Turkish Journal of Physiotherapy and Rehabilitation; 32(3)
ISSN 2651-4451 | e-ISSN 2651-446X

Estimation of Microbial Content and Concentration of some Chemical Contaminants in Samples of Imported UHT Milk in Iraqi Local Markets

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Abstract: The study was conducted with the aim of estimating the chemical and microbial contamination in samples of UHT milk in the local Iraqi markets, by estimating the microbial content and estimating the aflatoxin m1, melamine and heavy metals in four samples of UHT milk from different origins. It was found that the total count of bacteria is 0 - 1.46 log cfu/ml and spore-forming bacteria from 0 - 1.36 log cfu While the presence of yeasts and molds, coliform bacteria and Psychrophilic bacteria were not detected, the concentration of melamine was between 0.29 - 15.01 mg / L and the concentration of aflatoxin M1 between 11.5 - 105.7 ng / L, and the concentration of nickel, chromium and zinc was between 1.42 - 34.03, 4.66 - 64.29 and 0.18 - 0.55 mg/L each, respectively, while all samples were free of lead, cadmium, mercury and cobalt.

Keywords: Heat Treated Milk, UHT Milk, Melamine, Aflatoxin M1, Heavy Metal.

1. Introduction

Milk is a widely distributed multi-ingredient nutrient-rich natural food that is essential in the diet of a large number of people all over the world as it provides energy, macro and micro nutrients to the body (Ritota et al., 2017).

Milk is a suitable medium for the growth and activity of microorganisms due to its high-water content, pH close to 7, and appropriate chemical composition, Raw milk contains different types of microorganisms with different characteristics and characteristics. Bacteria are the predominant type of microorganisms in milk, including pathogenic and spoilage bacteria, which may be Psychrophilic, Thermophiles, or spore-forming bacteria (Moatsou and Moschopoulou, 2015).

In order to ensure the microbial safety of the milk and to prolong the storage period, the milk is subjected to heat treatment, and the milk is used by Ultra-high temperature of 135 - 145 °C for several seconds and then cooling directly to reduce thermal damage and prevent the growth of heat-resistant spore-forming bacteria remaining in the milk after heat treatment, thus obtaining a sterile product Commercially sterilized (Liao et al., 2018).

The Ultra-high temperature treatment process aims to destroy all microorganisms including spore-forming bacteria that grow under normal storage conditions, however the total count of bacteria in UHT milk should not exceed 10 log cfu/ml when milk was stored for 15 days at 30°C (Kelly et al., 2012).

Contaminants are substances that are not supposed to exist naturally in the food that reaches the consumer, and contamination occurs in food due to microbial or environmental pollution, or as a result of the wrong use of food additives, or because of the interaction of the components of the packaging with food, or the transfer of some components of the packaging to the food, and the residues of pesticides and antibiotics in Food is a pollutant (Al-Jubouri, 2015).

The problem of adulteration and contamination of milk became a global problem after the discovery of contamination of infant formula in China in 2008 with melamine. Milk is a food item that can be easily adulterated. One of the possible causes of milk adulteration is the high susceptibility to milk spoilage, the need to increase the storage period, the low purchasing power of consumers, and the lack of appropriate detection tests (Kamthania et al., 2014). The main motive for milk adulteration in general is economic, but the effects resulting from adulteration represent a source of great danger to public health, especially in developing countries, due to the lack of adequate food control. It is possible to qualitatively detect milk adulteration using chemical methods, but quantitative detection is more complex and diverse (Singh and Gandhi, 2015).

Milk and dairy products are the main sources of AFM1, as this toxin is excreted with milk after animals consume feed contaminated with AFB1 toxin, AFM1 is a metabolite formed in animal livers after hydroxyl group addition to AFB1 toxin, transported to the mammary glands and excreted with milk (Giovati et al., 2015).

Aflatoxin appears in milk 12 - 48 hours after animals consume contaminated feed, and aflatoxin excretion in milk stops 24 - 72 hours after the contaminated feed is removed from the animal's diet (Herrera et al., 2014).

