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Determination of fungi and some heavy metals in locally cheeses

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Abstract

In this study four cheese samples were randomly collected from local markets. These cheese samples were Ishaqi, Danone, Arab white cheese and Agricultural college cheese.

Results obtained revealed that all these cheese samples were contaminated by fungi in addition to the presence of same heavy metals under study which were Fe, Pb, Ni and Cr. All fungal contaminat were identified which were contaminated them in winter (January). However, fungal pollution in summer was 100% while in winter was 50% *Aspergillus niger* was polluted chees samples100% in summer while it was 75% in winter. *Aspergillus fumigatus* was polled cheese samples under study 50% in summer and 0% in winter .Results for heavy metals determination revealed that Fe was contaminated cheese samples followed by Ni, Pb and finally Cr and the percentage of their presence was (0.24, 0.006, 0.006, 0.001 ppm) respectively .

Keywords: Contamination, Pollution, Heavy metals Determination, Cheeses, infection

التحري عن الملوثات الفطرية وبعض العناصر الثقيلة في الاجبان المحلية

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الخلاصة

في هذه الدراسة تم جمع اربع نماذج من الاجبان المحلية وهي جبنة الاسحاقي ، جبنة دانون، جبن العرب الابيض وجبن كلية الزراعة. تم فحص هذه الاجبان من حيث تلوثها بالفطريات اضافة الى تلوثها بالعناصر الثقيلة المقترحة في هذه الدراسة وهي Cr, Pb, Ni, Fe.

اوضحت النتائج ان جميع الاجبان قيد الدراسة كانت ملوثة بالفطريات . تم تصنيف جميع الفطريات الملوثة لهذه الاجبان وتوضح ان التلوث يبهذه الفطريات كان خلال فصل الصيف (تموز) اعلى درجة من نسبة التلوث في فصل الشتاء (كانون الثاني) حيث ان التلوث بالفطريات كان 100% في فصل الصيف في حين كان 50% في فصل الشتاء. فطر *Aspergillus niger* كان ضمن الفطريات الملوثة للاجبان وبنسبة 100% في فصل الصيف و 75% في فصل الشتاء. في حين كان الفطر *Aspergillus fumigatus* يمثل 50% في فصل الصيف و 0% في فصل الشتاء. بالنسبة للعناصر الثقيلة التي رصدت في الاجبان قيد الدراسة اوضحت النتائج ان تركيز الحديد Fe كان يمثل اعلى نسبة تلوث مقارنة بالعناصر الاخرى وهي Ni، Pb واخيراً Cr وكانت ونسبها (0.24, 0.006, 0.006, 0.001) التوالي .

Introduction

Cheese is an important product that consumed in the word. About a third of the world's milk production is used in cheese making; it is highly valuable in nutritious product [1]. The origin of cheese is old as the human species and that is related with the taming of animals (10,000 B.C.).

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Cheese, like many other dairy products, provides vitamins, saturated and unsaturated fat, protein, minerals, and cholesterol. Also cheese is one of the valuable sources of calcium [2], and often it is expected to be healthy and stable food stuff. The world cheese production has increased by millions of tons since the beginning of this decade and in European Union an increasing fraction of all the milk produced used for cheese making [3]. Recently, milk prices on the world market have increased drastically due to a limited milk supply. More than ever, it would thus be an economic advantage if the cheese yield could be increased. The most important of all milk components for the manufacture of cheese and other fermented dairy products are the milk proteins [4]. It is major constituent of the diet, its quality assurance considered essential to the welfare of a community. In the last few years, the contamination of milk is considered as one of the main dangerous aspects [5].

Fungi are one of the biggest competitors for our food supply. We are constantly struggling to prevent food from spoiling. Spoilage is the result of the action of fungi and other microorganisms [6,7]. Milk and milk products are the most diversified of the natural foodstuffs in terms of composition, contains more than twenty different trace elements [8]. However is possible that not only valuable elements can appear in compose item, of food commodities.

Contamination of dairy products with such elements as Cr Fe, Ni, are essential for human body only in tiny amounts but could become harmful if quantities rise up to standard limits [9].

Cheese ripening is a complex and dynamic biochemical process that includes protein breakdown, fat hydrolysis and lactose metabolism. These processes are catalyzed by agents such as residual coagulant, indigenous milk enzymes, starter or nonstarter microflora and secondary organisms. The secondary organisms include moulds and presence of moulds on the surface of mould-ripened cheese gives them a different appearance and flavor from other cheeses. The moulds have more complex enzymatic systems than bacteria and their enzymes contribute in cheese maturing, i.e., to proteolysis and lipolysis which are more extensive in these cheeses. [10].

