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Replacement Effect of NaCl by KCl with Garlic Extract on *Escherichia coli* O157:H7 and *Salmonella typhimurium* Strains

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ABSTRACT

One of the most important purposes of NaCl and garlic uses is to control of microbial pathogens. NaCl has been replaced by KCl partially to lower the side effects of NaCl on the human health. In this research study focusing on the effect of replacement of NaCl by KCl mix with garlic extract on *Escherichia coli* O157:H7 and *Salmonella typhimurium* previously identified strains. Minimum inhibitory concentration (MIC) garlic extract NaCl and /or KCl alone and in combinations against *Escherichia coli* O157:H7 and *Salmonella typhimurium* strains in Brain Heart Infusion broth (BHIB) and in ground beef were determined. Acceptable concentrations levels from garlic extract, NaCl and KCl alone or in combinations were selected and used for killing time assay of both tested bacteria. *E. coli* O157:H7 exhibited more sensitive than *Salmonella typhimurium*, and in ground beef than in Brain Heart infusion broth (BHI) with all treatment of mixtures. Synergistic effects were detected in garlic extract and NaCl or KCl combinations at specific concentration. 50% NaCl replacement by KCl with garlic are the most effective combinations by reduction 54.4% in BHI 59.5% of *Escherichia coli* O157: H7 in ground beef, whereas 75% NaCl replacement by KCl with garlic extract are the most effective combinations by reduction *Salmonella typhimurium* with 36.2% in BHI and 37.9% in ground beef.

INTRODUCTION

Traditionally, garlic with salts grounded and mixed for enhancement food flavoring, or during some food processing in food manufacturing. There are benefits of NaCl and garlic, but also there are side effects. Decreasing sodium chloride intake to decrease high pressure and garlic intake to avoid serious allergic reaction. Potassium chloride (KCl) is white crystal appearance, similar to NaCl in the taste, and already used in food processing instead of NaCl according to FDA. NaCl salt has been replaced partially by potassium chloride for this purpose. Holm and Sherman (1921) stated that the effect of chloride salts on bacterial cell is on permeability of the cell. Action mechanism of NaCl on the bacterial cell is on the cell membrane called plasmolytic effect and its causes leakage of cytoplasmic water leading to cell shrinking (Shelef and Seiter 1993), in addition NaCl causes reduction water activity (Cayleyl *et al.* 1992 & Zarei *et al*. 2012). The most important factors affecting the growth rate of food borne pathogens are temperature, pH, and water activity (McMeekin *et al*. 2002, 1987).

Garlic exhibit broad spectrum activity against Gram positive and Gram negative (Khashan. 2014).

Garlic extracts inhibit growth of Gram positive and Gram negative bacteria, such as *Staphylococcus, Streptococcus, Enterobacter, Escherichia*, *Klebsiella*, *Lactobacillus*, *Pseudomonas*, *Shigella, Salmonella, Proteus*, and *Helicobacter pylori* (Tsao and Yin, 2001, Kumar and Berwal., 1998). Antibacterial activity is mainly due to the presence of allicin produced by the enzymatic activity of allinase on alliin. Allicin is an inhibitory substance present in garlic by 0.3 up to 0.5% (Shelef 1983).

The antibiotic activity of 1mg of allicin, is equated to that of 15 IU of penicillin (Han, *et al*. 1995), but it can be unstable (Hahn, 1996). Mechanism of action of allicin is inhibits RNA synthesis (Feldberg *et al*., 1988). The use of garlic for tuberculosis bacilli infected people is useful through the public health system (Catia *et al*. 2011). The efficacy of garlic juice was higher than chlorhexidine against oral pathogenic bacteria and could be used as an effective mouthwash (Amin *et al*. 2012). Among the tested bacterial pathogens against essential oil of garlic, *Staphylococcus aureus* was found to be highly sensitive followed by *E. coli*. *L. monocytogenes* and *S. pyogenes* were found to be less sensitive (Jagadeesh *et al*. 2011). This research study aimed to evaluate replacement of NaCl with KCl partially or totally in mix with garlic against *Escherichia coli* O157:H7 and *Salmonella typhimurium* to minimize NaCl and garlic intakes

