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Determination of some microbial and chemical pollutants in cooling raw milk and ice-cream found in the local markets of Salahaldin province

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Abstract

The study was conducted in the laboratories of food science Dept. College of agriculture- Tikrit University, which included the collection of samples of cooling raw milk and ice-cream of several trade markes and three replicates for each sample of different markets and areas in the province of salahaldin: Tikrit, Baiji, Sharqat and Alalam. The samples were taken to the laboratory, stored in the freezer at -18 ^{0}C until the test of microbial and chemical analysis. microbial analysis included the total bacterial count (T.B.C) of coliform & total bacterial count (T.B.C) of yeasts & molds also the isolation and diagnosis of listeria monocytogenes, and then conducted chemical analysis, wich included estimation of concentrations of four heavy metals: cadmium, lead, zinc and cupper. The results of microbiological tests showed that the total bacterial count (T.B.C) coliform, yeasts, molds and Liseria are:6.6,4.7,4.38,2.92 log^{10} cfu/g for ice-cream samples respectivaly, and 6.89,5.93,6.28,2.95 log^{10} cfu/g for cooling raw milk respectivaly. The results of the percentage of *L.monocytogenes* from the samples of ice-cream were 3.3% and cooling raw milk 18.75% .The results showed that *L.monocytogenes* was sensitive towards antibiotics:

Ampicllin, Chloramphinicol , Erthromycin, Nitrofuranotion, Amikacin, and Tobbramycin,while it was resistant towards Naldix acid, Novoblocin and cefotaxame .The results showed also that the high concentration of heavy metals for cadimium, lead, zinc ,and copper found in ice-cream were 0.174, 0.17, 4.25, and 1.086 mg/g respectivily and for cooling raw milk samples were 0.1, 0.3, 2, 1.08 mg/lit respectivily .

Key words: Cooling raw milk,ice-cream, heavy metals, *L.monocytogenes*.

Introduction

The spread of epidemics and diseases duo to food pollution and bad eating habits has become a feature of the times. Food pollution is caused by the presence of microorganisms and food poisoning , causing many injuries , including: food poisoning, and the infection is caused by eating large amount of microbes that attack the body tissues.

Human beings may play a major role in delivering these germs to food , and this role increases if the level of health awareness and hygiene of the workers in the preparation , and handling of food **Weagant, *et al,*(2001)**.

Heavy metals will transport to food, and from food will take their way to the tissue **Abbas, *et al,* (2006)** , and it caused many hygienic hazards, so in recent years, researchers have been conducting several studies to study the deposition of concentrations of heavy metals in different foods, and because of the increased risk to human health **Farkas, *et al,* (2003)**. Heavy metals are characterized by the ability to decompose and break down to the simplest of them, therefore they are different from hydrocarbon pollutants with a changing chemical structure, which lose part of their toxic properties and change their chemical composition, making them easily removed from the environment by natural processes such as most organic pollutants **Agbozu, *et al,* (2007)**. Heave elements contamination is also a serious threat due to the toxicity and bioaccumulation of these elements **Hashmi, *et al,* (2002)**.

Listeria monocytogenes is a wide spread germ in nature, and its an opportunistic germ that possesses many qualities that enable it to live within the body of a living organisms far from the immune system, it is found inside the immune cells or disappears in certain sites and foci of the body and it is one of food pollution causes in the field of various food industries **Schlech, *et al,* (1983)**. This bacteria affects both human and animals, causing (Listeriosis), which is a transitional disease with significant impact on public health **Ellin M. (2001)**. *L. monocytogenes* are widely spread in rural societies, so it is considered one of the most important contaminants of raw food used in the manufacture of ready- made food such as milk and its derivatives, the germ possesses good protection techniques, including the ability to tolerate high salt concentrations and high pH level, they can survive and reproduce at low temperatures reach 4^{0}c **McLauchlin & Rees (2008)**.

L. monocytogenes causes infections known as listeriosis and usually occurs by eating contaminated food, this bacteria, which is ingested with food from intestinal lumen through the blood stream, causing more systemic injuries that intestinal infections, meningitis

appears to be the most common clinical manifestation of listeriosis **Hitchins, *et al,* (1998)**.

These bacteria are transmitted from animal to human either through direct contact, as the case with veterinarians, butchers, animal breeders, or indirectly by eating contaminated food. The animals are infected with this germ as a result of the sudden change in the diet and the consumption of fodder contaminated with the faeces of the infected animals, and the soil, water, and feed, as well as the mammals and birds are the repository of this germ **Bala & Broome (1991)**.

