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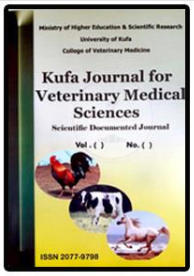

The investigate of Diarrheagenic *Escherichia coli* (DEC) by Conventional Methods from Cheese of Awassi ewes milk and its Effect in Public Health

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Abstract

The study conducted to investigate of the Diarrheagenic *Escherichia coli* (DEC) microbial load in which contaminate the locally produce cheese from milk of local Awassi breed ewes . And to indicate the effect of different types and concentrations of mixture' s of Emulsifying salts to choose the best mix of them that reduce the Diarrheagenic *Escherichia coli* (DEC) microbial load in this locally produce cheese . A 60 sample Cheese locally produced from milk of Awassi ewes were been collected randomly from Local Awassi Flock of College of Agriculture at Baghdad University (Iraq) , (30 samples to each winter and spring season) to investigate their microbial load . Both of all winter and spring samples were in high significant ($p<0.01$) microbial count . The Bacteristatic & Bactericidal effect of Emulsifying salts on microbial activity was confirmed when each of the Total Bacterial Count (TBC), Total Coli form Count, Total *Escherichia coli* Count and Total Diarrheagenic *Escherichia coli* (DEC) Count were highly significant ($p<0.01$) reduced in the cooked Cheese Samples with 3% Emulsifying salts composed of (90% ($Na_5P_3O_{10}$) + 10% ($Na_3C_6H_5O_7$)) were being added

Key Words :- Cheese , DEC , Emulsifying salts , Awassi ewes.

استخدام الطرق المخبرية التقليدية للتحري عن الأيشريكيا القولونية المسببة للأسهال الملوثة للجبن المصنع من لبن نعاج العواسي المحلي وتأثيرها في الصحة العامة.

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الخلاصة :

استهدفت هذة الدراسة قياس درجة تلوث عينات الجبن المصنع من اللبن المنتج محليا في بغداد (العراق) من نعاج العواسي بأعداد جراثيم الايشريكيا القولونية المسببة للأسهال (DEC) والملوثة لعينات هذا الجبن المحلي وتأثيرها في الصحة العامة وتأثير استخدام أنواع وتراكيز مختلفة من أملاح الاستحلاب في تصنيع هذه الاجبان وثاثيرها في هذة الحمولة الجرثومية . حيث جمعت بصورة عشوائية 60 عينة من الجبن المصنع محليا من ألبان نعاج قطيع أغنام العواسي التابع لكلية الزراعة / جامعة بغداد , العراق , وبمعدل 30 عينة لكل من الموسم الشتوي من بداية كانون الاول إلى نهاية كانون الثاني والموسم الربيعي من بداية آذار إلى نهاية نيسان , لدراسة حمولتها الجرثومية من هذه الجراثيم التي تميزت بالارتفاع العالي المعنوية ($P<0.01$) في عينات الموسم الشتوي والربيعي . وأثبتت النتائج التأثير القاتل أو المثبط لنشاط الجراثيم بواسطة أملاح الاستحلاب المستخدمة في عملية الطبخ حيث انخفض معدل العد الجرثومي الكلي في الجبن المطبوخ بفرق عالي المعنوية ($P<0.01$) بعد إضافة

وخلط 3% من خلطة أملاح أستحلاب متكونة من 90% الفوسفات المتعدد الثلاثي الصوديوم + 10% سترات ثلاثي الصوديوم .

كلمات مفتاحية -: جبن , الايشريكيا القولونية , أملاح استحلاب , نعاج عواسي

Introduction

In the various countries of world, Zoonotic diseases among humans and animals become widely scattered, and animal products is an important reason for the transmission of these diseases to humans, The suggest local estimates of the national survey of livestock for the year 2008 by the Central Statistical Org**. (CSO)** and Ministry of Agriculture, Iraq (1) that the number of sheep in 2008 amounted to (7.722.375) constitute 63.86% of the number of total animals of the country , and *Awassi* breed constitute 60-65% of this total local sheep breeds (2), where there is widespread in some northern areas of the central region of the country and one of the most local breeds of sheep number in Iraq . The cheese factory in sheep breeders houses from the raw milk of ewes have a big role in the transfer of many of the pathogens to humans because of the high content of bacteria resulting from the non-use of thermal treatment or these transactions are insufficient to eliminate pathogenic bacteria in milk intended for the manufacture, or as a result of the pollution due to primitive methods used during production, transportation and trading. the incorrect thermal treatment and determine the quality of Emulsifying salts typically and appropriate proportions of emulsification cheese manufactured locally will lead to the elimination of this bacteria that resides in it and work on the reduction of germ payload that have a significant role in determining the quality and lengthen the period of validity for human consumption (3;4;5;6;7;8).