Fungi are significant spoilage microorganisms of food stuffs during the storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Fungal growth on cheese is a common problem for the cheese manufacture during ripening and curing as well as for the retailer and consumer during refrigeration storage. Species of *Penicillium* and *Aspergillus* are common contaminants of cheese [11].By the searching in the medical references , it was observed that, most of this fungi had the ability to human and animal pathogenicity or produced toxins [12,13]. The growth of toxigenic fungi during ripening of Ras cheese must be considered as a problem of safety for human consumption. During the ripening of ras cheese, non-toxigenic strains of fungi should be avoid, moreover, fungi growth on the cheese surface causes economic losses and quality problems. The isolated fungi will tested for their mycotoxin production, selected and will used as an inoculum in an attempt to control ras cheese contamination during storage period. Studies have also shown that adventitious Fungi contaminant milk and cheese to contribute the ripening of specialty cheeses [14-16].

Milk and its products already historically are known as valuable human daily nutrition components, in particular as source of amino acids, vitamins and minerals. Calcium and phosphorus are the main major elements that make dairy products irreplaceable for growing organisms of children in process of bone and teeth formation. Other essential elements (e.g., F, Fe, K, Mg, Na, S, Si) also can be found in milk products and are coupled in such chemical formations that could be easily absorbed by human body [17-19].Heavy metals are persistent contaminants in the environment that can cause serious environmental and health hazards. They are released into the environment from natural as well as man-made activities. Some heavy metals (like Cu and Fe) are essential to maintain proper metabolic activity in living organisms; others (like Pb and Cd) are non-essential and have no biological role [20,21]. However, at high concentrations, they can cause toxicity to living organisms [22].

The main sources of heavy metals to milk and its products are the cow that grazed near motor ways, roads with heavy car traffic, industrial pollutants including: coal or oil combustion, sewage effluents, over- use of pesticides, using some types of plastics and/or phosphate fertilizers and sludge used in agricultural land [23]. With respect to human health impacts, such as Cadmium and Lead, Cadmium and Lead are of primary concern because of their toxicity for human, while Copper is the metal of secondary concern [24].

Materials and Methods

Samples collection and identification:

Four locally produced soft cheese samples were collected randomly during period in January and July (2015). All soft cheese samples were transported to the laboratory in ice-cooled box. Microbiological analysis was performed on arrival of samples to determine the average fungi with special emphasis on the isolation and identification of isolates through their biochemical characteristics and other mycological analysis [25].

Preparation of media for growth of fungi

Potato dextrose agar (PDA) and Modified Dixons agar (mDA) were two of the solid culture media selected for the growth and characterization of Fungi. After the media was prepared, Fungi were activated in media and the contents were thoroughly mixed and pour in sterile petri dishes.

Determination of heavy metals concentration in cheeses

Determination of heavy metals in cheeses were carried out according to [26]. Samples of cheeses were rated with 15% nitric acid and rinsed with distilled water then kept in the oven at 110 ℃ till needed. 0.5gm of each cheese samples was taken in 50mL Pyrex digestion flak and digested with 5mL of concentrated nitric acid and the content of flask was heated on electric hot plate at 80℃, for 2–3 h, till the clear transparent digest was obtained. Then, the excess acid was evaporated to semi dried mass on a heating plate. After cooling at room temperature, the final solution was diluted to 25mL with 0.2mol/L nitric acid and filtered through 0.45mm Whattman filter paper in polyethylene flask for end determination. Quantitative determination of heavy metals were conducted by using atomic absorption spectroscopy.

Statistical Analysis

Data were analyzed using the Statistical Analysis System [27] and the significant differences were determined at ($p< 0.05$) .The statistical analysis of the data was performed by F-test analysis (ANOVA) and then the least significant differences (LSD) were conducted to find the significant differences among the different mean values.

Results and Discussion

Fungal Contamination

All the cheese samples which were collected in this study were contaminated with Fungi Table-1. These contaminants were identified and listed in Table-2. The results revealed that fungal contaminants were appeared in summer (July) in all cheese samples and winter (January). These cheese samples could be contaminated due to the way of dealing in cheese manufacturing which were not suitable or could be contaminated during the transportation and stringing particularly in Baghdad, which has shortages in electricity power. However, contamination might be get in to the animals, cheese samples from external surfaces of animals, cheese equipments or from personal if adequate hyaline practices are not observed.

