CONTROLLING THE GROWTH OF LOCAL ISOLATES OF *LISTERIA MONOCYTOGENES* BY USING SOME CHEMICALS PRESERVATIVES

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ABSTRACT

This study include using of some chemical preservative against local isolates of *Listeria monocytogenes* , the effects of different concentrations of some generally regarded as save (GRAS) preservatives were studied on growth and survival of *L. monocytogenes*. These preservatives included salts of organic acid (sodium acetate and sodium benzoate) which inhibit growth of the bacterium at (25%) w/v concentration, also the effects of different concentrations of inorganic salt food additives sodium chloride were studied, which inhibit the growth of *L. monocytogenes* at (15%) w/v concentration . On the other hand the growth and survival of *L. monocytogenes* at different concentrations of two generally recognized as safe acids (lactic acid and acetic acid) was determined , the growth of bacterium was completely inhibited at (55mM) concentration of acetic acid and at (65mM) of lactic acid . Controlling of *L. monocytogenes* by tri-sodium phosphate (TSP) by dipping of artificially contaminated chicken meat in (10%) of TSP for (10) min., significantly reduced the population of the pathogen on the surface of meat to standard safe limit.So the study aimed to determining the effect of some chemical preservatives (acetic acid, lactic acid, sodium benzoate , sodium acetate and sodium chloride) on growth of local isolats of *L. monocytogenes* which isolated from different food sources in Erbil and Koya city.

INTRODUCTION

Control of *L. monocytogenes* is difficult due to its, wide spread in the environment, intrinsic physiological resistance, ability to adapt to external stress and ability to grow at a wide range of temperatures, in addition to other properties like resistance to heating, drying, freezing and defrosting are not sufficient to eliminate this potentially pathogenic microorganism (Kok,2009). A total elimination of *L. monocytogenes* from most foods will probably be unlikely, but it is possible to reduce and control this hazard in foods by hygienic measures and thus minimize the frequency and consequences of *Listeria* infection. However, as elimination of the bacterium is not possible, consumers will be exposed to low numbers of the pathogen despite the measures taken to control the hazard according to *Codex* (CAC, 2009). Antimicrobial agents that inhibit microbes are said to be static. Cidal antimicrobial agents destroy or kill microbes. Many chemicals used for sterilization and disinfections are cidal, killing `microbes. However, food preservatives must not be toxic to the consumer and then tend to be static agent (Beverly, 2004). The addition of antimicrobial agents is an effective method for controlling of *Listeria monocytogenes* in ready to eat food. In addition, preservation suppresses undesirable chemical and biochemical changes and helps to maintain the products desirable physical and sensory properties (Lou & Yousef, 2007). There are many chemical that used as preservative. The use of any antimicrobial agent depend on many factors like desired effect ,legal limits of use and effect on food (Pundir &Jain ,2011).

MATERIAL AND METHODS

Bacterial Preparation for Antimicrobial test

The bacteria were isolated from different sources of food in Erbil and Koya city, the isolated bacteria was transferred to Tryptic Soy Broth and incubated at 37°C for 24 hr. Bacterial cells were collected by centrifugation at 3000 rpm for 20 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match that of 0.5 McFarland standard to obtain a final concentration of 10^5 cells/ml (Nanasombat, and Chooprang, 2009).

Effect of Preservatives on the Growth of *L. monocytogenes*

TSB-YE was prepared with different NaCl concentration (0 %, 5 %, 7 %, 9%, 11 % ,13 % , 15%) , sodium benzoate eand sodium acetate (0%,5%,10%,15%,20%,25%,30%) , lactic acid and acetic acid concentrations were (01, 20, 30, 40, 45, 55, 60, 65, 70, 75, 80 mM). and sterilized by autoclaving then 0.1 ml from *L. monocytogenes* culture was transfered to test tubes containing 10ml of TSB-YE with different NaCl concentration and incubated at 35˚C for 24 hr. The effect of salt determined through calculating the number of colonies by plating

(0.1ml) of each different NaCl concentration into sterile petridish. Then TSA-YE was poured into plates, the plates were incubated at 35˚C for 24hr. after incubation, results were recorded and the percentage of the death determined comparing with the control (0%NaCl) treatment (Tienungoon *et al*., 2000).

