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INHIBITORY EFFECT OF ENTEROCIN WITH SOME FOOD ADDITIVES ON *ESCHERICHIA COLI* O18 IN UHT MILK

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ABSTRACT

Biopreservation is the oldest method for food preservation by addition of natural compounds to the food to increase its shelf-life and inhibit the growth of foodborne pathogens. Enterocin is a protein produced by *Enterococcus faecium* which have a great effect on Gram-positive bacteria, but it has limited effect on Gram-negative bacteria. The aim of this work is to study the addition of food additives as honey and EDTA to increase the inhibitory effect of enterocin against *Escherichia coli* O18 in UHT milk stored at ambient and refrigerator temperatures. The UHT milk inoculated with *E. coli* O18 was divided into 8 parts as the following, the first part without any additives as control, the second with 10% honey, the third and fourth parts contained 10% honey with different concentrations of enterocin (150 and 300 µg/ml), respectively. Each part of these prepared four parts was divided into two groups; the first group was stored at ambient temperature (30±2 °C) while, the second group was stored at refrigerator temperature (4±2 °C) for 24 hours. The obtained results showed that, the most effective treatment was the 10% honey and enterocin (300µg/ml) and stored in the refrigerator where the count of *E. coli* O18 reduced from $6x10^6$ cfu/ml to $7.1x10^4$ cfu/ml at the end of 24 h. Also, another trail was done as the previous experiment except the replacement of the honey with EDTA (20 mM). The highest inhibitory effect of the treatments obtained by the addition of EDTA (20mM) with enterocin (300 µg/ml) which was stored at the refrigerator temperature. The count of *E. coli* O18 was reduced from $1.5x10^5$ cfu/ml to $3.6x10^4$ cfu/ml by the end of 24 h. In conclusion, the addition of honey or EDTA with enterocin and preservation of milk in refrigerator temperature reduced *E. coli* O18 count. In addition, this method can be used as a safe method for preservation of milk which consumed directly or used in manufacture the milk products.

Key words: Enterocin, honey, EDTA, *E. coli* O18, UHT milk.

INTRODUCTION

In the recent years, food safety has become an important issue in many countries. Inhibition of food poisoning and spoilage bacteria by using natural compounds is of a great interest to the food industry due to its public health and economic concerns. Biopreservation can be defined as addition of natural compounds which have antibacterial effect to the food to increase its shelf life and inhibit the growth of foodborne pathogenic bacteria (Stiles, 1996).

Bacteriocins are ribosomal synthesized antimicrobial peptides produced by bacteria including lactic acid bacteria (LAB). LAB are Gram-positive bacteria as Lactobacillus spp., *Lactococcus spp.*, *Streptococcus spp.* and *Enterococcus spp*. *Enterococcus spp.* include *E. faecium* and *E. faecalis*, which are natural inhabitant in the gastrointestinal tracts of humans and animals. These bacteria have great beneficial health effects in the host, which include the inhibition of the tumor cell lines (Ohasi *et al*., 1992; Park *et al*., 1999), treatment of diarrhea and reduction of hypercholesterolaemia (Reuter, 1997; Agerholm-Larsen *et al*., 2000).

Moreover, *E. faecium* produces bacteriocins, that inhibit foodborne bacteria and intestinal pathogens, and are used as biopreservatives in fermented dairy products. The class II enterococcal bacteriocins which include enterocins A, B, P and L 50 share the characteristics of low molecular weight, heat stability and non-lanthionin-containg peptide structure (Kang and Lee, 2005).

