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EC MICROBIOLOGY
Research Article

Determination of Microbial Isolates Contamination of Yoghurt from Different Factories in Dohuk City

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Received: March 28, 2023; **Published:** April 10, 2023

Abstract

This study was conducted to the identification of microbial species isolates that contaminated yoghurt samples and to determine the viable microbial total counts, also the coliform counts, *Staphylococcus*, lactic acid bacteria (LAB) and the fungi counts, in addition to the pH levels after storage the samples for 7 or 30 days in refrigerator temperature at 5ºC from eight dairy factories in Dohuk city.

The results were shows that the total viable microbial counts significantly increased ($p < 0.05$) and appeared between log 6.25 to 9.25 CFU/ml after the end storage period, while, the LAB significantly decreased in all yoghurt samples and became between log 4.23 to 4.60 CFU/ml, compared with the counts at initial storage at between log 8.14 to 12.20 CFU/ml. The coliform, *Staphylococcus* and fungal isolates were found only at the end of yoghurt samples storage periods and some of its samples were not to detected in its. The range of pH for all yoghurt samples at the end of storage periods was 3.62 to 4.05 compared with the range at the initial storage periods at 3.97 to 4.40. The microbial isolates from yoghurt samples were the *Staphylococcus sp.*, *E. coli*, *Klebsiella sp.* and *Pseudomonas luteola*, *Lactobacillus bulgaricus, Aspergillus sp., Penicillium sp.,* and the yeast in all of the samples. Also, it that appears some bacterial isolates were completely resistant to antibiotics.

Keywords: *Yoghurt; Microbial Contamination; Dairy Factories; Safety; Dohuk*

Introduction

Yoghurt is the oldest dairy product used by a human, it is an exclusive food, that is consumed worldwide, without limitation from any tradition or religion [1]. Milk or dairy products were composed of rich nutrients, such as protein, lipids, lactose, vitamins, calcium, phosphorus, magnesium, zinc, etc. which are necessary for the healthful living of humans of all ages and both gender [2]. The yoghurt was prepared by milk fermentation using some lactic acid bacteria, especially the pure culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus* which are the most species used in the yoghurt fermentation process [3]. The fermentation process concluded with the fermenting of milk sugar as lactose to produce lactic acid, and some agents, to give the yoghurt of sour taste [4]. Yoghurt served as an essential food and its plays an important role in human nutrition, health maintenance, and therapeutic and dietetic functions [5]. The yoghurt nutrients contained were made an optimal medium for microbial growth and toxins production [6]. The microbial contami-

nation of yoghurt was occurring in various stages, including the production process, selling or storage period. Also, both the professional and manual process of yoghurt production at different species counts for the microbes which cause yoghurt as unhygienic food through its ability to produce microbial toxins or by changing some yoghurt ingredients to harmful agents. The contamination occurred depending on the microbial species that arrived in milk or yoghurt produce which were also related to the quality of milk, supplement agents, water, machine status and the manufacturing environment, It may contain gram-positive or negative bacteria, yeast or moulds [7].

According to a previous mention, the goal of this study was to detect the microbial total counts, assay the pH levels and identify the microbial species contamination of yoghurt samples, tools, the building of different dairy factories in Dohuk city, also to evaluation the resistant ability of bacterial isolates against some types of antibiotics.

Materials and Methods

Sample collection

The eight-dairy factory was selected to cover the Duhok city area, The yoghurt samples were taken at 30 cans for each dairy factory. In addition, the swabs samples were taken from the worker, tools, building and cooler rooms at three replicates for each dairy factory at the same time for yoghurt sampling. All samples were labelled and transferred to the microbial laboratory in the ice box, and the swab samples were carried out for microbial assay. While the yoghurt samples move to the refrigerator at 4ºC and kept for one month to determine the pH and total microbial counts every 10 days and microbial species diagnosis after 30 days of storage [8].