L. monocytogenes is a cold resistant germ (Psychrotrophic) that has the potential to grew at a temperature of 2-4 ^{0}c , as it resists salinity of 18-20% at 4 ^{0}c for 8 weeks.

The aim of this study was to know the total count of aerobic bacteria and coliform bacteria and yeasts & molds count in ice cream and cooling raw milk , as well as to isolate and diagnosis *L. monocytogenes* , in addition to estimating heavy metals such as : cadmium, lead, zinc, and copper .

Materials and Methods:

1-Sample collection:

The study was conducted on a group of samples of ice cream of six different samples and different brands taken from the local markets of Tikrit city and three replicates of each type **Alshamary, *et al,* (2009)**. Samples of cooling raw milk were taken from different areas in the district and areas of Salah al-Din province : Tikrit, Al-Alam, Shirqat, Baiji . The samples were withdrawn from the milk containers and transported to laboratory and stored in the refrigerator for 3-7 days, after which the microbiological and chemical tests were conducted.

2-Microbiological tests:

After the arrival of the samples to the laboratory they were stored at frozen temperature -18^{0}C for ice cream and 4^{0}c for raw milk and the method of A.O.A.C (2005) was followed. The total bacterial count was estimated using Nutrient agar medium, and the total count of coliform by using MacConkey agar medium, as well as an estimate of the total count of yeasts & molds using pour – plate method by using Saubourid dextrose agar and Malt extract agar medium.

3-Isolation and diagnosis of *Listeria monocytogenes* :

25 g of the sample or 25 ml of the raw milk samples with 225 ml of the enrichment medium (Trypticase Soy broth), incubated at 35^{0}c for 24hr., after that 1 ml of the sample was taken and spreated by glass role on the Listeria selective agar media, is also called Oxford Agar (OXA) and incubated at 35^{0}c for 24 hr.

4-Biochemical analysis:

These tests included, Indol test, Methyl red test, Vogas-Proskaur test, Citrate test, and sugar fermentation using : manitol, arabinose, raminose, and xylose. The antibiotic sensitivity test was done using the media Muller Hinton broth, and Muller Hinton agar, and using antibiotics: Ampicillin, Chloramphenicol, Erythromycin, Nitrofuranotion, Amicacine, Tobbramycin, Naldixic acid, Novoblocin, and Cefotaxime.

5-Determination of heavy metals:

using the Atomic Absorption type Leo, which was in the Department of Chemical Engineering – Tikrit University and according to the method used in A.O.A.C (2004).

Results and Discussion:

Table (1) shows concentration of heavy elements in raw milk, the results showed that the highest concentration of cadmium in raw milk obtained from Baiji area was 0.15 mg/l. The results were agreed with **Jianfing,*et al* (2012)** they found that the highest concentration of cadmium in raw milk (0.078-0.174) mg/l., and with **Anastasio,*et al* (2006)**, which they found that the highest concentration of cadmium 0.10 mg/l., and the lowest concentration 0.05 mg/l. in raw milk and differed with the results of **Farid, *et al* (2012)**, as they found that the concentration of cadmium in raw milk 4.7 mg/l.

Table 1. Concentration of heavy elements (mg/l) of raw milk samples

Area	Cadmium	Lead	Zinc	Copper
Tikrit	0.05	0.15	0.15	0.086
Baiji	0.15	0.5	1.57	0.026
Shirgat	0.07	0.014	2	1.08
Al-Alam	0.03	0.4	1.88	0.22

The lead component had the highest concentration 0.5 mg/l. in raw milk obtained from Baiji area, the results agreed with **Farid, *et al*(2012)**, that they found the highest concentration of lead in raw milk 0.16 mg/l., and agreed with **Jigam, *et al*(2011)** they mentioned that the concentration of lead in raw milk is 0,21 mg/l.

The results showed that the highest concentration of zinc in raw milk 2 mg/l. was founed in the raw milk

samples taken from Shirqat region, the results were agreed with **Jigam, *et al* (2011)** stating that the concentration of zinc in raw milk was 4.92 mg/l. and with **Farid, *et al* (2012)** which found that the concentration of zinc in raw milk 1.26 mg/l.

The results about copper showed that the highest concentration was found in the samples taken from Shirqat region 1.08 mg/l., which is higher than the **E.C. (2001)** in milk and its products 0.04 mg/l. The results differed with **Jigam, *et al* (2011)**, they found that the concentration of copper in raw milk 0,024 mg/l.

Table(2) shows that the highest concentration of cadmium in the ice cream samples was in the Reef/ Syrian brand 0.174 mg/g., the results were agreed with **Farid, *et al* (2004)** which indicating that the concentration of cadmium in milk powder 3.1 mg/g., ice cream is often made from dried milk.