Enterocin causes antimicrobial activity by forming specific potassium ion-conducting pores in the cytoplasmic membranes of target cells, which causes a rapid and drastic efflux of the

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interacellulary accumulated potassium ions thus impairing the electrochemical transmembrane potential (Herranz and Driessen, 2005). However, generally most LAB bacteriocin are not active against the Gram-negative bacteria as the outer membrane of*E. coli*act as an impermeable barrier (Ruhr and Sahl, 1985; Kordel *et al.,* 1989; van Belkum *et al.,* 1991; García Garcera´ *et al.,* 1993), so it prevents molecules as antibiotics and detergents from reaching cytoplasmic membrane (Nikaido and Vaara, 1987). Therefore, the combination of bacteriocin with natural compounds, chemical and physical treatments lead to damage of the bacterial outer membrane and reduce of the foodborne pathogenic and spoilage bacteria significantly (Stevens *et al*., 1991; Kalchayanand *et al*., 1994; Schved *et al*., 1994; Cutter and Siragusa, 1995a and 1995b, Boziaris *et al*., 1998, Ananou *et al*., 2005; Osmanağaoğlu, 2005).

According to the aforementioned, the present study is directed to determine the efficacy of enteroicin on *E. coli* O18 in combination with natural compound as honey and chemical treatment as EDTA in the UHT milk stored in ambient and refrigerator temperatures during 24 h.

MATERIALS AND METHODS

1. Organisms identification:
E. faecium strain was isolated from cheddar cheese and identified by microbiological and molecular identification in Molecular Biology Research Unit, Assiut University, Egypt. The molecular identification based on *16S rRNA* gene sequencing using 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1392R (5' GGTTACCTTGTTACGACTT 3') primer pairs (Applied Biosystem, USA) (Srinivasan *et al.,* 2015). *E. coli*O18 strain was isolated from ice cream sample from a previous study by Ewida and Hussein (2018). Comparison of the *16S rRNA* gene sequences with entries in the updated GenBank database (www.ncbi.nlm.nih.gov/ pubmed) was conducted using the Blast program. In addition, the two isolated strains were submitted (in 2018) in the Genebank after complete identification.

2. Materials used:
UHT milk was purchased from the dairy shops in Assiut city, Egypt. Citrus flower honey was purchased from the Faculty of Agriculture, Assuit University, Egypt, while, the EDTA was used in the experimental obtained from WINLAB, UK, Cat. No. E 10203.

3. Culture conditions:
E. faecium was propagated in De Mann Rogosa and Sharp (MRS) broth (TM Media, India) at 37ºC for 24 h and *E. coli* O18 was grown in Enterobacteriaceae Enrichment Broth (EE broth) (Himedia, India) at 37 ºC for 24 h.

4. Enterocin preparation:
After *E. faecium* propagation in MRS broth, the culture was centrifuged at 10000 rpm for 20 min under cooling (4ºC) using cooling centrifuge (Jouan, UK) to remove cells. The cells free supernatant (CFS) was passed through membrane filters with pore diameter of 0.22 µm and then stored at 4ºC till use as pure enterocin (Djadouni, 2017).

5. Bacteriocin activity assay:
A well diffusion assay procedure was used for determination of enterocin activity (Schillinger and Lucke, 1989). 100 µl of the pure enterocin was placed in 5 mm-in-diameter well of the plates seeded with *Staphylococcus aureus* strain (Animal Health Research Institute, Assiut, Egypt) as bioassay strain and *E. coli* O18as tested strain (pre-cooled at 40°C of molten nutrient agar medium was inoculated with overnight culture obtained from single colony). After 24 h of incubation at 37 °C, the clear zones of inhibition appeared and recorded.

6. Effect of enterocin in combination with EDTA and honey on *E. coli* O18 in UHT milk:
E. coli O18 suspensions of($8.9X10^9$ CFU/ml) inoculated into UHT milk were exposed to the following treatments:(1) honey (10%), (2) honey (10%) with enterocin (150 µg/ml), (3) honey (10%) with enterocin (300 µg/ml), (4) EDTA (20mM), (5) EDTA (20mM) with enterocin (150 µg/ml), (6) EDTA with enterocin (300 µg/ml) and the last part of milk was inoculated with *E. coli* without any treatments. During the incubation at ambient and refrigerator temp for 24 h, samples were periodically taken and viable cell counts were determined using violet red bile agar (VRBA) plates at 37°C for 24 h.

7. Measurement of pH value:
The pH value of each sample was determined according to the standard methods of A.P.H.A. (2004) with pH meter (Hanna, Portugal) previously standardized with buffer solution of pH 4.0 and 7.0.