Controlling of *L. monocytogenes* by tri-Sodium Phosphate

The samples prepared by weighting 1 grams of breast chicken meat and sterilized by dipping in 70% of ethanol for 5min, then washed with sterile distilled water for each treatment. Chicken meat was recontaminated by dipping in (20ml) of 10^5 cfu /ml of *Listeria monocytogenes* for (10) min. , then the chicken meat dipped in 10% of TSP for (5,10,15) min., and one of them put in normal saline as control, after that one gram of each treatment was put in normal saline and homogenized for several seconds then diluted to10^{-3} and (0.1ml)was put in sterile petridish and TSA –YE poured for plate count.

RESULT AND DISCUSSION

The effect of different lactic acid concentration (mM) on the growth of *L. monocytogenes* is shown in table (1), the strong inhibitory effect was(65) mM against *L.monocytogenes* growth when the pH was (4.3) in the culture media. Ray(2004) reported that the minimum pH at which growth of *L. monocytogenes* occurs is (4.6), also Young and Foegeding (1993) reported that growth of *L.monocytogenes* inhibited at (50 mM) of lactic acids, at 37˚C, when pH was 4.7, but not when pH was (6.0) .

Dipping of poultry leg in 0.55 mol /L Lactic acid reduced population of *L.monocytogenes* on poultry but not completely eliminated the bacteria therefore lactic acid can be used as an additional hurdle contributing to extend the shelf life of raw poultry (Gonzalez-Fandos and Dominguez, 2006). Organic acids can interact with other preservatives to enhance their effects. Lactic acid increased the susceptibility of *L. monocytogenes* to heat shock in culture media (Jorgensen et *al*, 1999). When lactic acid at (1.5-4%) concentration were sprayed on contaminated beef carcass or beef trim, large numbers of inoculated *L. monocytogenes* persisted and grew on the meat stored under refrigeration (Conner *et al .,*1997 *;* Dorsa*. et al.* ,1998). Organic acids (1-3%) used as dips are usually more efficacious than carcass washes because some residual activity remains on the meat. These acid concentrations generally cause no adverse effect on the sensory properties of the meat (Greer& Dilts ,1995; Smulders &Greer, 1998) **.** The tolerance to severe acid stress (pH 3.5) can be induced in *Listeria monocytogenes* following a 1-hr adaptation to mild acid (pH 5.5), a phenomenon termed the acid tolerance response (ATR), this process take place through a programmed molecular response which ensures cell survival under unfavorable conditions **(** Conte *et al*., 2002 ; Driscoll *et a*l., 1996) . Park *et al*., (2011) studied the effect of organic acid on *L. monocytogenes* on whole red organic apples and lettuce , they inoculated apples and lettuce with *L.monocytogenes* and treated with 1%and 2% of lactic acid for 0.5 ,1.5 and 10 min. after 10 min. treatment with lactic acid the population of the pathogens reduced from (1.69 to 3.42 log).

Table (1) Effect of lactic acid concentration on growth of L. monocytogenes

Lactic acid concentration mM	pH	mean*log cfu/ml ± SE
0	7.2	7.2800^{a} ±0.00577
10	6.75	7.2500^{a} ± 0.00577
20	6.24	7.1867^{b}± 0.00333
30	5.87	6.1133^{c} ± 0.01667
40	5.66	6.0633^{d} ± 0.01202
50	5.47	4.9500^{e} ± 0.00577
55	5	3.7267^{f} ± 0.02404
60	4.85	1.2033^{g} ± 0.01453
65	4.6	$.0000^{h}$
70	4.3	$.0000^{h}$
75	3.9	$.0000^{h}$
80	3.3	$.0000^{h}$

***The results represent the average of three replication . P< 0.01;a, b, c, d, e, f, g, h :different letters in the same column are significant.**

Effect of Acetic Acid on Growth of *L.monocytogenes*

As shown in table (2) the effective acetic acid concentration against *L.monocytogenes* was (55 mM) when the pH was (4.1), and the growth of the organism was decreased when the acid concentration was increased**.**