Table 1- fungal presence in cheese sample used in this study

Cheese Name	**Fungi**	
Name	January	July
Ishaqi	+	+
Danone	Nil	+
Arab white	+	+
Agricultural college	Nil	+

Table 2- Fungal whith identified as cheese contaminants

Cheese Name	Fungi	
	Jan.	July
Ishaqi	*Aspergillus niger*	*Aspergillus niger* *A. wentii*
Danone	Nile	*Aspergillus wentii* *A.fumigatus*
Arab whte	*Aspergillus niger* *A.fumigatus*	*Aspergillus fumigatus* *A niger* *Penicillium brevicompactum*
Agricultural college	Nil	*A. Niger* *A. fumigatus*

The contamination proved to be official as an indicator of good and poor sanitation techniques in cheese production.

In this results Fungal contaminants were represent 50% in cheese samples in winter while they were 100% in summer.

Aspergillus niger contaminated 75% of cheese samples in winter while they were contaminated by this fungus 100% in summer. However, *Aspergillus fumigatus* contaminated 50% of cheese samples in summer while it was 0% in winter.

Statistical analysis showed a significant value ($p \leq 0.05$) between the contaminants of fungi samples in both summer and winter Table-3. The percentage of contamination was more in summer comparing with winter which might be due to temperature increase during summer in addition to what mentioned above.

Table 3- Correlation coefficient of fungi in summer and winter

	Correlation Coefficient	r- value	Significant	p- value
Cheeses	Summer and winter all cheeses	0.847701184	Significant	$P \leq 0.05$
Fungi	Summer and winter all fungi	0.744781537	Sig.	$P \leq 0.05$

Fe Concentration in Studied Samples:

The Fe was detected in Ishaqi cheese as shown in Figure-1. The results showed that the Agricltural cheese was highly contaminated with Fe (0.24 ppm) in summer and decreased during winter as shown in Figure-3. The same results were obtained in other cheese sample under study and Fe concentration was between(0.14- 0.24 ppm).

The lowest Fe concentration was detected in Arab sheese was (0.14ppm) in summer Figure-2.

Pb Concentration in Studied Samples:

The minimum amount of Pb concentration was determined in Ishaqi and Agricultural cheese (0.001 ppm) followed by Danone cheese (0.008ppm) and finally Arab white cheese which was highly contaminated by pb (0.05ppm) as shown in Figures (1-4).

Ni Concentration in Studied Samples:

The highest value of this heavy metal was presence in Danone cheese (0.1ppm) followed by Arab white cheese and agricultural college cheese (0.045 ppm) the lowest Ni contamination was detected in Ishaqi cheese (0.02ppm) as shown in Figures (1-4).

Cr Concentration in Studied Samples:

This heavy metal was determined in Arab white cheese and agricultural college cheese with (0.009ppm) concentration followed by Ishaqe cheese (0.008ppm) and finally was deticted in lowest concentmation in Danone cheese (0.006 ppm) as shown in Figures (1-4).