RESULTS

The strain of *E. faecium* was submitted in the Genbank and had accession number MH748622, while, the *E. coli* strain had accession number MH748624.

The enterocin activity was tested against *Staph. aureus* and the diameter of the inhibitory zone was 20 mm. On the other side, the entrocin didn't give any inhibitory effect on *E. coli* O18.

Table 1: The count and reduction% of *E. coli* O18 inoculated in UHT milk with 10%honey and enterocin at ambient temperature (30±2 °C).

Period	UHT milk Control (additive free)	UHT milk with honey 10%		UHT milk with honey & enterocin (150 µg/ml)		UHT milk with honey & enterocin (300 µg/ml)	
	Count cfu/ ml	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %
0 h	$6.0x10^6$	$6.0x10^6$	_	$6.0x10^6$	_	$6.0x10^6$	_
6 h	$2.0x10^8$	$9.0x10^6$	95.50	$5.0x10^6$	97.50	$1.0x10^6$	99.50
12 h	$9.4x10^{10}$	$2.9x10^{10}$	69.15	$1.1x10^8$	99.88	$3.0x10^6$	99.99
24 h	$1.9x10^{12}$	$3.7x10^{10}$	98.05	$2.5x10^{10}$	98.68	$5.0x10^7$	99.99

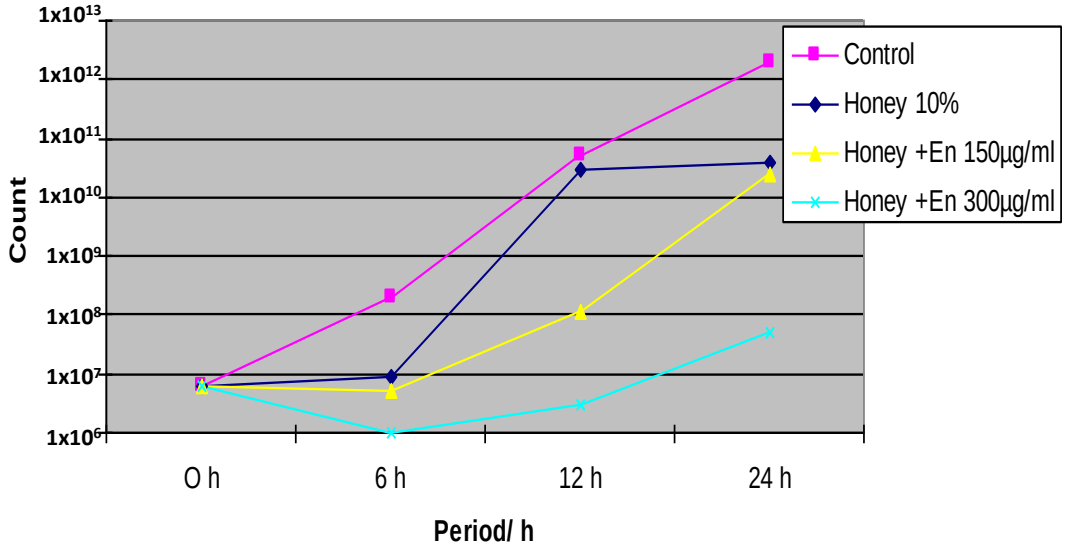

Figure 1: Chart showing *E. coli* O18 count that grown in UHT milk with 10% honey and different concentrations of enterocin stored at ambient temperature (30 ±2 °C).

Table 2: pH values of *E. coli* O18 inoculated in UHT milk with10%honey and different concentration of enterocin at ambient temperature (30 ±2 °C).

Period	UHT milk Control (additive free)	UHT milk with honey 10%	UHT milk with honey & enterocin (150 µg/ml)	UHT milk with honey & enterocin (300 µg/ml)
0 h	7.16	7.07	6.54	6.26
6 h	7.17	6.98	6.54	6.27
12 h	6.51	6.52	6.36	6.27
24 h	6.21	6.08	5.93	6.13

Table 3: The count and reduction % of *E. coli* O18 inoculated in UHT milk with 10% honey and enterocin at refrigerator temperature (4 ±2 °C).