Media preparation

The cultural media were prepared according to the company's manufacturer for each medium. It was boiled using the hot plate with a stirrer at 100ºC, then sterilized using an autoclave at 121ºC with 1.5 psi/inch^3 for 15 minutes. The media agar plates were incubated overnight for sterility test, then kept in the refrigerator till used in microbial isolation and identification [9].

Samples pH assay

The sample's pH was detected using the pH meter system (Hanna, Germany).

Microbial isolation and identification

Twenty-five (25) ml from each yoghurt sample were dissolved in 225 ml of normal saline until makes the optimal serious solutions at 10^3 and from the last dilute of each sample were taken 0.1 ml and separated on each plate containing optimal medium (Nutrient agar (NA), MRS agar, MacConkey agar (MA), Eosin Methylene Blue (EMB) Agar and Mannitol Salt Agar (MSA) then incubated aerobically at 35ºC for 24hrs and anaerobically for MRS agar media at 37ºC for 48hrs used anaerobic jar (Rod well, England) according to [8,10]. Different Isolates on bacterial medium agar were selected and sub-culturing on the same medium. For the identification of bacteria, the suspected colonies were stained using the gram stain method and their shapes, colours, and arrangements were observed under a light microscope. Then identification is complete species using the biochemical tests according to [11]. The fungal isolation was conducted after spreading 0.1 ml from the last dilution of each yoghurt sample on the Potato-Dextrose Agar media then incubated at 28 ± 2ºC for 3 to 5 days, while the diagnosis of fungal was conducted according to the identification key in Watanabe [12].

Antibiotics sensitively assay

Bacterial isolates from *E. coli*, *Klebsiella spp*, *Staphylococcus spp*, and *Pseudomonas spp*, were tested for sensitive susceptibility to the different antibiotics were used the Kirby-Bauer technique [13]. The antibiotics that are used were contains bacitracin, nisin, natamycin, azithromycin, clindamycin, amikacin, trimethoprim, ciprofloxacin and nalidixic acid. A single colony from each bacterial isolate were transferred to a new test tube containing 5 ml nutrient broth and incubated at 37°C for 24 hours to make a bacterial inoculum, then adjust

the balances by comparing it to McFarland tube No. 0.5 A sterile cotton swab is dipped in the inoculum and uniformly separated across the surface of a Muller-Hinton agar plate. The antimicrobial-containing disks are then put into the agar using forceps squeezed firmly to establish contact with the agar, and the plates are inverted and incubated at 37°C for 18 hours. Following incubation, the inhibition zone diameter (IZD) surrounding each disk is measured in (mm), and the isolates are classified as sensitive, intermediate, or resistant to a specific medication based on comparisons with conventional inhibition zone diameters [14].

Statistical analysis

The general linear model's procedure (ANOVA) of SAS [15] was used to analyze the data. Significant treatment differences were evaluated using Duncan's multiple-range test [16]. All statements of significance are based on the 0.05 level of probability.

Results

Microbial total counts

The results of total counts of microbial that's contamination of yoghurt samples taken from eight dairy factories distributed in Duhok city and stored at 4 (± 2ºC) in a refrigerator for 30 days were illustrated in table 1. The results indicated that the microbial total counts of yoghurt samples from each factory were significantly ($p < 0.05$) increased with the continuation to the end of cooling storage periods. The microbial total counts in the yoghurt samples at 30 days storage of factory Rabben (F1), Mateen (F2), Kave (F3), Besere (F4), Yaljeda (F5), Kahe (F6), Rawan (F7) and Baraka (F8) factories were appeared at log 8.30, 8.90, 9.20, 7.10, 7.80, 8.64, 7.90 and 6.84 compared with the 4.30, 6.25, 5.74, 5.0, 6.30, 6.0, 5.20 and 5.30 CFU/ml respectively at the first-day storage. While the lactic acid bacteria (LAB) counts were shown to be significantly increased in previous factory samples at the same period's storage and appeared at log 11.41, 12.20, 10.14, 10.22, 8.75, 9.60, 10.15 and 8.14 respectively compared with the log 4.48, 4.48, 4.48, 4.23, 4.60, 4.52, 4.48 and 4.54 CFU/ml at the first-day storage.

