The antimicrobial role of emulsifying salts against *E.coli* O157:H7 contaminated local markets soft cheese of Baquba city

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Summary

The aim of this study was to investigate the degree of contamination of locally produced soft cheese samples by *Escherichia coli* O157:H7 and to determine the ideal emulsifying salts and their appropriate ratios for emulsification of soft cheese and their impacts on microbial load of that influence on public health. Samples were collected randomly from five popular markets affiliated to the city of Baquba: 60 samples of the local soft cheese produced by farmers at a rate of 30 samples for each of the summer season, from the beginning of July to the end of August, while the other thirty samples of winter season were collected from the beginning of December to the end of January to study their bacterial load of coli form and Escherichia coli especially *E. coli* O157:H7. The result showed total bacterial count was characterized by high significance ($P<0.01$) in the local cheese samples of both summer and winter seasons. The results proved the bactericidal and bacteriostatic effect of emulsifying salts on microbial activity was confirmed when the total bacterial count was significantly ($P<0.01$) reduced in soft cheese with (2.5%) of emulsifying salts added. There was no growth of *E. coli* and *E. coli* O157:H7 after adding (2%) of emulsifying salts to nutrient broth and the results confirmed that the best mixing of the components of emulsifying salts that is made up of (90% Sodium tripolyphosphate + 10% Trisodium citrate).

Keywords: Cheese, *E. coli*, Emulsifying salts.

Introduction

Higher microbial prevalence in the soft cheese was owing to primitive techniques in the manufacturing process, transfer and circulation of this as well as the non-arrival of heat treatment used to sufficiently work to eliminate pathogenic microbes that may be present in the raw milk used in manufacturing process. So it is appropriate to determine the quality and the ratio of emulsifying salts that are used in this emulsifiers of manufactured locally typical soft cheese will lead to the elimination of some pathogenic microbes and these will lead to a reduction of microbial level that have a role in the quality and shelf life of the consumed cheese (1-3). In order to focus on the role of contamination of milk in a scientific manner and cheese products produced locally, in particular in the epidemiological of *Escherichia coli* O157:H7, and its relationship to public health of the consumer, studies have shown that dairy cows are the main reservoir for this bacteria (4). This study was conducted following the isolation and diagnosis techniques of *Escherichia coli* O157:H7 from the local cheese products over the summer and winter season. Estimated annual incidence by *Escherichia coli* O157:H7 in Iraq is 6.810 as the population of (25.374.6912) million (5-7).

The largest recorded infection by *Escherichia coli* O157:H7 in (1996) was that outbroke infection which occurred in Japan and infected 6300 School children and caused two deaths, while centers specialized medical recorded in recent years approximately (500) cases of plague annually, included (73000) cases combined with the death of 60 of these cases and thus increases the risk of these strain that consumer infection occur with low doses and estimated between (10 to 100 cells) and the period saw the latest in the various countries of the world increased In the incidence of this bacteria as a result of translated through a number of vectors contaminated food stuffs, particularly through milk and milk products (8-10).

The most reactions of *Escherichia coli* O 157:H7 is ideal except for being a non-fermented sugar sorbitol and non-fermented sugar ram nose and free from enzyme (Gelosoronidase) and grow this serotype on the infected gastrointestinal cells and characterized by its ability to move from one

person to another and that this serotype differs all other styles being produced Verotoxins for its effects on pathogenic cells natural vero of Green kidneys of monkeys and also called Shiga-like Toxin (SLT) represented by toxin (SLT1) or (SLT2) or both (11).

These bacteria concentrated in the gastrointestinal tract of the human being as a result of their ability to acidic tolerance of the food and that ability back to the exposure of the intestinal flora to low level of pH, making it adapted to living in the food acid or that this ability back to own of these bacteria for a particular gene, which encodes a factor responsible for their ability to live in as low acid up to less than (pH>2.5) therefore it have the ability to tolerant the stomach acidity up to the colon, which is the area of infection and of symptoms of diarrhea, where these bacteria attach themselves to the surface of the intestinal tract and caused attaching and effecting lesion of it is minute Valais, or it may produce Verotoxin and the relevance of toxin-cell targeted absorbed into it happen deaths and damage of this the cells and revealed the presence of high concentration of toxin receptors (SLT) in the renal tubules epithelial cells of Human kidney, which leads to increased toxin combination and finally kidney failure due to cells distraction and thus increases the efficiency of the pathogenesis of these bacteria as a result of it is a low-dose compared to the rest of other bacteria related to the same species (12).