About zinc the results showed that the highest concentration was 4.25 mg/g. in the samples bearing the brand Fistiqa / Iraq, the results were agreed with **Harmankaya, *et al* (2012)** when studying the levels of zinc and copper in samples of ice cream 6.75-13.52 mg/g. in samples of ice cream filled with plastic packages. The average of zinc in ice cream samples filled with biscuit were 0.66-9.04 mg/g.

The copper component was found to have the highest concentration (1.086)mg/g. and found in the samples of the brand Corentodesk/ Turkey, the results were agreed with **Harmankaya, *et al* (2012)** stating that concentration of copper in samples of ice cream filled with plastic packages 1.77-6.82 mg/g., while samples filled with biscuit indicated that average concentration of copper 0.27-4.27 mg/g.

Table 2. Concentration of heavy elements (mg/kg) of ice cream samples

Trade brand	Cadmium	Lead	Zinc	Copper
Galaxy	0.03	0.11	2.78	0.025
Nice	0.01	0.17	2.73	0.84
Corentodesk	0.15	0.14	1.2	1.086
Reef	0.174	0.5	1.57	0.72
Vanilla	0.09	0.3	2.01	0.18
Fistiqa	0.01	0.3	4.25	0.1

As for the total count of bacteria, coliforms, yeasts & molds, and Listeria, the results showed in table(3) that the highest total count of aerobic bacteria was found in the brand Galaxy/ Salah din (Iraq), where it reached 6.61 $\log_{10}$ cfu/g., and the lowest total count of bacteria founed in ice cream samples of brand Corentodesk/ Turkey 3.1 $\log_{10}$ cfu/g. When dintifiedlimit of the total count of bacteria 10 and that the maximum (2.5x10)cfu/g., the samples of brand Nice, Galaxy, and Fistiqa exceeded the microbial limits.

The results showed that the highest total count of coliform bacteria was found in samples of ice cream belongs to brand Galaxy/ Salah din(Iraq) 4.7 $\log_{10}$ cfu/g., and the lowest count founed in Turkish ice cream with the brand Corentodesk 3 $\log_{10}$ cfu/g. The microbial limit of coliform bacteria identified by the **Syrian standard of the year,(2000)** showed that the permissible microbial limit is 10 $\log_{10}$ cfu/g, and the maximum allowed is 10 $\log_{10}$ cfu/g.

The results showed that the highest count of yeasts & molds was found in the samples of ice cream bearing the brand Nice / Erbil 4.38 $\log_{10}$ cfu/g. and the lowest count of yeasts & molds was found in the samples carrying the brand Corentodesk/ Turkey 3 $\log_{10}$ cfu/g., and the highest count of Listeria it was found in the samples of ice cream of the brand Galaxy 2.92 $\log_{10}$ cfu/g., while the lowest count were found in the brand Corentodesk, the results were agreed with **Saleeq, *et al* (2004)** when they studied some of the characteristics of the local ice cream, indicating that the total count of aerobic bacteria in the samples of ice cream was **2.1x10 – 4x10** and that the total count of coliform bacteria was **1.6x10 – 1.8x10** .

The study agreed with **Movassagh, *et al* (2011)** that they found that the total count of aerobic bacteria for North-Western regions of Iran was 6.28-8.77 $\log_{10}$ cfu/g. and the total count of coliform bacteria was 3.58-4.42 $\log_{10}$ cfu/g. The results was also agreed with **Caglayanlar, *et al* (2009)** stating that the total count of aerobic bacteria in the non – coated ice cream was 6.9-27x10 $\log_{10}$ cfu/g. and the total count of coliform bacteria ranged from less than 10 to 84x10 $\log_{10}$ cfu/g, and in the coated less than 10.

The total count of yeasts & molds ranged from less than 10 to 1.5x10 cfu/g. in coated samples, while the count of molds in non-coated samples was less than 10 to 21 $\log_{10}$ cfu/g.

Kanbakan, *et al* (2009) said that the total count of yeasts & molds in ice cream ranged from less than 10 to 3x10 $\log_{10}$ cfu/g.

Table 3. Average total count of microorganisms contaminate ice cream samples log_{10} cfu/g.

Brand&Source of ice cream	T.B.C	Colifororm bacteria	Yeasts & Molds	***L. monocytogenes***
Galaxy / salahdin	6.61	4.7	3.27	2.92
Nice / Erbil	5.46	3.45	4.38	2.77
Fistiq / Mousil	5.66	3.40	3.89	2.81
Corentodesk / Turkey	3.11	3	3	2.6

Table 4. Average total count of contaminated microorganisms of raw milk samples taken from different regions (log_{10} cfu/ml.)