Melamine 2,4,6-triamino-1,3,5-triazine is a polar organic compound with molecular formula $C_3H_6N_6$, molecular weight 126 g/mol Rich in nitrogen, widely used in industry, containing a large amount Of nitrogen, 66.6% of its mass, and therefore a source of adulteration of milk and dairy products to obtain high readings of protein ratios, and the use of Kjeldahl method for the determination of total nitrogen without specifying the sources of nitrogen in the sample and does not show the true protein content and therefore it is not possible to distinguish melamine from protein substances other (Guo et al., 2014: Wu et al., 2016).

2. Materials and Methods

Sample Collection

Samples of UHT milk imported from the local markets in Iraq were collected from four different origins (Saudi, Kuwaiti, Syrian, and Iranian) and kept at room temperature until analysis.

Preparation of Samples for Microbial Analysis

The samples were mixed before the start of the microbial analysis by stirring the package 25 times quietly to avoid foam formation, and left for a period not exceeding 3 minutes of mixing, then 10 ml was withdrawn through a sterile pipette from each sample and mixed with 90 ml of the previously prepared sterile peptone solution and mixed with light stirring for 10 seconds and thus The first dilution was completed and the rest of the dilutions were prepared by transferring 1 ml of the first dilution into test tubes containing 9 ml of peptone solution to reach the appropriate dilution for each sample (Frank and Yousef, 2004).

Microbiological Analysis

Estimate the total count of bacteria in the samples using Plate Count Agar medium (ISO, 2014). The number of molds and yeasts was estimated by using Potato Dextrose Agar medium (Frank and Yousef, 2004). The number of spore-forming bacteria was estimated by heating the samples in a water bath to 80 °C for 12 minutes, then directly cooled to 10 °C, cultured by casting method using Nutrient Agar medium (Ranieri et al., 2009). Estimate the number of Psychrophilic bacteria using Nutrient Agar medium (Frank and Yousef, 2004). The number of coliform bacteria in the samples was estimated by using MacConkey Agar medium (Frank and Yousef, 2004).

Determination the Concentration of Aflatoxin M1

The concentration of aflatoxin M1 in milk samples was estimated using an analysis kit supplied by the Chinese company (Shenzhen Lvshiyuan Biotechnology), following the instructions supplied by the company, and using an ELISA device at a wavelength of 450 nm.

Determination of Melamine Concentration

The concentration of melamine was estimated using a melamine determination kit (Shenzhen Lvshiyuan Biotechnology) supplied by the Chinese company (Shenzhen Lvshiyuan Biotechnology), following the instructions supplied by the company, and using an ELISA device at a wavelength of 450 nm.

Determination of the Concentration of Heavy Metals

Heavy metals in milk samples were estimated using wet incineration method according to Nielsen (2017), which included 3-4 g weight in a digestion tube, adding 5 ml of nitric acid and 4 ml of 30% hydrogen peroxide. The sample was digested using a Keldal digester at 175 °C and left to cool for 30 minutes, the sample was diluted with 25 ml of deionized distilled water and then the elements were quantified using an atomic absorption device.

Statistical Analysis

The results of the experiments were analyzed using the Linear Model General within the ready-made statistical program (SAS, 2015) to study the effect of factors on the complete random design (CRD). Also, The

Duncan test was conducted to determine the significance of the differences between the means of the factors affecting the studied traits at the level ≤ (0.05).

3. Results and Discussion

Microbial Quality of UHT Milk

The results of the microbial analysis in the samples of UHT milk shown in Table (1) showed that only the sample S3 contained microorganisms and the total count of bacteria was 1.46 log cfu/ml, and the number of spore-forming bacteria in it was 1.36 log cfu/ml, while all samples were free of coliform bacteria, Psychrophilic bacteria and molds and yeasts.

The results are in agreement with Deeth and Lewis (2017) who stated that samples of UHT milk should have a total number of microorganisms not to exceed 10. log cfu/ml /ml except for Form S3. Also, the results are in agreement with what was mentioned by Ansari and Sahoo (2018) and Hamad et al. (2017), who indicated that the UHT milk samples did not show any microbial growth, and this is due to the efficiency of the heat treatment used and the high temperatures used. As well as the absence of microbial contamination after heat treatments. As for the S3 samples, the presence of microbial growth in it is attributed to the inefficiency of heat treatment or the occurrence of contamination after heat treatment during various processes, and this is in agreement with what was mentioned by Nascimento et al. (2020) who mentioned that the number of bacteria in UHT milk did not exceed 15 log cfu/ml.