The coliform bacteria counts appeared in factory yoghurt samples at the same time of storage in F3, F4, F5, F6, and F8 at log 4.18, 3.70, 4.26, 4.11 and 3.90 CFU/ml respectively while they cannot detect these bacteria in the yoghurt samples of F1, F2 and F7, also that's were not detected the coliform bacteria in all samples at the first-day storage.

The *Staphylococcus* bacteria count also was not shown in four yoghurt factory samples at 30-days storage of F2, F3, F4, and F6 and detected its colony was in other factories of F1, F5, F6, F7 and F8 at log 4.54, 4.40, 4.30, 4.52 and 4.53 CFU/ml respectively, compared with no growth of these isolates at first-day storage unless the F7 which appeared at log 2.08 CFU/ml.

Furthermore, the other microbial contamination of yoghurt samples was the fungal species that appeared at the same period storage in samples of F1, F4, F5, F6, F7 and F8 at log 4.78, 4.54, 4.60, 4.38, 3.30 and 4.48 CFU/ml respectively while it's not found in the yoghurt samples of F2 and F3 factories, compared with not fungal isolates in all samples at first-day storage just in the yoghurt sample of F5 which contains 1.90 CFU/ml.

The pH samples average assay

The results in a table 2 show the average pH value of yoghurt samples from the dairy factories separated in Duhok city that were stored at 4 (± 2ºC) in the refrigerator for 30 days. The average pH level was significantly ($p < 0.05$) lowered in each yoghurt sample and became at the factories F1, F2, F3, F4, F5, F6, F7 and F8 at the end of period storage at 4.05, 3.91, 3.83, 3.85, 3.70, 3.62, 3.87 and 3.93 respectively compared with the pH level of yoghurt samples at the first-day storage which appeared at 4.19, 4.06, 4.04, 3.97, 4.14, 4.37, 4.40 and 4.26 respectively.

Yoghurt Factory	Time test (Day)	Different Microbial total counts log (CFU/ml) after 30-day storage periods				
		Microbial counts	LAB counts	Coliform counts	*Staphylococcus* counts	Fungi counts
F1	1	4.30f ± 0.21	4.48e ± 0.70	-	-	-
	30	8.30b ± 1.12	11.41a ± 1.45	-	4.54a ± 1.01	4.78a ± 0.21
F2	1	6.25d ± 0.58	4.48e ± 0.87	-	-	-
	30	8.90a ± 1.03	12.20a ± 2.50	-	-	-
F3	1	5.74d ± 0.32	4.48e ± 0.73	-	-	-
	30	9.20a ± 1.22	10.14b ± 1.33	4.18a ± 0.83	-	-
F4	1	5.00e ± 0.45	4.23e ± 0.26	-	-	-
	30	7.10c ± 0.94	10.22b ± 1.47	3.70b ± 0.41	-	4.54a ± 0.45
F5	1	6.30d ± 0.24	4.60e ± 0.44	-	-	1.90c ± 0.46
	30	7.80b ± 0.86	8.75c ± 0.98	4.26a ± 0.73	4.40a ± 0.63	4.60a ± 0.24
F6	1	6.00d ± 0.28	4.52e ± 0.45	-	-	-
	30	8.64a ± 0.85	9.60c ± 2.46	4.11a ± 0.94	4.30a ± 0.56	4.38a ± 0.28
F7	1	5.20e ± 0.63	4.48e ± 0.11	-	2.08b ± 0.65	-
	30	7.90b ± 0.55	10.15b ± 2.54	-	4.52a ± 0.83	3.30b ± 0.63
F8	1	5.30e ± 0.72	4.54e ± 0.51	-	-	-
	30	6.84c ± 0.52	8.14d ± 1.11	3.90a ± 0.67	4.53a ± 0.95	4.48a ± 0.72