Period	UHT milk Control (additive free)	UHT milk with honey 10%		UHT milk with honey & enterocin (150 µg/ml)		UHT milk with honey & enterocin (300 µg/ml)	
	Count cfu/ ml	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %
0 h	$6.0x10^6$	$6.0x10^6$	_	$6.0x10^6$	_	$6.0x10^6$	_
6 h	$1.1x10^8$	$1.8x10^5$	99.84	$7.0x10^4$	99.94	$1.1x10^5$	99.90
12 h	$1.5x10^7$	$4.2x10^5$	97.20	$2.6x10^5$	98.27	$2.0x10^4$	99.87
24 h	$2.7x10^7$	$2.0x10^6$	92.59	$9.9x10^5$	96.33	$7.1x10^4$	99.74

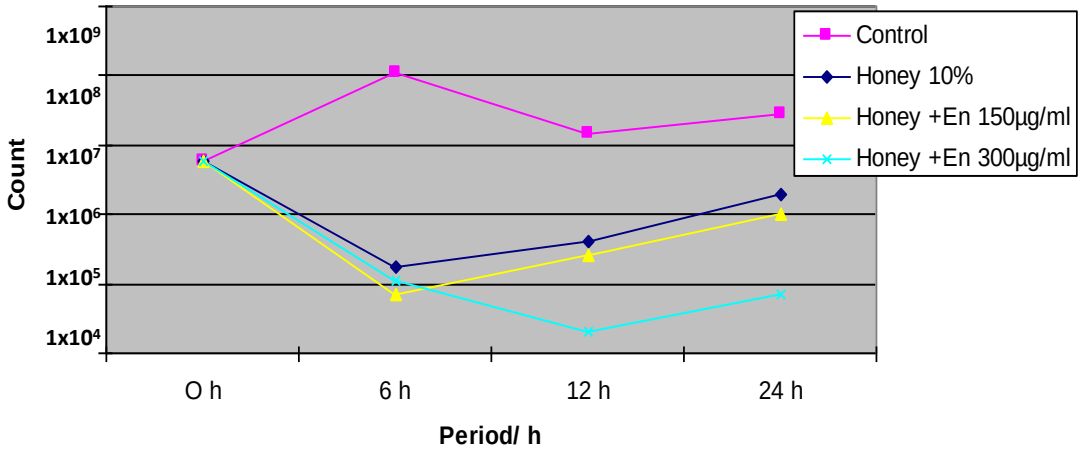

Figure 2: Chart showing *E. coli* O18 count that grown in UHT milk with 10% honey and different concentrations of enterocin stored at refrigerator temperature (4 ±2 °C).

Table 4: pH values of *E. coli* O18 in UHT milk inoculated with10% honey and different concentration of enterocin at refrigerator temperature (4 ±2 °C).

Period	UHT milk Control (additive free)	UHT milk with honey 10%	UHT milk with honey& enterocin (150 µg/ml)	UHT milk with honey& enterocin (300 µg/ml)
0 h	7.16	7.07	6.54	6.26
6 h	7.25	7.07	6.66	6.35
12 h	7.17	7.01	6.62	6.33
24 h	7.30	7.00	6.61	6.31

Table 5: The count and reduction % of *E. coli* O18 inoculated in UHT milk with EDTA (20 mM) and enterocin at ambient temperature (30±2 °C).

Period	UHT milk Control (additive free)	UHT milk with EDTA (20mM)		UHT milk with EDTA & enterocin (150 µg/ml)		UHT milk with EDTA & enterocin (300 µg/ml)	
	Count cfu/ ml	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %
0 h	$1.5x10^5$	$1.5x10^5$	--	$1.5x10^5$	--	$1.5x10^5$	--
6 h	$9.0x10^8$	$7.3x10^7$	91.89	$7.3x10^6$	99.19	$1.1x10^5$	99.99
12 h	$1.0x10^9$	$2.0x10^8$	80.00	$1.0x10^6$	99.90	$2.0x10^4$	99.99
24 h	$1.3x10^{10}$	$1.2x10^9$	90.76	$3.3x10^6$	99.97	$7.1x10^4$	99.99