On this basis and because of the lack of such a cheese with health conditions, the determining quality will remain dire because of the weakness of strict sanitary measures. *Vis* , the application of the health measures and conditions would lead to the elimination of pathological bacteria that exist within it and to the reduction of microbial load that have a significant role in the quality and prolong its Shelf-Life for human consumption ,focusing on the role of the contamination of milk products in general and cheese produced locally in particular, in the process of epidemiological Diarrheagenic *Escherichia coli* (DEC) spp. And its relationship to Public Health and for the purpose of the consumer to know the true size of the problem locally, this study was carried out by following techniques to isolate and diagnose from the local milk products over the winter and spring season. (9;10).

In Iraq, the estimated annual incidence rate of default *E.coli* stands at 6.810, considering that the population 25,374,691 people (11;12). *E.coli* Isolated for the first time from the feces of children sampled in Germany in (1885) by (Theodor Von Escherich), While (Lareull) was first postulated its Pathogenesis in (1889) (13), *E.coli* is the most important causes of coli-form non relatively pathogen or opportunistic pathogen and exists naturally in colon of human and animal warm-blooded. Preparing unhealthy products and with inefficient thermal treatment and pollution-winning post-production to make the cause known to spread (8;9;10). There is a strain of the Diarrheagenic *E.coli* serotype (O157:H7) also called EHEC or STEC due to producing of the toxin. Its infection happening by drinking contaminated water or the consumption of contaminated milk products untreated thermally. Knowing infection of this strain for the first time in 1982 in the states of Michigan and Oregon

in America, as the biggest pathogenic infection recorded in 1996 at Japan (14). Most reactions *E.coli* O157:H7 pattern is ideal and that this pattern differs from other styles being produced toxins (Verotoxin) and effects of pathogenic cells and natural Vero College of green monkeys, also called Shiga-like Toxin (SLT) and include toxin (SLT1) and (SLT2) , In recent period in various countries around the world increase in the incidence of this bacteria as a result of this transition through a number of vectors contaminated food products with this pathogen (5;6;7;12).

Studies reported that the incidence and deaths associated with *E.coli* O157:H7 bacteria is focus on them through the impact of the disease, toxin-producing as well as the clinical symptoms, found that all ages of both sexes are susceptible to newly infected with this bacteria but children, the elderly and persons with low level of immune system are most susceptible (12).It occurs when eat food contaminated with the result of the presence of the bacteria itself or because of the presence of toxins produced by show of infection within hours and up to days (5;6;7;12).

International health organizations deemed of human infection with this pathogen of health and economic importance for being a top cause severe or serious medical complications or both intestinal injury (15). Through studies and reports issued by the FDA and CDCs, it shows that the incidence of annual infection with *E. coli* O157:H7 germs be high, but the seriousness of the injury and the death rate is low (which is 0.94 deaths/100 cases) compared to other germs the incidence of infection is low, but the high rate of death (16;17;18;19).

By the experiences of researchers working in the Cheese industry, a result of the search for a way to save the dry and semi-dry Cheese 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 protein in emulsion (3;4;20) . Habicht at 1934 used part of Emulsifying salts known at the present time (21), which are organic compounds with mono-valent roots and positively charged and many other parity negatively charged, where its works as a dispersant of protein and thus help to dissolve as well as the emulsification of fat, but found that some of them not suitable for use in the Cheese industry and for reasons that economic considerations . **Joha** E.salt found in 1936 and used in the cheese industry (22) there are about 20 species of these salts to fit all kinds of cheese industry, they vary salts in the capacity to bring about the spread of the protein and its effect on the pH of the mixture of Cheese the user and its ability to resist pH changes ([1%] solutions) and has been divided into: acidic, alkaline and neutral E.salts , its works to stop calcium effectiveness, which affects the stability of the gel Cheese , the first property of E.salt is the ability to dissolve the casein to produce a homogeneous fluid (23). its has the ability to influence the properties of keeping the product of the bacteriological through its (Cidal or Static) antibacterial impact (24).