Burgess et al. (2017) mentioned the presence of many bacterial genus in raw milk that can resist high temperatures, as it was possible to isolate different types of UHT milk. The process of contamination constitutes after treatment, another source of spore-forming bacteria is due to poor cleaning and sanitation or the use of insufficiently sterile containers.

The Concentration of Melamine and Aflatoxin M1

The results of estimating the concentration of melamine and AFM1 in UHT milk samples shown in Table (2) showed that the melamine concentration in UHT milk samples ranged between 0.29 - 15.01 mg/L, while the concentration of AFM1 in UHT milk samples was between 11.5 - 105.7 ng/L.

The results of melamine are in agreement with Al-Jubouri (2015) who found that the concentration of melamine in UHT milk is between 4.4 - 33 mg / liter, and agree with what was mentioned by Alawi and Thalj (2013), who stated that the concentration of melamine in milk is between 5.9 - 17.5, and the results were less from what Deabes and El-Habib (2012) found, the concentration of melamine in milk was between 29.1 - 39.7 mg/kg.

Table 1. Microbial quality of UHT milk

	Samples	Total count of bacteria	Molds and yeasts	Psychrophilic bacteria	spore-forming bacteria	coliform bacteria
S1	Saudi UHT Milk	ND^{Bb}	ND^{Aa}	ND^{Aa}	ND^{Bb}	ND^{Aa}
S2	Kuwaiti UHT Milk	ND^{Bb}	ND^{Aa}	ND^{Aa}	ND^{Bb}	ND^{Aa}
S3	Syrian UHT Milk	1.46^{Da} 0.03 ±	ND^{Aa}	ND^{Aa}	1.36^{Ca} 0.03 ±	ND^{Aa}
S4	Iranian UHT Milk	ND^{Bb}	ND^{Aa}	ND^{Aa}	ND^{Bb}	ND^{Aa}

- The numbers in the table are in duplicate.
- The different letters in the same column indicate significant differences at the level (P≤0.05).
- ND: not detected

The presence of melamine in milk is due to the use of this substance to raise the nitrogen content of milk. It is also added to animal feed to raise nitrogen levels in it, which explains its presence in milk even when it is not added directly to milk. Allowing the use of melamine in the manufacture of food packaging materials allows by transferring proportions of it to food (Amaral and Mafra, 2018). Wen et al. (2016) stated that the World Health Organization (WHO) stated that the permissible daily limits of melamine are 0.2 mg per kg of body weight, as defined by standards in the United States and European Union countries The concentration of melamine in food should not exceed 2.5 mg/kg as a maximum. Thus, it was found that 3 samples exceeded the maximum permissible limits in milk.

The results of AFM1 are in agreement with Najim and Jasim (2014) who reported that the concentration of AFM1 in raw milk samples was between 15 - 86.96 ng/L, and agreed with what was reported by Al-Mossawei et al (2016) who found that the concentration of AFM1 in UHT samples was Milk was between 1.6 - 251.57 ng/L, and it agreed with Daou et al. (2020) who reported that the AFM1 concentration in UHT milk samples were between 13 - 219 ng/L.

Due to the seriousness of AFM1, most countries of the world and food organizations have set limits for mycotoxins allowed in food, and in general, the permissible concentration is between 50 - 500 ng / L in milk and dairy products (Matabaro et al., 2017), and thus the aflatoxin concentration in all samples did not exceed the maximum allowed in all the samples under study.

The presence of AFM1 in milk is due to the use of feed contaminated with AFB1 in the feeding of milk-producing cows, as AFM1 is the product of AFB1 metabolism in cows and is excreted with milk. The advantage of AFM1 is that it is stable in milk and does not break even when treated with high temperatures, and this explains its presence in UHT milk (Turkoglu and Keyvan, 2019).

Several studies have indicated contamination of feed with aflatoxin, which are natural contaminants of agricultural commodities, and various studies have indicated contamination of raw milk with different percentages of AFM1 and it may sometimes exceed the permissible percentages in the standards (Permingo et al., 2016).