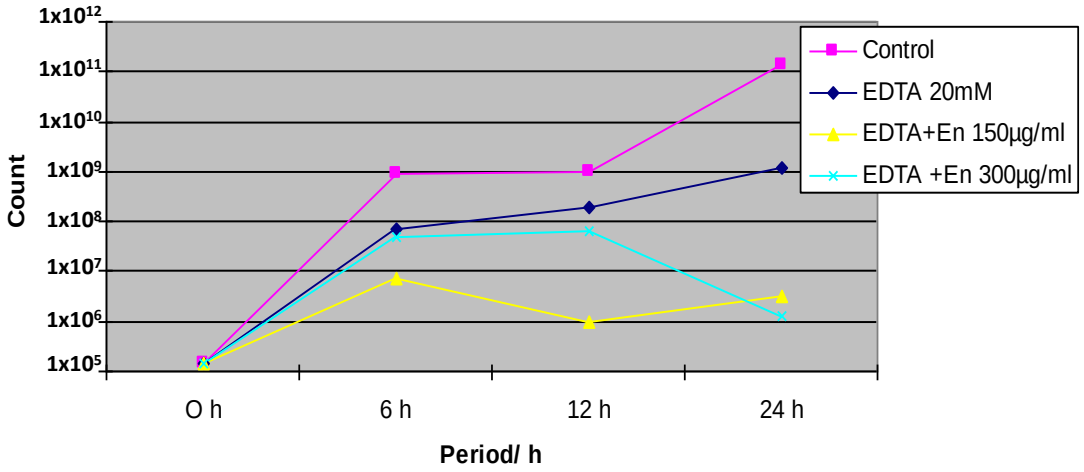

Figure 3: Chart showing *E. coli* O18 count that grown in UHT milk with EDTA (20mM) and different concentrations of enterocinstored at ambient temperature (30 ±2 °C).

Table 6: pH values of *E. coli* O18 in UHT milk with EDTA (20 mM) and different concentration of enterocin at ambient temperature (30±2 °C).

Period	UHT milk Control (additive free)	UHT milk with EDTA (20mM)	UHT milk with EDTA & enterocin (150 µg/ml)	UHT milk with EDTA &enterocin (300 µg/ml)
0 h	7.22	6.24	5.94	5.73
6 h	7.01	6.18	5.95	5.77
12 h	6.69	6.10	5.95	5.82
24 h	6.26	6.04	5.93	5.82

Table 7: The count and reduction% of *E. coli* O18 inoculated in UHT milk with EDTA (20mM) and enterocin at refrigerator temperature (4 ±2 °C).

Period	UHT milk Control (additive free)	UHT milk with EDTA (20mM)		UHT milk with EDTA & enterocin (150 µg/ml)		UHT milk with EDTA & enterocin (300 µg/ml)	
	Count cfu/ ml	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %	Count cfu/ ml	Red. %
0 h	$1.5x10^5$	$1.5x10^5$	_	$1.5x10^5$	_	$1.5x10^5$	_
6 h	$3.0x10^5$	$2.0x10^5$	33.33	$1.5x10^5$	50.00	$1.0x10^5$	66.67
12 h	$1.0x10^6$	$4.0x10^5$	60.00	$2.2x10^5$	78.00	$1.5x10^5$	85.00
24 h	$2.6x10^6$	$3.8x10^5$	85.38	$2.3x10^5$	91.15	$3.6x10^4$	98.62