- Materials&Methods:

- The Samples Preparation :

a-The process of the local soft cheese manufacture: - It was, according to the (3;4): by putting the milk drained in a vase and warms to the point of 38C and add the rennet at a rate of 2.5g/50kg milk and mix well for 5m. and cheese-forming process occur during 2-3h. and drain the clot formed with a piece of muslin cloth to get rid of whey and placed in molds refinery clot pressing weights.

b-Processed cheese manufacturing locally: - It was, according to the (3;4): Using a

manufacture soft cheese where the local soft cheese cut into slices and mince electric with machine and put in the cooking pot and added 2% E.salts the mixture no.(2) in table (1) then was cooked at 85C/30m. and then was packed in 100g. capacity containers .

- The Micro-biological tests:

a- Total Bacterial Count (TBC) by the way of Standard-Plate-Count (SPC).Procedure ,(11 grams) of the sample was taking 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 (5 min.) to be obtained on the sample liquid. Conducted on the sample series dilution and using two petri dishes for each dilution and then transfer of 1 ml. and 0.1 ml. of dilution to each dish and pour Cefixim Tellurite (CT)-Sorbitol MaCconky (SMAC) media to diluted dish sample and the laying of 1 ml. of the diluted and incubated in (37 C/24 h.) for the purpose of isolating the *E.coli* , the growth of *E. coli* in the form of colonies of pink color to red while non fermented sorbitol sugar, which including the DEC if found , will grow in the form of colorless colony to almost gray and smooth with a hazy center and its diameter (1-2 mm). Then select cultured petri dish 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) . Selection of colonies and cultured this colonies on the nutrient agar and incubated in 37C/24 h. (25;26;27;28;29;30).

b- confirmatory testing (Bio-chemical ractions): - Hold the modalities of serotype using slid agglutination test as checking my assurance to diagnose DEC using special kit for this serotype described in (7;20).

c- The effect of Emulsifying salts investigate after confirmation of installing of bacteria by took one standard platinum loop of colonies of bacteria Pure grown in nutrient broth and incubated in 23C/24 h. 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 Emulsification salt powder of the mixture No. (2) in the (Table,1) 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.

-Statistical Analysis:

The data were analyzed according to statistical software, Statistical Package for the Social Sciences (SPSS, version 21, 2013),

- Results & Discussion

The percentages of the components of four Mixes of Emulsification salts used in the experiment and the results of the change in pH are showed in table No. (1) .

The proven Micro-Biological results analysis that cheese samples locally produced from the milk collected at random from the herd sheep Awassi of the Faculty of Agriculture / University of Baghdad, Iraq, and at a rate of 30 samples for each of the winter season from the beginning of December to the end of January and the spring season from the beginning of March to the end April , all of these samples were of a low level of health and in terms of quality and not in conformity with local and international standards ,(31,32), and "The European Union directives (33). in which states that Total Bacterial Count (TBC) does not increases for (10^5 bacteria/ml) " .

The results of table (2) shows the number of positive samples to the total number of samples and the proportions of the isolation in Cheese samples in winter seasons

(months 12 and 1) and spring (month 3 and 4), and we find that the overall average of the rates of the presence of the bacteria CFU / g ratios in these products was 50% .

The results of table (3) shows the effect of the seasons of the year on each of the total bacterial count (TBC) , total coliform count (TCC) , *E.coli* count and the Diarrheagenic *E.coli* (DEC) count , where the statistical test results showed a significant difference (P<0.05) in the (TBC) and (TCC) rates CFU/g. of the spring season for the winter season , this high count attributed to many reasons including an appropriate degree air heat to the growth and reproduction of these germs, an increase subtracting the germs shedding with the droppings of cattle during the spring season, the lack of efficiency of the thermal treatment or pasteurization of raw milk, misapplication of health law at the production, marketing and supply in addition to the rapid multiplication of this bacteria in the local Cheese products when they become in a temperature close to the optimum for their growth through spring season where exposed to conditions of repeated cooling and thawing because of a power outage during storage. in addition to survival for long periods in the retail and not consumed in shortly time exposing them to these conditions for longer periods. It also notes from the results table (3) there is a significant difference (P<0.05) in the count rates CFU/g. of the spring season for the winter season and attributed this rise to advanced reasons as there is a relationship between growth and reproduction of this bacteria in milk and temperatures different note during the seasons of the year in addition to the increased incidence in cows affected by the Clinical and sub-clinical Mastitis, .