Through the employed researchers in the cheese industry experiences and as a result look for a way to save the dry cheese and semi-dry observed the separation of cheese ingredients (water, fat and protein) from each other during cooking, which led to the search for chemical compounds have the ability to prevent separation in addition to its ability to spread the protein in the emulsion. Use Habicht in year (1934) part of emulsifying salts known at the present time (13), which are organic structures with a membership with mono roots compounds parity and positively charged and many other parity negatively charged which working as a dispersant of protein and thus helps to dissolve as well as the emulsification of fat and also found that some inappropriate for use in the cheese industry and for reasons and considerations of economic. Joha emulsification salts was found in (1936) and used in the cheese industry (14) with great success, and there are about 20 of these types of salts suitable for the manufacture of all kinds of cheeses, and vary these salts in the ability of them to bring the spread of the protein and the impact on the pH of the mixture of cheese the user and the ability to resist changes in pH (their solutions to a concentration of 1%) and divided into acidic and alkaline salts and neutralized. The work of emulsifying salts is to stop calcium effectiveness, which affects the stability of the gel cheese and first characteristic of salt emulsifying ability to break the casein through a homogeneous mass production (15) and salts emulsifying ability to influence the product properties of the bacteriological through its impact bactericidal or bacteriostatic for growth of bacteria (16). Emulsifying salts used in cheese are tri-sodium citrate salts, sodium monophosphate salts, sodium pyrophosphate, Penta sodium tripolyphosphate and Graham salts, Hexametaphosphate, sodium trimeta phosphate and sodium tetra meatphosphate.

The aim of this study was to investigate the degree of contamination of locally produced soft cheese samples by *Escherichia coli* O157:H7 and to determine the ideal emulsifying salts and their appropriate ratios for emulsification of soft cheese and their impacts on microbial load of that influence public health.

Table, 1: The percentage of the four components of mixtures of emulsifying salts used in the experiment and the results of the change in pH.

Components of mixture of emulsifying salts%	Mixture No.(1)	Mixture No.(2)	Mixture No.(3)	Mixture No.(4)
Sodium tripolyphosphat	90	90	95	100
Trisodium citrate	5	10	---	---
Sodium carbonte	5	----	5	---
pH	10.23	10.23	10	9

Materials and Methods

Total count of *Escherichia coli* O157:H7 First: Standard-Plate-Count (SPC). Taking 11 grams of a sample to be tested and put in a blender and add (99 ml) of 2% sodium citrate solution its temperature of (45 ˚C) and mix on high speed for a period of (5 min.) to be

obtained on the sample liquid. Conducted on the sample series dilution and using two petridishes for each dilution and then transfer of 1 mL and 0.1 mL of dilution to each petridish and pour Cefixim Tellurite (CT)-Sorbitol MaCconky (SMAC) media to diluted petridish sample and the laying of 1 ml of the diluted and incubated at a temperature of (37 °C) for (24 hours) for the purpose of isolating the bacteria *Escherichia coli* O157:H7. Growth of *E. coli* in the form of colonies of pink color to red while non fermented sorbitol suger, which including the *E. coli* O157:H7, if any, will grow in the form of colorless colony to almost gray and smooth with a hazy center and its diameter (1.2 mm). Than selection petridish Zorai a decimal mitigation optimization which range from the preparation of the developing colonies and scrupulous diagnosed between (15-150) colony. Calculated prepare total colonies and takes the rate, and then multiply the inverted dilution for the number of colonies/g of cheese sample (CFU/g) (17-19).

Selection of colonies and cultured this colonies on the nutrient agar and incubated for 24 hours at a temperature (37 °C). Hold the modalities of serotype using slid agglutination test as checking my assurance to diagnose *Escherichia coli* O157:H7 providing counter-worshiper specific antigen physical (O157) and antigen flagellate (H7) of this serotype. Using several special for this serotype (Oxoide @ LatexO157 Serotyping Kits) which includes four reagents which are: (Control+Ve, Control-Ve, O157 test latex, O157 Control latex). According to the result of positive to own isolation bacterial antigen O157, provided you do not receive the correlation with the detector control. The effect of emulsifying salts on the *E. coli*: After confirmation of installing of bacteria (18 and 19) took one standard platinum loop of colonies of bacteria Pure grown in nutrient broth and incubated at a temperature 1 ± 23°C for 1 ± 24 hour. Then been taking (1 ml) of nutrient broth-grown and underwent serial dilution with salt solution or phosphate buffer solution and calculate the number of bacteria in each (1 mL) of the broth, then was added (2, 2.5 and 3) grams of powder emulsification salt of the mixture No. (2) in the (Table,1) above and used in the experiment per (100 ml) of the broth-grown and then by preparing colonies of bacteria developing after the addition of emulsifying salt. Means were compared using Unpaired *t*. test while the proportions were compared using Chi-square test. $P<0.05$ considered significant.