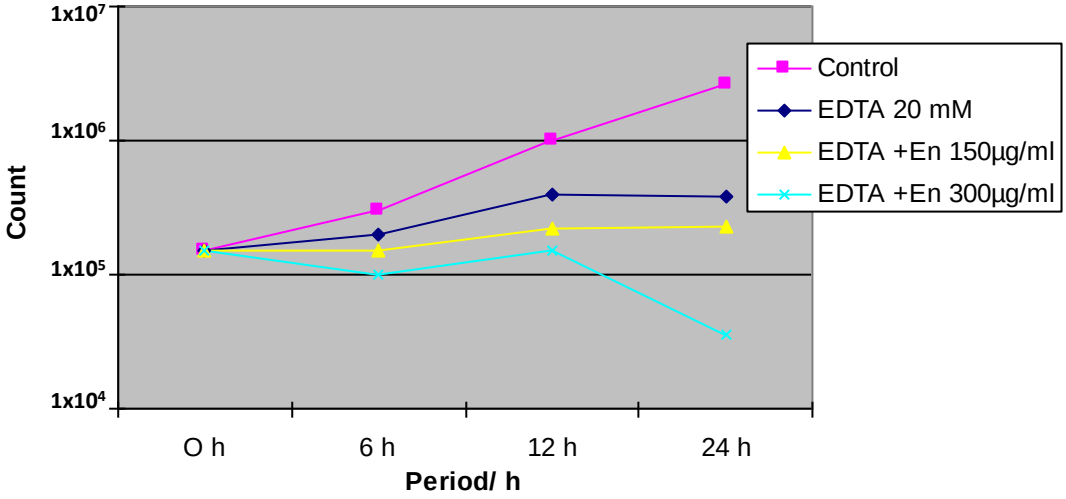

Figure 4: Chart showing *E. coli* O18 count that grown in UHT milk with EDTA (20mM) and different concentrations of enterocin stored at refrigerator temperature (4±2 °C).

Table 8: pH values of *E. coli* O18 inoculated in UHT milk with EDTA (20mM) and different concentration of enterocin at refrigerator temperature (4±2 °C).

Period	UHT milk Control (additives free)	UHT milk with EDTA (20mM)	UHT milk with EDTA & enterocin (150 µg/ml)	UHT milk with EDTA &enterocin (300 µg/ml)
0 h	7.22	6.24	5.94	5.73
6 h	7.23	6.29	5.95	5.78
12 h	7.26	6.31	6.01	5.80
24 h	7.29	6.31	6.01	5.85

DISCUSSION

Enterococci are found in wide range of environment including milk products and they play an important role in organoleptic characteristics of these products (Giraffa, 2003). They are also known to produce one or more bacteriocin which inhibit the growth of a wide range of foodborne pathogens as *E. coli*, *Salmonella typhimurium*, *Staph. aureus* and *Listeria monocytogenes* (Kang and Lee, 2005). *E. coli* is one of the enteropathogenic bacteria which cause gastrointestinal tract disease. *E. coli* O18 is one of the enteropathogenic *E. coli* strain, which harbor many virulence factors as intimin (*eaeA*) and antibiotic resistant genes as bla_{TEM} and $bla_{CTX\text{-}M1}$ (Ewida and Husssein, 2018).

In this study, the inhibitory zone occurred due to the inhibitory effect of enterocin, it was 20 mm for *Staph. aureus* which was used as an indicator strain in the antimicrobial activity assay, while, it didn't give any inhibitory zone for the tested *E. coli* O18. These results agreed with that postulated by Vimont *et al*. (2017) as they tested the bacteriocin produced by *E. faecium* LCW isolated from camel's milk.

Honey has been used as a medicine in many cultures for long time as in ancient Egypt, the Egyptians considered the honey as the nectar of the gods. Dustmann in 1892 demonstrated the first bactericidal activity of honey (Dustmann, 1989). It is used in the treatment of different pathogenesis caused by bacteria as diarrhea-causing bacteria including *E. coli* (Adebolu, 2005). Moreover, it is also used in topical treatment of infected wounds and burns (Molan, 2001; Adeleke *et al*., 2006). The bactericidal action of honey is due to its normal acidity, osmotic effect, high sugar content, enzymes, nitrogenous and other compounds. Also, most types of honey generate hydrogen peroxide, which produced by the activation of glucose to gluconic acid and hydrogen peroxide, which is toxic to bacteria (Jeffrey and Echazarreta, 1996).