MATERIALS AND METHODS

Bacterial Cultures

Escherichia coli O157:H7 called E-A and *Salmonella typhimurium* called (S-A) was obtained from Bacteriology lab, Botany and Microbiology department faculty of Science, Al-Azhar University, Assiut. Isolates were previously identified. Two strains were sub cultured and confirmed the purity by two specific selective media. MacConkey sorbitol agar medium for *Escherichia coli* O157:H7 and Xylose lysine deoxycholate agar (XLD agar) for *Salmonella typhimurium* and characterized by API20 rapid method kit.

Preparing the Garlic Aqueous Extract

Fresh garlic (*Allium sativum*) was purchased from a local market in Assiut city, Assiut, the garlic bulbs were peeled, weighed (100 g), washed and surface sterilized using ethanol. The ethanol was allowed to evaporate in a laminar flow chamber, then the garlic was homogenized aseptically using a sterile blender. The homogenized garlic was filtered through What man No.4 filter paper. Sterile stock solution were stored at -20°C.

NaCl and KCl preparation

Appropriate amounts of NaCl and or KCl were placed in individual test tubes and dissolved with s Brain Heart infusion broth (BHI) to provide the range of NaCl and or KCl solutions to be used for susceptibility tests. Test tubes containing various NaCl or KCl solutions at various concentrations were sterilized (121 ^{0}C/ 15 min) and stored at refrigerator for not more than one weak.

Measurement of inhibitory effect of mixture

Set of test tubes contain Brain Heart infusion (BHI) with different concentrations of NaCl (1% up to 10%) Set of BHI with different concentrations of KCl (1% up to 10%) , set of of tubes containing mix of NaCl and KCl in mix with different concentrations partially replaced of both NaCl and KCl (w/v), autoclaved for 15 minutes ,after cooling then add different concentration of aliquot portions of fresh garlic extract (v/v). The tubes containing the various combinations of garlic, NaCl and/or KCl were inoculated with 1ml overnight broth culture of either *Escherichia coli* O157:H7 or *Salmonella typhimurium*. Inoculated tubes were incubated for 24h at 37^{0}C. Minimum inhibitory concentrations (MICs) of the treatment measurement were depend on turbidity method (Michael *et al*. 2005).

Assessment of killing time of Escherichia coli* O157: H7 and *Salmonella typhimurium* challenged by *NaCl, KCl, garlic extract alone and in combination

Escherichia coli O157: H7 and *Salmonella typhimurium* were grown overnight in BHI broth medium. 0.2 ml of inoculums was added to 20 ml BHI flasks or 20 gm. ground beef containin acceptable amounts of antibacterial agents, NaCl and KCl under condition salt not exceed 5% while garlic extract concentrations selected according to the result of the MICs experiments as following:

1- Garlic (G)6% for *E.coli* O157:H7
2- Garlic (G)8% for *Sal.typhimurium*
3- NaCl 5%
4- KCl 5%
5- NaCl 5%+ G-extract
6- NaCl 2.5%+ KCl 2.5%+ G-extract
7- NaCl3.75%+ KCl 1.25%+ G-extract
8- NaCl1.25%+KCl 3.75%+ G-extract

For ground beef, 20 g of the inoculated ground beef sample with treatment were transferred into sterile 250-stomacher bag together with 80 ml of sterile 0.1-peptone water. The sample was thoroughly homogenized by using hand squeezing. Serial dilutions of the homogenate were prepared by using 0.1-peptone water as diluents. Inoculated BHI and ground beef. Inoculated tubes were incubated up to 24h at 37^0C. Flasks and stomacher bags were incubated at 37°C. After 6 h, the flasks and stomacher bags were strongly agitated and stomacher bags mixed by hand squeezing. A 0.1ml sample was diluted and immediately plated; the flasks and stomacher bag were immediately returned to incubation.