Source of milk	T.B.C	Coliform count	Yeasts & molds	***L. monocytogenes***
Tikrit	5.56	5.92	5.42	2.44
Al-Alam	6.43	5.93	6.28	2.69
Shirgat	6.89	5.16	5.36	2.95
Baiji	6.85	5.09	5.03	2.85

Table (4) shows the total count of contaminated microorganisms of raw milk samples taken from different sources. The results showed that the highest total count of bacteria was found in raw milk taken from Shirqat region with a total count 6.89 log_{10} cfu/ml., while the highest count of coliform bacteria found in the samples of raw milk taken from Al-Alam region 5.93 log_{10} cfu/ml. The study showed that the highest total count of yeasts & molds were found in raw milk obtained from Baiji region (5.03)cfu/ml. The highest count of *L. monocytogenes* was found in raw milk samples taken from Tikrit, which count to 2.44 log_{10} cfu/ml. The results were agreed with **Meshref (2013)** when studying the microbial quality of raw milk taken from cows and fresh cream. The average total count of aerobic bacteria was 2.1x10 – 4x10 cfu/ml., and the average total count of coliform bacteria ranged from less than 3 to 1.5x10 . The results also agreed with **Beukes, *et al* (2001)** when they studied the total count of raw milk in South Africa, where they found that the total count of aerobic bacteria was 8.6x10 cfu/ml. The results also agreed with **Edward &Inya (2003)** when they studied the microbial quality of raw milk in four regions of Nigeria, where they found that the mean total count of aerobic bacteria was 9.8x10 – 1.1x10 cfu/ml. and the average total count of coliform bacteria was 5.4x10 - 9.5x10 cfu/ml. and the average count of yeasts & molds was 6.4x10 -9.2x10 cfu/ml.

AL-Kwzaiy,(2011) reported when she studying some of the microbial contaminants of raw milk and its products traded in the markets of Diwaniyah city, the mean total count of aerobic bacteria of raw milk was 5.1x10 – 8.1x10 and the rate of 6.6x10 cfu/ml. and the mean total count of coliform bacteria was 4.9 – 7x10 and the rate 5.9x10 cfu/ml., and the average count of yeasts & molds 3.3 -5.5x10 and the rate 4.4x10 cfu/ml.

About the isolation of *L. monocytogenes*, three isolates were obtained from 35 raw milk samples with an isolation rate of 8.57% and one isolation from 30 samples of ice cream with 3.33% isolation rate. The results were agreed with **Beukes, *et al* (2001)** in South Africa as it isolated from ice cream samples and by 6% isolated rate. **Molla& Roman(2004)** mentioned when they studying the isolation and diagnosis of *L. monocytogenes* from raw milk and its products taken from the single sale shops in Addis Ababa, Ethiopia, where the percentage of isolation was 19.6% of the samples examined. The results agreed also with the study of prevalence of *L. monocytogenes* in raw milk taken from livestock and their sensitivity to antibiotics, this bacteria was isolated from 4 samples of 67 samples and with isolation rate 5.3%. **AL-Shamary,& Najim, (2009)** obtained to isolate this bacteria from 10 samples out of 68 of raw and imported milk in the city of Baghdad and by isolation rate 14.7%. While **Abbas,& Jabar, (2012)** in their study of the presence of *L. monocytogenes* in raw milk for ruminants in Basrah province as the study included the collection of 300 samples of raw milk, as the results showed that the number of isolates of this bacteria **Slik, *et al,* (2004)** and by isolation rate 7.3%.

The results of ice cream were agreed with **AL-Shamary, A. H. A., (2010)** when he studied *L.monocytogenes* in ice cream samples in the city of Baghdad, the results showed isolation and diagnosis of this bacteria in **Ellin M, (2001)** isolates with isolation rate 14% from 50 samples.

L. monocytogenes is wide spread in the environment as it present in soil, faeces, water, and sewage water, in plants, animals, and fodder. Milk is contaminated directly and indirectly by these environmental media. This bacteria has the potential to grow at low temperatures.