The growth of *L.monocytogenes* inhibited at (50 mM) acetic acid, at 37°C, when the pH was 4.7, but not when it was 6.0 (Young and Foegeding, 1993) ,also Vermeulen *et al* ., 2007 reported that *L. monocytogenes* was not able to grow at pH < 4.3 or a total acetic acid concentration > 0.4% in brain heart broth at 30°C. Acidification of foods with short-chain organic acids, either by fermentation or by deliberate addition, is an important and widespread mechanism for controlling food-borne pathogens in a variety of food. The antimicrobial effects of organic acids such as acetic acid, lactic and propionic is due to both the depression of pH below the growth range and metabolic inhibition by the undissociated acid molecule by diffusing through the cell membrane, which is permeable to nondissociated, nonprotonated, and lipophilic weak acids. This leads to an accumulation of the acid within the cell cytoplasm, acidification of the cytoplasm, disruption of the proton-motive force, and inhibition of substrate transport(Alvarado and McKee**,** 2007**).**

Table (2): Effect of acetic acid on growth of *L. monocytogenes*

Acetic acid concentration(Mm)	PH	mean*log cfu/ml ±SE
0	7.0	$7.2800^{a} \pm 0.00577$
10	6.39	$7.2300^{a} \pm 0.01155$
20	6.1	$6.1867^{b} \pm 0.01202$
30	5.83	$5.1633^{c} \pm 0.00882$
35	5.56	$4.1200^{d} \pm 0.00577$
40	5.1	$3.8600^{e} \pm 0.01155$
45	4.7	$2.5900^{f} \pm 0.19519$
50	4.5	$1.1733^{g} \pm 0.03480$
55	4.13	$.00000^{h}$
60	3.7	$.00000^{h}$
65	3.3	$.00000^{h}$
70	2.8	$.00000^{h}$

***The results represent the average of three replication .**
P< 0.01;a, b, c, d, e, f, g, h :different letters in the same column are significant

Effect of Sodium Acetate on Growth of *L. monocytogenes*

The minimum inhibitory concentration of sodium acetate was (25%) as shown in table (3),the growth of *L.monocytogenes* was decreased as sodium acetate concentration increased . Nanasombat and Chooprang (2009) reported that *L.monocytogenes* was inhibited at (210 mg/ml) of sodium acetate when the range of pH was (5-7), and when pH was 4.5 the inhibition occur at (52.5mg/ml)**.** Sodium acetate used to control *L.monocytogenes* on sliced vacuum-packaged bologna stored at 4°C for up to 120 days (Samelis *et al*., 2001).

Table (3) :Effect of sodium acetate on the growth of *L. monocytogenes*

Sodium acetate concentration(%)	mean*log cfu/ml ± SE
0	$7.2833^{a} \pm 0.00333$
5	$6.1833^{ab} \pm 0.00333$
10	$5.9700^{bc} \pm 0.01000$
15	$4.8833^{c} \pm 0.00333$
20	$3.6774^{d} \pm 0.00120$
25	$.0000^{g}$
30	$.0000^{g}$

***The results represent the average of three replication .**
P< 0.01;a,a b, bc, d, , g:different letters in the same column are significant .

Effect of Sodium Benzoate on Growth of *L. monocytogenes*

The effect of different concentration of sodium benzoate on growth of *L. monocytogenes* is shown in table (4), *L. monocytogenes* growth was inhibited at (25%)of sodium benzoate . Growth of *L. monocytogenes* on the surface of frankfurters dipped into 15 to 25 % sodium benzoate (pH ~7.8), was inhibited at 4°C for 14 days . When the contaminated frankfurters stored at 13 and 22°C, slow growth was observed after 10 and 3 days, respectively(Islam *et al.,*2002**)**,and Seman *et al.,* (2008) reported that (0.1%sodium benzoate and 0.1% sodium diacetate). *Listeria* cells sublethally injured in presence of benzoate have impaired mRNA synthesis (Buazzi and Marth, 1992) The temperature and pH are most important factors influencing the efficiency of sodium benzoate on growth of *L. monocytogenes*. Thus, generation time of the organism influenced by temperature, pH and concentrations of sodium benzoate **(**Elci &, AKpolat , 2003**).** The allowed concentration of sodium benzoate in food is (0.1%-0.2%), therefore sodium benzoate used in combination with other preservative in food to inhibit *L.monocytogenes* (Beringer *et al* ., 2006)

Table (4): Effect of sodium benzoate on growth of *L.monocytogenes*.