Results and Discussion

Health international organizations have considered humans to this pathogen are important health and economic paramount because they cause acute intestinal illness or serious satisfactory complications or both and infection occur when eating contaminated food either as a result of the presence of the bacteria itself or due to the presence of toxins produced by show infection within hours and up to days. Through studies and reports issued by the medicines and food (20) and the Centers for Disease Control (21) shows that the incidence of annual casualty by *E. coli* O157:H7 may be high (which are 1.06 cases/10000), but the seriousness of the infection and death rate are low (which are 0.94 death ratio /100 cases) compared to other bacteria the proportion of low infection but incidence the death ratio is high. We have provided the EU Directives (22) that the number of total bacterial count not more than 100,000 cells per ml of milk or milk products. But microbiological analysis result proved that the cheese factory samples locally and collected randomly from five popular parties subordinate markets of the city of Baquba, at a rate of 30 appointed by each of the summer season, from the beginning of July to the end of August and the winter season from the beginning of December to end of December and all of these samples were of a low level in terms of quality, health and non-conforming domestic and international. It was found the results (Table, 2) the number of positive samples to the total number of samples and the proportions of the isolation and the rates of the total count of bacteria E. coli serotype (O157:H7) in the local cheese for two seasons, summer (the month of July and August) and winter (the month of December and January), while showing results (Table, 3) the number of positive samples to the total number of samples and isolation ratios and rates of the total count of bacteria *E. coli* O157:H7 in the local cheese in popular areas

that have been the samples were collecting them for the summer season and the winter in both (Tables, 2 and 3), It find that the overall rate of rates of presence of bacteria *E. coli* O157:H7 in these products was 50% and consistent results presented with what it referred to both (23 and 24), where there was a rise in the overall rate ratios for the presence of bacteria *E. coli* in the local cheese products but difference of nearly a decade between the date of the research referred to.

Table, 2: Isolation ratio and the rate of bacterial count of *E.coli* O157:H7/g in stores popular areas.

Popular areas	Total sample count/(+ve) sample in summer	Total sample count/ (+ve) sample in winter	Total sample count/Total (+ve) sample	Isolation ratio (%)	Mean of microbial count of *E.coil* O157:H7
1	6/4	6/3	12/7	34	1.4×10^{6}
2	6/4	6/3	12/7	34	1.8×10^{5}
3	6/2	6/2	12/4	32	3.7×10^{6}
4	6/4	6/2	12/6	50	4.2×10^{5}
5	6/3	6/3	12/6	50	4.2×10^{6}
Total	30/17	30/13	60/3o	50	5.1×10^{5}

Table, 3: Bacterial isolation of *E. coil* O157:H7/g of local cheeses sample during the probationary period.

Month	Total sample No./ (+ve) sample No.	Isolation ratio (%)	Mean of microbial count of *E.coil* O157:H7/gm
July	15/9	6o	2.7×10^{6}
August	15/8	53.3	3.2×10^{6}
December	15/7	46.6	7.5×15^{5}
January	15/6	40	6.8×10^{5}
Total No.	30	50	5.1×10^{5}

While confirming the results (Table, 4) that the seasons of the year effect on the total bacterial count of soft cheese and both coliform bacteria and *E. coli* and *E. coli* O157:H7 as shown statistical results of the scan and there is a highly significant difference (P<0.01) in the rates of total bacterial count in the summer season than the winter season. This rise in number is elevated due to many reasons including the appropriate degree air to the growth and reproduction of these bacteria and the increasing incidence of mastitis and an increase subtracting the bacteria with the droppings of cattle during the summer season and poor application of sanitary laws when production and marketing and supply in addition to the rapid multiplication of bacteria in cheese products locally when the temperature becomes close to the optimum for their growth during the summer season, where exposed to conditions of cooling and thawing repeated because of a power outage during storage in addition to keeping them for long periods in the retail and not consumed shortly before the citizen, which offered these circumstances and length of periods and these result are consistent with what he referred to both (23 and 24). It also notes the existence of a highly significant difference (P<0.01) in the rates of total coliform count in the summer season for the winter season, while there was no significant difference in the high counting rates of coli form bacteria and *E. coli* O157:H7 through the seasons and attributed this to continue in the high count to many reasons which influenced cows to clinical and subclinical inflammation of the udder which caused by *E. coli*, and continue to eliminate *E. coli* O157:H7 with cows droppings being longer repository president of these bacteria, in addition to the mentioned above, and thus these results are consistent with what he referred to both (23 and 24).

Table, 4: compared to the bacterial count of the local soft cheese samples for both summer and winter season rates.