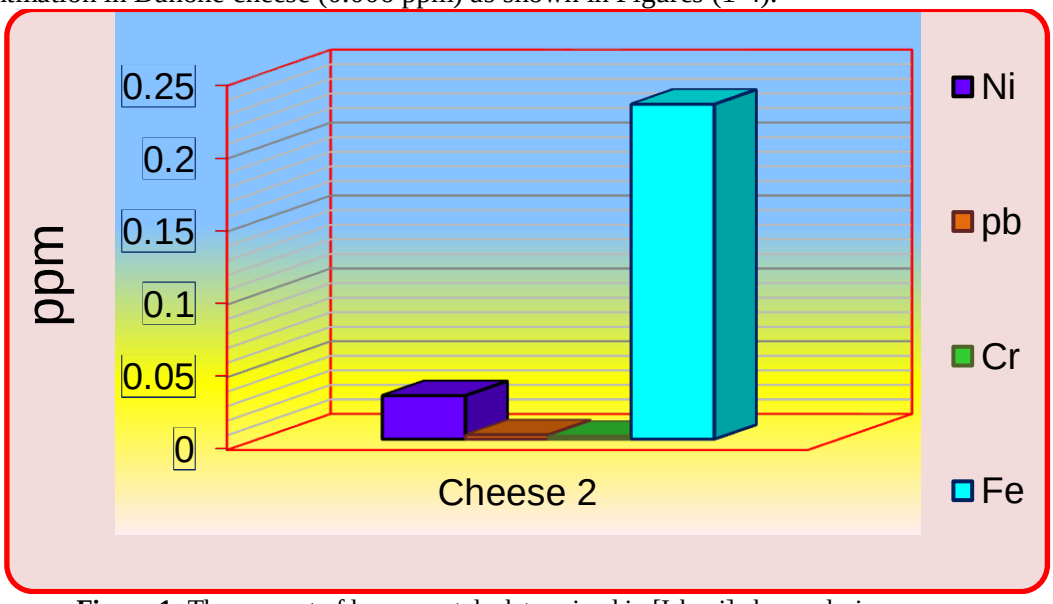

Figure 1- The amount of heavy metals determined in [Ishaqi] cheese during summer

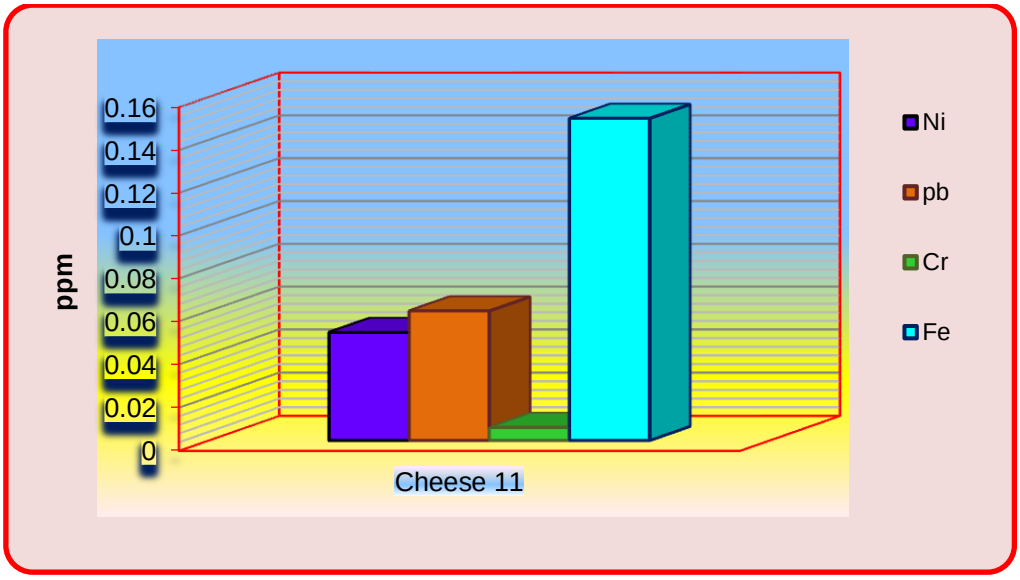

Figure 2- The amount of heavy metals determined in [Arab White cheese] during summer.

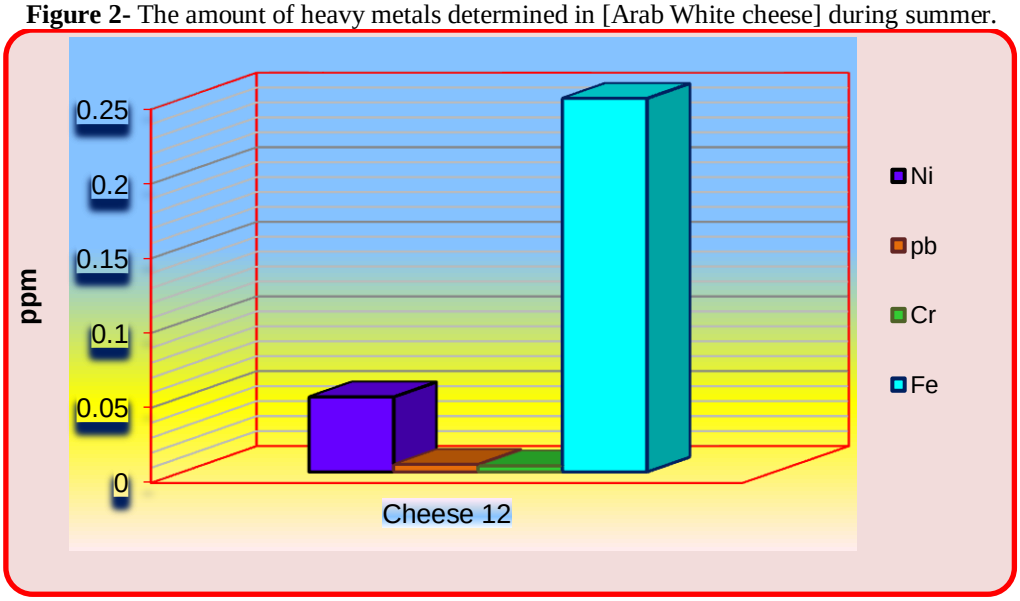

Figure 3- The amount of heavy metals determined in [Agriculture college cheese] during summer