Sodium benzoate concentration (%)	mean*log cfu/ml ± SE
0	$7.2833^{a} \pm 0.00333$
5	$6.8767^{a} \pm 0.33338$
10	$5.1233^{b} \pm 0.00882$
15	$3.9233^{c} \pm 0.00882$
20	$2.3133^{d} \pm 0.02404$
25	$.0000^{h}$
30	$.0000^{h}$

***The results represent the average of three replication . P< 0.01;a, b, c, d, h :different letters in the same column are significant Effect of Inorganic**

Salt Food Additive(Sodium Chloride) on Growth of *L .monocytogenes*

The effect of sodium chloride on growth of *Listeria monocytogenes* is shown in table (5). The growth of *L. monocytogenes* decreased with an increase in salt concentration ,the growth of *L. monocytogenes* completely inhibited in (15%) w/v. *Listeria monocytogenes* is considered a salt tolerant and has been known to survive in commercial cheese brines (23.8% NaCl, pH 4.9) at 4ºC for 229 days (Larson et al., 1999) and grow in media containing up to 10% NaCl. Survival of *Listeria*, in the presence of > 12 % NaCl decreases with increasing salt concentration or incubation temperature. Presence of 16 % NaCl was listeriostatic for at least 33 days at 4˚C. and survived after exposure to 20% NaCl or higher (Guillier *et al.*, 2005; Tiganitas *et al.*, 2009). Furthermore, studies of survival after a pre-treatment at a low level of salt, followed by an increase to 25% NaCl (Duch & Labadie., 2003) or to 20% NaCl (Adriao *et al.*, 2008) have been reported. Therefore, use of high salt concentrations should not be considered as a permanent and reliable method with which to eliminate *L. monocytogenes*. NaCl in growth media or foods can be a source of osmotic stress by decreasing water activity (aw).

The presence of sodium chloride in growth media also partially protects *L. monocytogenes* from other stresses such as heat in ground pork (Yen et *al.,* 1993), and hydrogen peroxide in culture media (Lis-Balchin and Deans , 1997,Vignolo, *et al*., 1998).

Table (5):Effect of NaCl on Growth of *L. monocytogenes*

Sodium chloride concentration (%)	mean*log cfu/ml ± SE
0	7.2867^{a} ± 0.00333
3	6.2167^{b} ± 0.00333
5	5.1700^{c} ± 0.02000
7	4.1200^{d} ± 0.00577
9	3.6800^{e} ± 0.24007
11	2.7233^{f} ± 0. 00882
12	2.1900^{g} ± 0.4933
15	$.0000^{h}$
17	$.0000^{h}$

***The results represent the average of three replication . P< 0.01;a, b, c, d, e, f, g, h :different letters in the same column are significant**

Control of *L. monocytogenes* by Tri-Sodium Phosphate

The effect of tri-sodium phosphate TSP at (10%)concentration reduced *L. monocytogenes* population on the surface of meat artificially contaminated with 10^5 cfu/ml of *L. monocytogenes* from (4.10-1.46 log) as shown in table (6), the count of bacteria was reduced as the dipping time increased. (10%) TSP for (10) min was appears to be an effective treatment in reducing populations of *L. monocytogenes* on meat surface which is reduced the population but not completely removed the bacteria and this concentration is the allowable use which is(8-12%)solution used as a dip or spray, with (30) seconds allowed for giblets and (15)seconds for carcasses which is used in the poultry processing as an antimicrobial for raw, unchilled carcasses and giblets . In a study when broiler carcasses were dipped in different concentration of TSP, after 7min the results showed significant differences between the treated groups and control. A significant reduction to < 100 cfu/g after 10 min (Ahmed and Abd El-Atti, 2010, Sallam and Samejima, 2004) . The effect of TSP may be due to its high pH (pH 10), which affects the cell wall and the adherence of bacteria, also may repress enzyme synthesis and inhibit enzyme activity of bacteria (Ahmed and El-Atti ,2010). European countries set acceptable limits below 100 cells per gram for products intended to be consumed after cooking). TSP solutions (10%) for 10 min. dipping reduced *L. monocytogenes* populations below the acceptable limits set by European countries.

Table (6) : Efficacy of(10%) of TSP in Reduction rate of *L. monocytogenes* in the Surface of Chicken Meat in (5,10 and 15nim .)