Table 1: *The microbial total counts log (CFU/ml) contamination of yoghurt samples stored at 4ºC (± 2ºC) for 30 days from the different factories in Duhok city.*

The different letters mean significant differences in each column at probability 0.05. (-) mean not detected. F1= Rabben factory, F2= Mateen factory, F3= Kave factory, F4= Besere factory, F5= Yaljeda factory, F6= Kahe factory, F7= Rawan factory, F8= Baraka factory.

Yoghurt Factory	Time test (Day)	Yoghurt pH level after 7- or 30-day storage periods
F1	1	4.19a ± 0.24
	30	4.05b ± 0.35
F2	1	4.06a ± 0.51
	30	3.91b ± 0.35
F3	1	4.04a ± 0.15
	30	3.83b ± 0.21
F4	1	3.97a ± 0.23
	30	3.85ab ± 0.24
F5	1	4.14a ± 0.42
	30	3.70c ± 0.33
F6	1	4.37a ± 0.37
	30	3.62d ± 0.27
F7	1	4.40a ± 0.17
	30	3.87c ± 0.35
F8	1	4.26a ± 0.51
	30	3.93c ± 0.28

Table 2: *The pH level of yoghurt samples was stored at 4ºC (± 2ºC) for 30 days from different factories in Duhok city.*

The different letters mean a significant difference in each raw at probability 0.05. -F1= Rabben factory, F2= Mateen factory, F3= Kave factory, F4= Besere factory, F5= Yaljeda factory, F6= Kahe factory, F7= Rawan factory, F8= Baraka factory.

The microbial species contaminated yoghurt and factories partition

The microbial isolates that were isolated from yoghurt samples and the factories partition include, *Staphylococcus sp, Streptococcus sp, Klebsiella sp, and Pseudomonas sp, Acinetobacter sp, Enterobacter kobei, E. coli, Sphingomonas paucimobilis*, furthermore the lactic acid bacteria species. The high bacterial load in yoghurt was attributed to inadequate hygienic measures in production.

The presence of Coliforms species, *Staphylococcus* sp., and *Candida* sp., in most yoghurt samples as contamination, referred to the lack of cleanliness during processing. That's because these microbes were not found in yoghurt due to high temperature, short-duration pasteurization, adequate washing, and appropriate sanitary measures. The presence of these microbes is a significant risk to consumer health and suggests a lack of care on the side of the processors or yoghurt sellers. These species are considered natural flora of the human and animal digestive tracts, and their presence suggests direct fecal contamination. The presence of the same microbe species on the workers, tools and the factories building indicate that they are dependent as the sources of yoghurt contamination.

