the analyzed samples.

Results

This work was carried out to estimate the concentrations of some essential major elements and essential trace elements in different types of processed milk, gathered from different markets in Benghazi city, during 2017. All processed milk samples were undergone a digestion process and analyzed by spectroscopic methods [23-25], for detection of phosphorus, calcium, magnesium, copper, iron and zinc. The contents of these elements in examined milk samples were recorded in Table (2) and Table (3).

Table 2: Results of some major essential elements (mg/kg) in various processed milk products gathered from Benghazi Markets

Milk Type	NO.	Phosphorus		Calcium		Magnesium	
		Mean ± SD	Range	Mean ±SD	Range	Mean ±SD	Range
powder	7	5979 ±1609	3322 -7692	4262 ±2771	1050-8500	560.1 ±82	452.7-681.7
Infant formula	6	4184 ±1058	2622 -5490	5006 ±896	3809-6449	419.4 ±153	245.1-623.9
Evaporated	8	2640 ±1043	1381 -4720	1276 ±319	822.7-1589	249.7 ±7.1	237.5 -257.5
Sterilized	5	1484 ±583	1049-2326	1231 ±154	1048-1455	127.7 ±21	108.2-164.4

Table 3: Results of some minor essential minerals (mg/kg) in various processed milk products gathered from Benghazi Markets

Milk Type	Copper		Iron		Zinc	
	Mean ± SD	Range	Mean ±SD	Range	Mean ± SD	Range
Milk powder	2.13 ±0.84	0.93-3.30	27.60*	0.75-97.49	33.48 ±17.4	16.46-66.05
Infant milk	1.15 ±0.95	0.21-2.45	62.25 ±10.2	51.23-79.48	51.16 ±8.0	35.02-55.88
Evaporated milk	0.19 ±0.09	0.02-0.34	0.80 ±0.48	0.10-1.43	3.97 ±1.0	2.80-5.77
Sterilized milk	0.15 ±0.05	0.06-0.20	0.50 ±0.12	0.34-0.66	0.77 ±0.3	0.42-1.15

*The concentrations of iron in powdered milk samples have a very great variation in concentrations

As presented in Table (2), the mean value of phosphorus concentrations in powdered milk products was 5979 ±1609mg/kg, these results were in the range between 3322-7692mg/kg. In the powdered milk samples the calcium and magnesium were detected at mean concentrations 4262 ±2771mg/kg and 560.1 ±82mg/kg, respectively. The essential trace elements, included copper, iron and zinc, were also detected in the selected milk powder products. In these samples the concentrations of copper ranged from 0.93 to 3.30mg/kg, Table (3). The Results recorded in Table (3) show that the mean value of iron concentrations in milk powder products was 27.60mg/kg, with a minimum concentration value of 0.75mg/kg and a maximum concentration value of 97.49mg/kg. While zinc levels in milk powder products were distanced from 16.46 to 66.05mg/kg with a mean value of 33.48 ±17.4mg/kg.

Phosphorus element was found in all infant formula products with a mean value corresponding to 4184 ±1058mg/kg, Table (2). While the calcium concentrations in gathered infant formula were varied from 3809 to 6449mg/kg with a mean value corresponding to 5006 ±895mg/kg. The magnesium concentrations ranged between 245.1-623.9mg/kg, while the average value of magnesium concentrations was detected at 419.4 ±153mg/kg. In examined infant formula samples, the concentrations of copper ranged from 0.21 to 2.45mg/kg, and iron concentrations varied between 51.23 to 79.47mg/kg with a mean value 62.25 ±10.25mg/kg, Table (3). While, zinc in infant formula products detected at 51.16±8.0 mg/kg, and ranged between 35.02-55.88mg/kg, Table (3).

The evaporated milk products under investigation, contained phosphorous element in the range between 1381 and 4720 mg/kg, Table (2). While the levels of calcium and magnesium metals ranged between 822.7-1589 mg/kg and 237.5 to 257.5 mg/kg, respectively. The results recorded in Table (3), revealed that the concentrations of copper in evaporated milk samples varied from 0.02 to 0.34mg/kg, with a mean value of 0.19 ±0.09mg/kg. While, the concentrations varied between 0.10 to 1.43 mg/kg for iron and 2.80mg/kg to 5.77mg/kg for zinc.

As illustrated in Table (2), the concentrations of major essential minerals in sterilized milk products ranged between 1049-2326mg/kg for phosphorous, with a mean value of 1484 ±584mg/kg. While the concentrations ranged between 1048-1455mg/kg for calcium and 108.2-164.4 mg/kg for magnesium. On another hand, the contents of copper, iron and zinc in five products of sterilized milk, were recorded in Table 3. The mean value of copper and iron concentrations recorded at 0.15 ±0.05mg/kg and 0.50 ±0.12mg/kg, respectively. While, the concentration of zinc in sterilized milk samples varied from 0.42 to 1.15mg/kg, with a mean value of 0.77 ±0.33mg/kg.

Discussion

In the present work, we were focused on the estimation of six of essential minerals in four different types of processed milk products that frequently purchased from Benghazi markets, by local inhabitants. The concentrations of phosphorous, calcium, magnesium, copper, iron and zinc were estimated by measuring the absorbance of all samples using spectrophotometer for phosphorous and FAAS for the other investigated minerals. In milk, these minerals form complexes with organic matter such as proteins, peptides, carbohydrates and fats [23]. The organic matters and other ingredients in the samples would interfere with the analytical process unless they were removed. Therefore, all samples undergo destructive processes, using some oxidizing acids, before instrumental analysis [20-22].