About the antibiotic sensitivity test, this bacteria, as shown in table (5), which were isolated from the raw milk and ice cream samples, were shown to be sensitive to the antibiotics: Ampicillin, Chloramphenicol, Erythromycin, Nitrofuranotion, Amikacine, and

Tobramycin. While it was resistant to antibiotics: Naldixic acid, Novoblocin, and cefotaxim, the results were agreed with **Mauroconter, *et al* (2007)** when studying the effect of antibiotics on *L. monocytogenes* isolated from different sources, and agreed also with **Abbas, *et al,(2012)*** when they studying the presence of this bacteria in raw milk, which was found to be sensitive to Chloramphenicol, Erythromycin, Nitrofuranotion, and Tobramycin. **Prescott, *et al* (2002)** confirmed that antibiotic Naldixic acid is used in some selective media for the isolation of *L. monocytogenes*, which its resistance to this antibiotic which inhibits the Gram – negative bacteria by influencing the process of building DNA. The differences in resistance to these antibiotics is due to misuse of antibiotics when added randomly to feed, or to the effect of transposons genes.

Table 5. The sensitivity of *L. monocytogenes*to antibiotics

Antibiotic	Symbol	Concontration (Mg/disc)	The result
Ampcillin	Am	10	S
Chloramphinicol	Ch	30	S
Erythromycin	E	15	S
Naldixic acid	NA	30	R
Nitrofarantion	F	30	S
Doxycycline	Do	30	R
Amikacin	Ak	30	S
Toblramycin	TOB	10	S
Novoblocin	NV	30	R
Cefotaxim	CTX	10	R

S = Sensitive.
R = Resistant.

A number of conclusions can be drawn from the results of this study, a large part of samples of ice cream and cooling raw milk are contaminated with a variety of pathogenic bacteria that pose a danger to the health of the consumers, and that the cooling and frozen foods have a major role in the transmission of *L. monocytogenes* to human and the occurrence of serious illness. All isolates of this bacteria have shown an absolute sensitivity to the antibiotics : Ampicillin and Chloramphenicol, while all isolates have shown resistance to the antibiotic Naldixic acid.

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تقدير بعض الملوثات الميكروبية والكيماوية في الحليب الخام المبرد والمثلجات اللبنية الموجودة في الأسواق المحلية لمحافظة صلاح الدين.

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كلية الزراعة–جامعة تكريت–العراق

الخلاصة

أجريت الدراسة في مختبرات قسم علوم الأغذية في كلية الزراعة _ والتي تضمنت جمع عينات للحليب الخام المبرد والمثلجات اللبنية ولعدة علامات تجارية وبواقع ثلاث مكررات لكل عينة من أسواق ومناطق مختلفة من محافظة صلاح الدين وهي تكريت , بيجي ,الشرقاط ,والعلم.وبعد اخذ العينات نقلت إلى المختبر وحفظت في المجمدة على درجة حرارة –18مه لحين أجراء الفحوصات الميكروبية والكيميائية ,إذ تضمنت الفحوصات الميكروبية معرفة العدد الكلي للبكتريا وبكتريا القولون والعدد الكلي للخمائر والاعفان وعزل وتشخيص بكتريا الـ L.monocytogenes وبعدها أجريت الفحوصات الكيماوية والتي تضمنت معرفة تركيز أربعة عناصر معدنية ثقيلة هي الكادميوم والرصاص والزنك والنحاس.وأظهرت نتائج الفحوصات الميكروبية أن معدل الأعداد الكلية للبكتريا الهوائية وبكتريا القولون والخمائر والاعفان ولبكتريا الليستريا هي 6.6, 4.7, 4.38 ,2.92 لو10 وحدة تكوين مستعمرة /غم لعينات المثلجات البنية على التوالي و 6.89 ,5.93 ,6.28 , 2.95 لو 10 وحدة تكوين مستعمرة /غم لعينات الحليب الخام المبرد على التوالي .

وبينت النتائج النسبة المئوية لعزل بكتريا L.monocytogenes من عينات المثلجات اللبنية والتي كانت 3.3% وللحليب الخام المبرد 18،75 %. وبينت النتائج حساسية بكتريا L.monocytogenes تجاه كل من المضادات الحيوية : Ampicillin و Chloraphinicol و Erythromycin و Nitrofuranotion و Amikacine و Tobbramycin في حين انها كانت مقاومة للمضادات الحيوية Naldix acid و Novoblocin و Cefotaxime.

وأظهرت النتائج إن اعلى معدل تركيز للعناصر الثقيلة لكل من عناصر الكادميوم والرصاص والزنك والنحاس والتي وجدت في المثلجات اللبنية هي 1.08, 4.25, 0.17, 0.174 ملغم /لتر على التوالي.

الكلمات المفتاحية :المثلجات اللبنية ,الحليب الخام المبرد ,بكتريا الليستريا ,العناصر الثقيلة .