YFS	Yoghurt microbial species at 30 days storage	Worker	Tools	Building
F1	*Staph. saprophytic, Staph. warneri, Candida spp*, LAB	*Staph. warneri, Staph. epidermidis Candida spp.*	*Staph. warneri, Staph. epidermidis Candida spp.*	*Staph. warneri, Staph. epidermidis Candida spp.*
F2	LAB, *Candida spp*	*Staph. vituluns, Staph. Cohnii, Staph. epidermidis, Burkhol. cepacia, Candida spp.*	*Staph. vituluns, Staph. epidermidis, Klebsiella oxytoca, Candida spp.*	*Staph. epidermidis, Candida spp., Penicillium spp.*
F3	LAB, *Ps. oryzihabitans*	*Staph. epidermidis Ps. oryzihabitans Candida spp.*	*Staph. epidermidis, Candida spp.*	*Staph. epidermidis, Ps. oryzihabitans Candida spp.*
F4	LAB, *E. coli, Candida spp, Sphingomonas paucimobilis, Candida spp.*	*Staph. saprophyticus, Staph. warneri, Staph. epidermidis, Candida spp.*	*Staph. epidermidis, Candida spp.*	*Staph. saprophyticus, Ps. oryzihabitans, Penicillium spp.*
F5	LAB, *Staph. epidermidis, Pseudo. luteola Candida spp.*	*Staph. aureus, Staph. captis, E. coli, Kellbsilla oxytoca, Pseudo. luteola, Candida spp.*	*Staph. epidermidis, Pseudo. luteola*	*Aspergillus spp., Staph. epidermidis, Pseudo. luteola*
F6	LAB, *Staph. epidermidis, Pseudo. luteola, Candida spp.*	*Staph. Erysi, Staph. epidermidis, Staph. lentus, Ps. oryzihabitans, Candida spp.*	*Pseudo. luteola, Staph. epidermidis, Candida spp.*	*Aspergillus spp., Candida spp.*
F7	LAB, *Staph. lentus, Staph. epidermidis, Burkhol. cepacia, E. coli, Pseudo. luteola, Candida spp.*	*Staph. sciuri, Staph. capitis, Staph. lentus, Staph. epidermidis, Burkhol. cepacia, E. coli, Ps. stutzeri, Ps. oryzihabitans, Candida spp.,*	*Staph. capitis, Staph. lentus, Pseudo. luteola, Candida spp.*	*Staph. lentus, Aspergillus spp., Candida spp.*
F8	LAB, *Staph. captis, Pseudo. luteola, E. coli, Candida spp.*	*Staph. capitis, Staph. epidermidis, Candida spp.*	*Staph. erysi, Staph. Epidermidis, Candida spp.*	*Staph. lentus, Aspergillus spp., Candida spp.*

Table 3: *The microbial species contaminated yoghurt samples, workers, tools, and building from the different factories in Duhok city. YFS= Yoghurt factory symbol. YFS= Yoghurt factory symbol, LAB= Lactic acid bacteria. F1= Rabben factory, F2= Mateen factory, F3= Kave factory, F4= Besere factory, F5= Yaljeda factory, F6= Kahe factory, F7= Rawan factory, F8= Baraka factory.*

Antibiotics sensitively to bacterial isolates

The antimicrobial sensitivity of some bacteria isolated from yoghurt samples is illustrated in table 4. The results investigated that the *E. coli* isolate appeared that able to resist all antibiotics, while intermediate resistant for each of azithromycin and amikacin, also, the *Enterobacter kobei* appeared resistant for all antibiotics except as intermediate for trimethoprim and appeared sensitive to each of azithromycin and ceftriaxone. The results also appeared that the *Pseudomonas luteola* and *Klebsiella sp.* were resistance rates for all antibiotics used in this study, and appeared sensitive at 58 and 60% for clindamycin antibiotic respectively. Moreover, show as intermediate resistance against ceftriaxone at 80 and 67% respectively. The gram-positive bacteria are *Staph. aureus* appeared to resistant to bacitracin, nalidixic acid and ceftriaxone at 95, 95 and 96% respectively, also were appeared intermediate resistant for each azithromycin and trimethoprim at 82 and 80% respectively while it was shown as sensitive for each clindamycin and amikacin at 55 and 65% respectively.

Isolates species	Microbial species sensitivity to antibiotics types (%)						
	B	DA	AZM	NA	AK	CRO	TMP
E. coli	R(100)	R(95)	I(60)	R(90)	I(40)	R(90)	R(90)
Enterobacter kobei	R(95)	R(95)	S(60)	R(95)	R(97)	S(60)	I(82)
Pseudo. luteola	R(100)	S(58)	R(90)	R(90)	R(95)	I(80)	R(95)
Klebsiella sp.	R(100)	S(60)	R(98)	R(90)	R(95)	I(67)	R(93)
Staph. aureus	R(95)	S(55)	I(82)	R(95)	S(65)	R(96)	I(80)

Table 4: *Antimicrobial sensitivity of some bacterial isolates from yoghurt samples.*

R= Resistance I= Intermediate, S= Sensitive, B= Bacitracin (10 µg), Ak= Amikacin (10 µg), CRO = Ceftriaxone (10 µg), NA= Nalidixic acid (30 µg), DA= Clindamycin (10 µg), AZM= Azithromycin (15 µg), TMP= Trimethoprim (10 µg).