Among the major minerals, calcium metal has the highest level followed by phosphorous and magnesium in all milk samples. In fact, milk and milk products are very rich source of calcium, which is responsible for many regulatory functions in human body [3]. The highest content of calcium was found in infant formula (3809 - 6449mg/kg) and milk powder (1050 - 8500mg/kg). The statistical analysis indicated that there is no significant different between infant formula and milk powder samples in the contents of calcium (P=0.340). However, the mean value of calcium concentrations in infant formula were at lower levels than the values reported for calcium in infant formula products analysed by Decastro *et al*. [7]. While, calcium levels in infant formula products gathered in this work were similar to the values of calcium in infant formula analysed by Alkalifa and Ahmad [9] and Joseph *et al*. [10]. In our work, the powdered milk products contained levels of calcium higher than the concentrations of calcium published for the milk powder products available in Nigeria [15] and Pakistan [18] markets.

On the other point of view, the least calcium content was recorded in sterilized milk samples (1231 ±154mg/kg) and evaporated milk (1276 ±319 mg/kg). There is no statistically significant difference in calcium contents between sterilized and evaporated milk products (P=0.955). The concentrations of calcium in the analyzed sterilized milk were higher than the level of calcium in sterilized milk products gathered from Brazilian markets [8].

Phosphorus is an essential nutrient for human being and animals' life. It is fundamental to growth, maintenance, and repair of all body tissues. Phosphorous occurs as organic and inorganic phosphate in all body tissue and fluid. Calcium phosphate compound is the main structure component of teeth and bones [3]. In the present work, the mean value of phosphorous element concentrations in analysed samples are in order: powder > infant formula> evaporated >sterilized milk. The obtained mean value of phosphorous concentrations was in agreement with the concentration recorded by Birghila *et al*. in powder and infant formula milk products [24]. However, in literatures, there is no data available to compare the concentrations of phosphorous in evaporated and sterilized milk samples.

Magnesium is essential in the synthesis of nucleic acids and proteins. Magnesium is an important cofactor for great numbers of enzymes and transporters. Also, magnesium has important effects on the cardiovascular system. Intracellular magnesium forms a key complex with adenosine triphosphate and has a main role in many other essential biological processes such as protein synthesis, cell replication, and energy metabolism [3]. In our study, magnesium was found in all processed milk samples. The contents of magnesium metal in the investigated milk products were statistically different. Although, the mean values of magnesium concentrations in sterilized and powdered milk samples were higher than the quantities of magnesium reported by Soares *et al*. [8] and Rehman and Tanveer [18]. In contrast, our results were lower than the quantities of magnesium detected in infant formula products gathered from Saudi Arabian [9] and Nigerian markets [10].

Although, milk and milk products are poor source of iron [3], it was observed that Infant formula and milk powder products have high quantities of iron. Vella and Attard measured the quantities of iron and zinc in infant formula samples from Maltese markets [17]. They found the mean levels of iron was 18.34 mg/kg, which were significantly low as compare to the levels of iron in the current study. In fact, the high quantities of iron in dried milk products were made by enrichment of milk powder with this metal, which is a common practiced of most companies. Even more, in the infant formula, the manufacture`s fortification of essential elements, especially iron, zinc and copper, resulted in concentrations many times higher than those in foods [12]. On the other point of view, high quantities of those minerals were also detected in infant formula and milk powder products. Our data were significantly higher than the zinc concentrations recorded in infant formula product gathered from Maltese markets [17].

Copper is, a minor mineral, important for the absorption of iron and as cofactor of different enzymes [16]. The highest copper content is recorded in powder milk samples, with a mean concentration 2.13 ±0.82mg/kg, followed by infant formula products with mean concentration 1.15±0.95mg/kg. The high copper contents were also observed in infant formula and milk powder products collected from Egypt and Malta [16], [17]. Whereas, the mean value of copper metal concentrations in the analyzed powder milk samples was exceeded the copper quantities reported from Palestine [12], Saudi Arabia [14] and Nigeria [15].

The quantities of minor essential minerals in evaporated and sterilized milk samples were in levels lower than 1mg/kg, except zinc in evaporated milk. Our results exceeded the levels of zinc in evaporated milk samples from Nigeria [6] and Saudi Arabia [14]. However, no significant difference was observed regarding the concentrations of copper (*P=0.918)*, iron (*P=0.977)* and zinc (*P=0.536)* between the samples of evaporated and sterilized milk products. The quantities of iron and copper in evaporated milk were similar to the samples of evaporated milk in Saudi Arabian markets in 2013 [14]. The mean values of copper, iron and zinc concentrations in sterilized milk were at lower levels than the values reported for these minerals in the same products samples analysed by Soares *et al*. [8] and Abdulkaliq [12]. However, copper and iron are essential minerals, but in excess, these minerals are able to produce free oxidant radicals [25].

Conclusion

The current work provided some information about the mineral contents in commonly purchased processed milk products, from Benghazi`s markets. The selected samples include; powdered milk, infant formula, evaporated milk and sterilized milk. Related to the results of the current study, the analysed samples contained appreciable amount of major essential elements (calcium, magnesium and phosphorous) and minor essential elements (iron, copper and zinc). Most of the obtained results agreed with recent reported studies which dealing with the mineral quantities in various different processed milk.

In literature, a rare data is available on phosphorous analysis in processed milk samples. However, it was also noted that high levels of zinc and iron found in dried milk, due to the minerals forting process that performed by the manufactured companies.

The mineral content of milk is very important for health of human. Therefore, future studies should include the estimation of minerals quantities in other processed milk products available in Benghazi`s markets. Also, the quantities of other nutritive minerals should be evaluated.

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