The data presented in Table 1&Fig. 1 showed that the inhibitory effect of the honey 10% and honey 10% with different concentrations of enterocin (150

and 300 µg/ml) in the UHT milk stored at ambient temperature (30±2 °C) during 24 h, as the highest reduction of *E. coli* count was obtained by the effect of honey with 300 µg/ml enterocin, where *E. coli* O18 count became 5 x 10^7 cfu/ml with percent reduction was 99.99 % at the end of 24 h of storage. The second reduction count of *E.coli* O18 occurred by the addition of honey 10% with enterocin 150 µg/ml, which became 2.5 x 10^{10} cfu/ml with inhibitory percent 98.68%. Moreover, the *E. coli* O18 count was gradually increased to $3.7x10^{10}$cfu/ml in milk containing honey 10% alone and the percent reduction was 98.05%. In case of the control UHT milk (additives free), the *E. coli* O18 count gradually increased until reaching to 1.9 x 10^{12} cfu/ ml by the end of 24h. In addition, the pH of the UHT milk treated with honey 10% and enterocin (150µg/ml) was the lowest pH value as 5.93 followed by honey 10% with enterocin (300µg/ml) as 6.13, then the UHT treated with honey 10% only as 6.08 (Table 2).

Results in Table 3 &Fig. 2 depicted the effect of 10% honey alone and honey 10% with two different concentrations of enterocin (150 and 300 µg/ml) in UHT milk containing *E. coli* O18 stored at refrigerator temperature (4±2 °C). The initial count of *E.coli* O18 was $6x10^6$ cfu/ml and it decreased to $7.1x10^4$, $9.9x10^5$ and $2x10^6$ cfu/ml in UHT milk treated with honey with (300 µg/ml) enterocin, honey with (150 µg/ml) enterocin and 10% honey alone, respectively, after 24 h storage at refrigerator temperature. Moreover, the inhibitory percents of the previous treatments were 99.74, 96.33 and 92.59%, respectively. However, after 24 h storage at refrigerator temperature, the count of the tested microorganism in control UHT milk samples (additives free) was increased to reach to $2.7X10^7$ cfu/ml, while, pH was slightly higher compared to that stored at ambient temperature (Table 4).

EDTA (ethylenediaminetetraacetic acid) is used in the food as antioxidant agent (Silva and Lidon, 2016) and it was added to the list of ingredients that are generally recognized as safe (GRAS) by FDA (Flood, 2016). Also, the current maximum acceptable daily intake (ADI) of EDTA is 1.9 mg $day^{-1}kgbw^{-1}$ (Wreesmann, 2014).

The outer membrane of Gram-negative bacteria act as a strong barrier to the cell. It prevents the entrance of the antibiotics and detergents to the bacterial cell. Magnesium (Mg) ions are the main ions responsible for stability of the lipopolysaccharide layer of the outer membrane of the bacteria. EDTA is a chelating agent which bind with Mg ions and removes these ions from the lipopolysaccharide membrane. Thus cause weakening of the outer membrane and the bacteria becomes more susceptible to the antibiotics and detergents (Nikaido and Vaara, 1987).

Regarding the results in Table 5&Fig. 3, UHT milk samples stored at ambient temperature (30 ±2 °C) for 24 hours containing EDTA (20mM), EDTA (20mM) with enterocin (150 and 300µg/ml), respectively, the counts of *E. coli* O18 were increased considerably from $1.5x10^5$ cfu/ml to $1.2x10^9$, $3.3x10^6$ and decreased to 7.1 $x10^4$ cfu/ml in UHT milk, in addition, the percent reduction of the previous treatment were 90.76, 99.97 and 99.99%, respectively. While, the count increased in control sample until reaching to $9x10^8$ cfu/ml by the end of 24 h. The most effective treatment was EDTA with enterocin (300 µg/ml), showing the highest reduction rate of the *E. coli* O18 during 24 h of storing the UHT milk at ambient temperature.