Time (min)	mean*log cfu/ml ± SE
0	**4.1050^{a} ± 0.01500**
5	**3.7750^{b} ± 0.02500**
10	**1.8550^{c} ± 0.03500**
15	**1.4600^{d} ± 0.03000**

***The results represent the average of three replication .**
P< 0.01;a, b, c, d :different letters in the same column are significant

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السيطرة على نمو عزلات محلية من بكتريا *Listeria monocytogenes* باستخدام بعض المواد الحافظة الكيمياوية

الخلاصة

اسهدفت الدراسة الى استعمال المواد الحافظة الكيمياوية المسموح باستعمالها في السيطرة على نمو عزلات محلية من بكتريا *Listeria monocytogenes* المعزولة من اغذية المحلية مختلفة في مدينتي اربيل وكويه.

تضمنت هذه الدراسة اختبارات السيطرة الكيمياوية على (*Listeria monocytogenes*) ، فقد اختبرت تأثير تراكيز مختلفة من بعض المواد الحافظة الآمنة عامة على نمو وبقاء جرثومة (*Listeria monocytogenes*). شملت المواد المستعملة على املاح الحوامض العضوية (خلات الصوديوم وبنزوات الصوديوم) والتي اظهرت القدرة على تثبيط النمو البكتيري عند التركيز (25 % وزن / حجم) . كما اختبرت تأثير ملح الطعام كأحد الاملاح غير العضوية على نمو وبقاء الجرثومة، اذ اظهر القدرة على تثبيط النمو البكتيري عند التركيز (15 % وزن/ حجم). وكذلك اختبر التأثير التثبيطي لحامض الخليك واللبينيك واللتان تعدان من الحوامض الحافظة الآمنة ، اذ اظهرتا القدرة على تثبيط النمو البكتيري لجرثومة (*Listeria monocytogenes*) عند التراكيز(55 ملي مول) لحامضِ الخليك و (65 ملي مول) لحامضِ اللبنيكِ. كذلك اختبر التأثير التثبيطي لفوسفات الصوديوم TSP والتي تستخدم لتعقيم السطوح الخارجية للحوم، فقد تم الاختبار بغمر عينات لحم الدجاج الملوث بجرثومة(

Listeria monocytogenes) ، اذ اظهرت القدرة على خفض اعداد الجرثومة إلى الحدِّ الآمنِ القياسي على سطح اللحم عند التركيز 10% ولمدة 10 دقائق معنويا، فقد انخفضت اعداد البكتريا الى الحدود الامنة .

پۆختە

ئەم لێکۆلینەوەیە بە کار هینانی هەندیك مەوادي پاریزەری بە متمانە کردە مەبەست بۆ کونترول کردنی گەشەی بکتریای *Listeria monocytogenes* جیاکراو لە خۆراکی ناوخۆی لە بازارەکانی هەولێر وکۆیە.

ئەم لێکۆلینەوەیە تاقیکردنەوەی کونترول کیمیاوی لە بەکتریای *Listeria monocytogenes* لە خۆ کرد ، هەندیك مەوادی کیمیاوی پارێزەی ناسراو بە دژە گەشەو و مانەوەی ئەم بەکتیریایە و بە خەستی جیا جیا بەکارهێنرا لە لەم تۆیژینەوەدا، کە بریتیبوو لە خویەکانی ترشە ئەندامیەکان (خەلاتی سودیۆم و بەنزەواتي سودیۆم)، کە کاریگەری دژە گەشەی بەکتیریایان هەبوە کەی دەرخست لە خەستی (25 ٪). هەروەها خویێ کلۆریدی سودیۆم بە خەستي (15 ٪) کاریگەري دژە گەشەي بەکتیریایەکەي دەرخست. هەروەها لێکولینەوەکان دەریان خست کە ترشی سرکە وە ترشی ماست کاریگەری دژە گەشەی بەکتیریایەکەی بوو لە خەستی (55 ملیمول) بۆ ترشی سرکە وە لە خەستی (65 ملیمول) بۆ ترشی ماست. هەروها لێکۆلینەوەی کاریگەری مادەی سودیۆمی سێ فوسفات (TSP) بەکار هاتبوو بۆ پاکردنەوەي روي گۆشت ، تواني ئەم مادەییێ دەرخست بۆ کەمکردنەوەي ژمارەي بەکتریایەکان بۆ ئاستی پەسەندکراو لە کاتی بەکارهینانی بە خەستی (10 ٪)بۆ ماوەی 10 خۆلەك.