Discussions

Consuming yoghurt is essential since it aids in digestion thanks to the good bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. They are regarded as the top probiotics suitable for human ingestion. However, because the raw milk, with its neutral pH and high-water activity, it is an excellent growth medium for various microorganisms, prepared yoghurts may be contaminated with a variety of infectious bacteria, molds and viruses, is the most serious and widespread concern about yoghurt safety [17].

The microbial contamination can happen at any step throughout the manufacturing process. the quality of yoghurt is decreased as a result of mistakes in production, packing, handling, raw material sources, bad sanitary conditions, poor personnel hygiene, and unsterilized equipment [18].

In this study, the microbiological quality of eight factories in Duhok city was assessed. Data obtained for the total viable count (TVC), *Staphylococcus* sp. (SC), counts, total coliform count (TCC), and total fungal count (TFC) of the yoghurt samples were shown significantly increased and become more the upper limited allows to consumed. In addition, the microbial species that diagnosis from the yoghurt samples were *Staphylococcus sp, Streptococcus sp, Klebsiella sp, and Pseudomonas sp, Acinetobacter sp, Enterobacter kobei, E. coli, Sphingomonas paucimobilis*, indicator that yoghurt samples produced in the non-suitable condition which may be referred to the lack of cleanliness, un-adequate washing, and un-appropriate sanitary measures during processing. These levels of microbial counts were indicated for those samples were not in safe to consume according to the Iraqi Food safety management systems [19].

The total of LAB count was found in the optimal count on the first day of production because they were used as starters culture and they were increased significantly in all yoghurt samples after the storage periods. This finding is consistent with prior research in which

Lactobacillus spp. and other bacteria involved in yoghurt fermentation were identified [20]. These results were in agreement with those results found by [21], who mention that the microbial count was increased with the increase of the storage periods.

The microbial contamination in terms of the number of colonies and the microbial species has enhanced is a change of the pH level and causing in decrease with increasing conservation period, which indicates a decrease in the quality of the yogurt product. These were happened as a result of the growth of microbial species and their production of enzymes to ferment lactose sugar and produce organic acids according to the ability it's to ferment, these were causing in a decrease in the pH level and low quality of yogurt.

The pH values obtained in this research were in agreement with a previous study by [22] while differing from the study by [23] who recorded that the pH of yoghurt samples at 4.48 in the second weeks storage. The optimal acidity of yoghurts should be recorded from 4.0 to 4.5 to become suitable for consumption [24]. In addition, [25] observed an increase in acidity with increased storage time.

The fermentation activity of the starters bacteria that make up the yoghurt acidity during the storage. These starter microbes can still digest lactose under refrigeration, though the process takes longer [26].

These results were in agreement with a previous study conducted by [27] who found that yoghurt samples were contaminated with different isolate species. These results were referred that the bacterial isolates that contaminated of yoghurt samples appeared as resistant to most of the antibiotics that are widely used in the treatment of infections, this status of bacterial isolates' ability to be resistant to antibiotics indicated its importance because they were capable to cause infections and diseases after transmission from contaminated yoghurt samples.

Conclusion

In this study we noted that locally made yoghurt samples obtained from factories in Dohuk city, were constitute a high risk of health hazard to the consumers because it was concluding a variety of pathogenic microbial which caused to become the yoghurt samples as an acceptable for human consumed. This suggests that it is necessary to apply strict hygienic actions during production, processing, and delivery of yoghurts to avoid contamination with unwanted resources and microorganisms.

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Volume 19 Issue 6 June 2023