The results of table (4) shows the existence of a highly significant difference (P<0.01) in each of the total bacterial count (TBC) , total coliform count (TCC) , *E.coli* count and the Diarrheagenic *E.coli* (DEC) count rates before and after the addition of (2, 2.5 and 3%) of Emulsifying salts into Nutrient Broth and attributed this difference is due to changes in the pH of the nutrient broth to become the baseline by Emulsifying salts are added and is not valid for the growth of these bacteria. Where it was pH 7.2 and became after the addition of salts (9.8 , 9.85 and 9.9), respectively. Resulting in a reduction of the growth of the bacteria in nutrient broth samples .

The results of table (5) shows that the (Cidal) or (Static) antibacterial effect of some Emulsifying salts. When 3% concentration of the mixture number (2) used in this experiment which composed of (90% Sodium tripolyphosphate + 10% Trisodium citrate) of this salt in made experiment Cheese, and there was a highly significant decrease (P<0.01) in the CFU/g. and this difference attributed as a result of inhibiting the bacteria bacterio-static effect by Emulsifying salts on the Bacteria .

Table (1) : Emulsifying salts % used in the experiment and the results of the change in pH .

Emulsifying salts %	Mix(1)	Mix(2)	Mix(3)	Mix(4)
(Na_2CO_3)	5	-	5	-
($Na_3C_6H_5O_7$)	5	10	-	-
($Na_5P_3O_{10}$)	90	90	95	100
pH	10.23	10.23	10	9

Table (2) : Isolation from cheeses samples during the probationary period .

Season	**Month**	no. of (+) samples/total no.	Isolation %	CFU/g
Winter season	December+January	**13/30**	**43.33**	**7.6×10^5**
	December	**7/15**	**46.6**	**7.5×10^5**
	January	**6/15**	**40**	**6.8×10^5**
Spring season	March+April	**17/30**	**56.66**	**3×10^6**
	March	**9/15**	**60**	**2.7×10^6**
	April	**8/15**	**53.3**	**3.2×10^6**
Overall	Dec,Jan,Mar,Apr.	**30/60**	**50**	**5.05×10^5**

Table (3) : Samples compared count rates for both winter and spring season.

CFU	**Winter season** TBC±SE	**Spring season** TBC±SE	significant level
TBC/g.	**$5.4 \times 10^7 \pm 0.9 \times 10^7$**	**$7.6 \times 10^7 \pm 0.8 \times 10^7$**	*
TCC/g.	**$3.1 \times 10^5 \pm 0.9 \times 10^5$**	**$3.2 \times 10^5 \pm 0.8 \times 10^5$**	*
***E.coli*/g.**	**$7.5 \times 10^4 \pm 0.4 \times 10^4$**	**$8.2 \times 10^4 \pm 0.3 \times 10^4$**	Non-significant
DEC/g.	**$4.8 \times 10^5 \pm 0.4 \times 10^5$**	**$5.4 \times 10^5 \pm 0.3 \times 10^5$**	Non-significant

- SE = standard error.

* Significant difference (P<0.05).

Table (4) : count rates in samples Nutrient Broth affected by adding Emulsifying salts%

Emulsifying Salt %	**0%**	**2%**	**2.5%**	**3%**	Significance level
pH	**7.2**	**9.8**	**9.85**	**9.9**	**-**
TBC/g.	**B** **6.5×10^7**	**B** **1.4×10^2**	**A** **1.1×10^2**	**A** **1.0×10^2**	**
TCC/g.	**B** **3.1×10^5**	**B** **1.4×10^2**	**B** **1.1×10^2**	**A** **1.0×10^2**	**
E.coli/g.	**B** **7.9×10^5**	**B** **1.8×10^2**	**B** **1.3×10^2**	**A** **1.0×10^2**	**
DEC/g.	**B** **5.1×10^5**	**B** **1.8×10^2**	**B** **1.3×10^2**	**A** **1.0×10^2**	**

- Large different letters within the same column indicate Highly significant difference (P<0.01).

-** Highly significant difference (P<0.01).

Table (5) : the effect of adding 3% of Mixture No. (2) to Cheese samples.

Emulsifying Salt %	**0%**	**3%**	Significance level
TBC	**1.2×10^6**	**6.7×10^4**	**
TCC/g.	**1.0×10^6**	**8.2×10^3**	**
E.coli/g.	**5.1×10^6**	**8.4×10^3**	**
DEC/g.	**5.1×10^5**	**8.0×10^2**	**

** Highly significant difference (P<0.01).

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