As presented in Table 7 & Fig. 4, the most effective processing to reduce the number of *E. coli*O18 was addition of EDTA (20mM) with enterocin (300µg/ml) and storage the treated UHT milk at refrigerator temperature. The number of *E. coli*at the begging was $1.5x10^5$ cfu/ml, while at the end of 24 hours of storage was $3.6x10^4$ cfu/ml with inhibitory percent 98.62%. The second effective treatment was EDTA (20mM) with enterocin (150µg/ml) followed by EDTA (20mM) treatment. In contrast, the number of *E. coli* was surge gradually in the control sample (additive free) till reach to $2.6x10^6$ cfu/ml by the end of 24 h. The lowest pH was obtained in the treated UHT milk with EDTA and enterocin (300 µg/ml), the pH value became 5.82 and 5.85 at the end of 24 h of storage at ambient and refrigerator temperatures, respectively (Tables 6&8).

In conclusion, the addition of honey as natural compound and as EDTA chemical treatment with biopreservative like enterocin had a great inhibitory effect on *E. coli* O18 growth in UHT milk when stored at refrigerator temperature.

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التأثير المثبط للإنتروسين وبعض الإضافات الغذائية على ميكروب الإيشيريشيا كولاي O18 في اللبن المعقم بالحرارة الفائقة

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الحفظ الحيوي هي طريقة من أقدم الطرق لحفظ الأغذية وذلك عن طريق إضافة مركبات طبيعية إلى الطعام لزيادة فترة صلاحية المنتج كما تساعد علي تثبيط نمو الميكروبات المسببة للتسمم الغذائى. الإنتروسين هو منتج بروتيني تفرزه بكتريا الإنتيروكوكاس فاسيزم التي لها تأثير مثبط كبير على البكتريا الموجبة الجرام بينما لها تأثير محدود على البكتريا السالبة الجرام. والهدف من هذه الدراسة هو إضافة بعض الإضافات الغذائية مثل العسل والإيدتا لزيادة التاثير المثبط للإنتروسين على الإيشيريشيا كولاي O18 فى اللبن المعقم بالحرارة الفائقة والمخزن في درجات حرارة الغرفة والثلاجة. وذلك عن طريق تقسيم اللبن المعقم بالحرارة الفائقة المحتوي على بكتريا الإيشريا كولاي إلى ثمان أجزاء على النحو التالي: الجزء الأول لا يحتوى على أي إضافات، والجزء الثاني يحتوى على عسل بتركيز ١٠%، والأجزاء الثالثة والرابعة تحتوي على عسل ١٠% مع تركيزين مختلفين من الإنتروسين (١٥٠ و ٣٠٠ ميكروجرام/ مل). والأجزاء الأربعة السابقة يعاد تقسيمها إلى مجموعتين المجموعة الأولى تم تخزينها في درجة حرارة الغرفة (٣٠ م) والأخرى في الثلاجة (٤ م) لمدة ٢٤ ساعة. وكان أكثر المعالجات تأثيرا هو الإنتروسين (٣٠٠ ميكروجرام/ مل) مع العسل المخزن في درجة حرارة الثلاجة حيث أن العدد البكتري إنخفض من ٦×١٠ ٦ إلى ٧×١٠ ٤ / مل في نهاية ٢٤ ساعة من الحفظ في الثلاجة. وقد تم إعادة نفس التجربة السابقة مع إحداث تغيير وهو إستخدام الإيدتا (٢٠ ميكرو مول) بدلا من العسل ١٠% وقد وجد أن أعلى معدلات التثبيط تمت بإستخدام الإيدتا مع الإنتروسين (٣٠٠ ميكروجرام/مل) مع حفظ اللبن في الثلاجة حيث أن عدد بكتريا الإيشيريشيا كولاي إنخفض في نهاية الأربع وعشرين ساعة إلى ٣،٦×١٠ ٤ / مل. ولذلك يفضل إضافة العسل والإيدتا مع الإنتروسين لتثبيط نمو ميكروب الإيشيريشيا كولاي في الألبان المعقمة والتي يمكن إستخدامها مباشرة بواسطة المستهلك أو إستخدامها في تصنيع منتجات الألبان مثل الجبن والأيس كريم.

الكلمات الكاشفة: الإنتروسين ، العسل، الإيدتا، اللبن المعقم بالحرارة الفائقة