CFU/gm	Summer Total bacterial count± (S.E)	Winter Total bacterial count± (S.E)	Significant ** High significant difference (P<0.01)
Total bacterial count	$10^{7}\times1.80$ $-10^{7}\times12.083$ $\pm10^{7}\times0.086$	$10^{7}\times1.20$ $-10^{7}\times9.5$ $\pm10^{7}\times0.087$	**
Total coliform count	$10^{4}\times5.1$ $-10^{5}\times1.35$	$10^{4}\times1.03$ $-10^{5}\times5.0$	**
E.coil count/gm	$10^{4}\times8.2$	$10^{4}\times7.5$	Non significant
E.coli O157:H7 count/gm	$10^{5}\times5.4$	$10^{5}\times4.8$	Non significant

The results of the (Table, 5) the presence of high significant difference (P<0.01) in the rates in the total coliform count and *E. coli* O 157:H7 before and after the addition of (2, 2.5 and 3%) of emulsifying salts into nutrient broth and this attributed difference is due to changes in the pH of the nutrient broth to become the alkaline by emulsifying salts additive and is not valid for the growth of these bacteria, where the pH of nutrient broth (7.2) add the pH become (9.8, 9.85 and 9.9),

respectively after adding emulsifying salts, which lead to a lack or prevent of growth of both *E.coli* and *E.coli* O157:H7 in nutrient broth samples, these findings are consistent with what he referred to both (23 and 24). The results of (Table, 6) that for some salts emulsifying salts have bactericidal or bacterostatic effect (16) so when adding concentration (2.5%) of emulsifying salts from the mixture (2) that is used in the experiment and there was low significant difference (P<0.01) in total bacterial count 1.2×10^6 to 6.7×10^4 and total *E. coli* 1.5×10^6 to 8.2×10 and also *E. coli* O157:H7 5.1×10 to 4.1×10 CFU/g, and attributed this difference as a result of bacteriostatic by emulsifying salts and also these results are consistent with what he referred to both (23 and 24).

Table, 5: Count rates *E.coil* O157:H7/ g samples nutrient broth Effect ratios emulsifying salts.

Emulsifying salts%	0%	2%	2.5%	3%	Significant level **High
pH	7.2	9.8	9.85	9.9	--
E.coli count/gm	B $10^6\times1.14$	A 0	A 0	A 0	**
E.coli O157:H7 count/gm	B $10^5\times5.1$	A 0	A 0	A 0	**

*Significant difference (P<0.01)

Table, 6: The effect of adding mixture (2) a ratio of (2 .5%) on total bacterial count and *E. coil* O157:H7 count.

Emulsifying salts %	0%	2.5%	Significant level **High
Total bacterial count/gm	$10^6\times1.2$	$10^4\times6.7$	**
E. coli count/gm	$10^6\times1.0$	$10^3\times8.2$	**
E.coli O157:H7 count/gm	$10^5\times5.1$	$10^3\times4.1$	**

*Significant difference (P<0.01)

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دور أملاح الإستحلاب كمضاد بكتيري لجرثومة الايشريكيا القولونية نمط (O157:H7) الملوثة للجبن الطري المحلي في أسواق مدينة بعقوبة

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الخلاصة

التجربة شملت دراسة تلوث عينات من الجبن الطري المحلي بأعداد الايشريكيا القولونية نمط (O157:H7) وتحديد أنواع الاستحلاب النموذجيه ونسبها الملائمة لاستحلاب الجبن الطري وتأثيرها في التلوث الجرثومي المؤثر في الصحة العامة. جمعت العينات جمعاً عشوائياً من أسواق خمسة أطراف شعبية تابعة لمدينة بعقوبة (60) عينه من الجبن الطري المحلي المنتج من الفلاحين والبقالين وبمعدل (30) عينه لكل من الموسم الصيفي من بداية تموز إلى نهاية آب والموسم الشتوي من بداية كانون الأول الى نهاية كانون الثاني لدراسة التلوث الجرثومي من بكتريا القولون وجنس الاشريكيا خصوصا نوع الايشريكيا القولونية نمط (O157:H7). تميز العد الجرثومي الكلي بالارتفاع العالي المعنوية ($P<0.01$) في عينات الجبن المحلي للموسم الصيفي والشتوي. وقد أثبتت النتائج التأثير القاتل أو المثبط لنشاط الجراثيم بواسطة أملاح الإستحلاب المستعملة حيث انخفض معدل العد الجرثومي الكلي بفرق عالي المعنوية ($P<0.01$) بعد إضافة وخلط (2.5%) من أملاح الإستحلاب مع الجبن الطري المحلي المفروم مباشرة وانعدام نمو الاشريكيا القولونية وجراثيم الايشريكيا القولونية نمط (O157:H7) بعد إضافة (2%) من أملاح الإستحلاب ألى المرق المغذي لنمو الجراثيم وتؤكد النتائج بأن أفضل خلطة لمكونات أملاح الإستحلاب للجبن الطري المحلي هي تتكون من 90% الفوسفات المتعدد لثلاثي الصوديوم مضافة إلى 10% من سترات ثلاثي الصوديوم.

الكلمات المفتاحية: جبن، ايشريكيا قولونية، أملاح الإستحلاب.