Table 2. Furosine concentration in laboratory and imported milk samples

Samples	Heat treatment type	Melamine mg/L	AFM1 ng/L
S1	Saudi UHT Milk	0.29^{e} 0.02 ±	11.5^{e} 0.04 ±
S2	Kuwaiti UHT Milk	8.97^{b} 0.04 ±	45.2^{d} 0.01 ±
S3	Syrian UHT Milk	7.05^{c} 0.04 ±	105.7^{a} 0.03 ±
S4	Iranian UHT Milk	15.01^{a} 0.04 ±	61.3^{c} 0.04 ±

- The numbers in the table are in duplicate.
- The different letters in the same column indicate significant differences at the level ($P \leq 0.05$).

Heavy Metal Concentration

The results of estimation of heavy metals in the heat-treated milk samples shown in Table (3) showed that the concentration of nickel in the UHT milk samples ranged between 1.42 - 34.03 mg/L, and the concentration of chromium in the samples of UHT milk ranged between 4.66 - 64.29 mg/L, it was found that the concentration of zinc in UHT milk samples ranged between 0.18 - 0.55 mg/L, while all samples were free of lead, cadmium, silver and cobalt.

The results agree with Soltan et al. (2017) that the raw and heat-treated milk models are free of cadmium, lead, cobalt and mercury elements. Elsherif et al. (2017) found that the chromium concentration in the milk models ranged between 0.1 - 5.77 mg / L and the zinc concentration between 0.12 - 216.95 mg / L. Also, Ahmad et al. (2017) reported that the concentration of nickel is between 0.13 - 1.15 mg / L and the zinc concentration is between 3.11 - 5.15 mg / L, and Anetta et al. (2021) found that the concentration of nickel in heat-treated milk samples is between 0.53 - 1.37 mg / L.

There are different and diverse elements in milk, and the concentration of these elements varies greatly. This difference is attributed to the production and packaging processes that milk is exposed to, as well as the environmental pollution of soil, water and fodder with heavy elements, which is transmitted to cows and from them to milk (Ziarati et al., 2018). Pérez-Carrera et al. (2016) mentioned that the pollution of plants and water greatly affects the concentration of heavy elements in milk. When studying the concentration of heavy elements in milk from different regions, the concentration of heavy elements in milk varied significantly and was directly proportional to the degree of water pollution and that the highest the proportions were in the milk produced on farms using deep well water.

Akhtar et al., (2015) mentioned that the concentration of heavy metals in heat-treated milk depends mainly on their concentration in raw milk and is not greatly affected by heat treatments. It was also found that the concentration of heavy metals in milk produced in polluted industrial areas is much higher than in less polluted areas. Therefore, the difference in the concentration of heavy metals in the milk samples under study and the group from different sources is due, as it was found that the concentration of nickel was higher than the permissible limits, which amounted to 0.43 mg/L, except for the samples prepared using raw milk.

As for chromium, it was found that its concentration in all samples was higher than the permissible limits, which amounted to 1.61 mg / L, and it was found that the concentration of zinc in the samples was within the permissible limits (Hasan et al., 2020).

Table 3. Microbial quality of UHT milk

	Samples	Total count of bacteria	Molds and yeasts	Psychrophilic bacteria	spore-forming bacteria	coliform bacteria
S1	Saudi UHT Milk	ND^{Bb}	ND^{Aa}	ND^{Aa}	ND^{Bb}	ND^{Aa}
S2	Kuwaiti UHT Milk	ND^{Bb}	ND^{Aa}	ND^{Aa}	ND^{Bb}	ND^{Aa}
S3	Syrian UHT Milk	1.46^{Da} 0.03 ±	ND^{Aa}	ND^{Aa}	1.36^{Ca} 0.03 ±	ND^{Aa}
S4	Iranian UHT Milk	ND^{Bb}	ND^{Aa}	ND^{Aa}	ND^{Bb}	ND^{Aa}

- The numbers in the table are in duplicate.
- The different letters in the same column indicate significant differences at the level (P≤0.05).
- ND: not detected

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