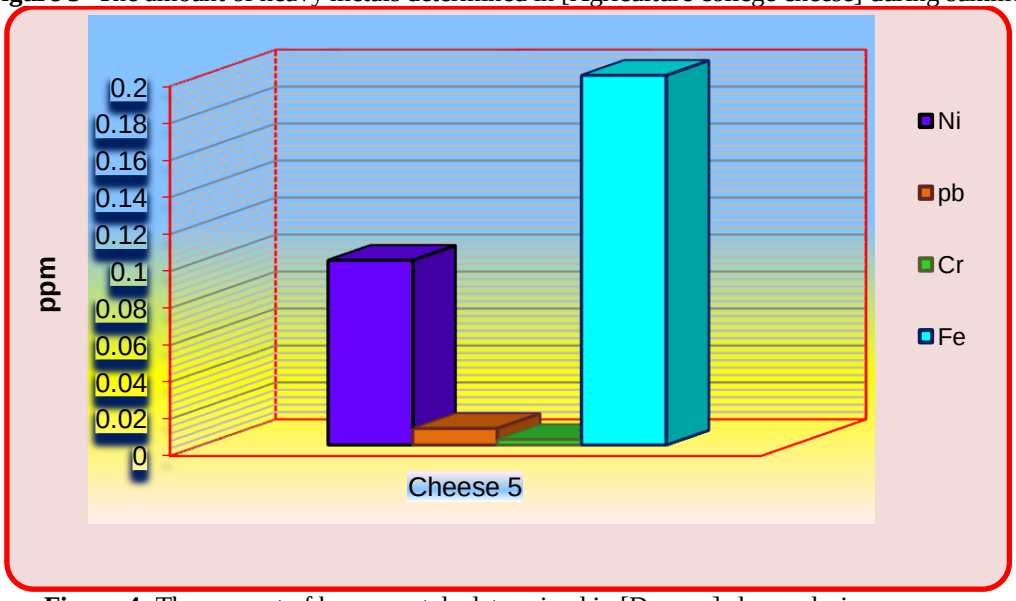

Figure 4- The amount of heavy metals determined in [Danone] cheese during summer.

Heavy metal are considered the most difficult problems to the environment nowadays due to their spread in nature due to their accumulation from industries remains agricultural pesticides or others such as Pb or Fe, etc. It is significance in their available with very low concentration no more than parts of millions in tissues of animals or humans and some of these heavy metals are necessary like Fe to perform biooperation as metabolism or growth or reproduction [28]. The increase of these elements more than natural limit makes them poisonous and damage or effect health of animals or man. They enter animal bodies by skin or digestive system or respiratory system and cause poisonous effects due to their interference with the biological metabolism processes in cells. Their risks appear in their analysis chemically or heating and lead to accumulation in environment and pollution of food minerals and infected by diseases such as cancer . Consequently reach to milk or to food animal products to man causes damages to the consmers.

Iron is from heavy metals that have significance and effects on health of man and animals, so it was focused on the poisonous effect caused by Cadmium or lead and Copper.

The animals receive heavy metals by air or water or food. The food is polluted by metals is considered of main ways for remains of metals in milk resulted to be more informed the level of heavy metals stays in the same level in milk during pasteurization. The products of diary remain level of heavy metals in diary products with the same original milk levels, therefore, more of researchers have made to study pollution of remains heavy metals in sample of checked milk because of their important pollutants in food .

Cheese was known since very old times and inhabitants of Mesopotamia and Mediterranean are regarded of the beginners to make cheese the spread by trade between Asia and Europe. Most of chees names were called according to the place when it is made in origin to be indicated to available more than 400 names or types of cheese were described but they were cleared that number of types of natural cheese.

In conclusion, all the examined cheese sample were contaminated with heavy metals with variable concentration that the continuous consumption of such cheese may constitute a public health hazard through progressive accumulation of these elements inside the consumer body, therefore the raw ingredients used in cheese manufacture including (raw milk, milk powder, salt emulsifier etc.) as well as water supply equipment's used and packaging materials should be free from any traces of heavy metals and all the cheese manufacturing procedures as well as the hygienic conditions of the cheese factory should be regularly checked by the local authorities at cheese factors. The cheese procedures should have educational programmes about hygienic disposal of their waters sewage drainage especially at water sources. Heavy metal are essential for correct body metabolism however the range between their effective and toxic levels are small. Heavy metals contamination raises environmental concerns such as enterance on the food chain which can be potentially harmfull to human's health .Trace elements are shown to have a multitude of toxic effects such as acute syndrome and neurotoxic effects [29].

Result reported in [30] showed the contamination by heavy metals in Egypt were in agreement with this study finding . However , [31] who studied the selected heavy metals in Diary products , had results about- heavy metals contamination which agreed with this study finding as well.

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