Counting of viable cells

Bacterial counts were performed on Plat count agar after dilution, ranging from 10^{-1} to 10^{-6}, in sterile saline solution, 0.1ml sample of each dilution was plated in to agar medium, and counts were made in triplicate.

Statistical analysis

All experiments were carried out with three replicates. Statistical tests Analysis of variance (ANOVA) was performed.

RESULTS

Determination of antibacterial activities of garlic, NaCl* and *KCl

Treatment by NaCl combined with garlic extract called T1. Totally replacement of NaCl by KCl with garlic extract combination called T2 while partially replacement of NaCl by KCl 50%, 25% & 75% with garlic extract combination called T3, T4 & T5 respectively.

According to the present results garlic (G) MIC for *Escherichia coli* O157:H7 exhibited 6%, whereas for *Salmonella typhimurim* exhibited 8%. MICs of NaCl and KCl exhibited 9% for *Escherichia coli* O157:H7 and 10% for *Salmonella typhimurium*. MICs of the combinations (garlic extract and NaCl or KCl) show garlic concentrations percentages decreased when increasing of NaCl or KCl concentrations, but concentration of garlic extract not changed at specific concentration of NaCl or KCl. MIC for *Escherichia coli* O157:H7 at NaCl and KCl 1 & 2 %, garlic extract was 5% (Table 1- columns 2, 4 rows 3 & 4), 4 & 5 % of NaCl or KCl concentrations , G was 3% (Table 1 column 2, 4 rows 6 & 7) while at 6 & 7% NaCl or KCl concentrations , G was 2% (Table 1, columns 2, 4 rows 8 & 9), also for *Salmonella typhimurium* concentration of garlic (8%) not changed at concentration of NaCl 0.5, 1 & 2% (Table 2, column 1& 2, rows 2, 3 &4), whereas garlic concentration was 7% at 0.5 & 1% KCl (Table 2, column 3 & 4, rows 2 & 3), also it was constant 3% at concentration 5 & 6% KCl (Table 2, column 3 & 4, rows 7& 8).

MICs for *Escherichia coli* O157:H7, garlic concentration in totally replacement of NaCl by KCl is the same at different concentration of NaCl or KCl (T1 & T2) (Table 1, Fig. 1). On the other hand, concentration of garlic in case of using KCl less than NaCl (1-2%) except in case 7 & 8% of NaCl and KCl, concentration of garlic was the same.

Table 1: MICS of garlic extract in combination with sodium chloride and / or potassium chloride (Totally or partially replacement) of *Escherichia coli* O157:H7

MIC in Combination %												
T1		T2		T3			T4			T5		
NaCl	G	KCl	G	NaCl	KCl	G	NaCl	KCl	G	NaCl	KCl	G
0	6	0	6	0	0	6	0	0	6	0	0	6
0.5	6	0.5	6	0.25	0.25	6	0.375	0.125	6	0.125	0.375	6
1	5	1	5	0.5	0.5	5	0.75	0.25	5	0.25	0.75	5
2	5	2	5	1	1	4	1.5	0.5	4	0.5	1.5	4
3	4	3	4	1.5	1.5	3	2.25	0.75	3	0.75	2.25	3
4	3	4	3	2	2	2	3	1	2	1	3	2
5	3	5	3	2.5	2.5	3	3.75	1.25	2	1.25	3.75	2
6	2	6	2	3	3	1	4.5	1.5	1	1.5	4.5	1
7	2	7	2	3.5	3.5	1	5.25	1.75	1	1.75	5.25	1
8	1	8	1	4	4	1	6	1	1	2	6	1
9	0	9	0	4.5	4.5	0	6.75	2.25	0	2.25	6.75	0

T1=NaCl + Garlic extract, T2=KCl + Garlic extract, T3=NaCl/KCl (1:1) + Garlic extract
T4= NaCl/KCl (3:1) +Garlic extract, T5=NaCl/KCl (1:3) + Garlic extract,
NaCl (w/v), KCl (w/v), Garlic extract (v/v).

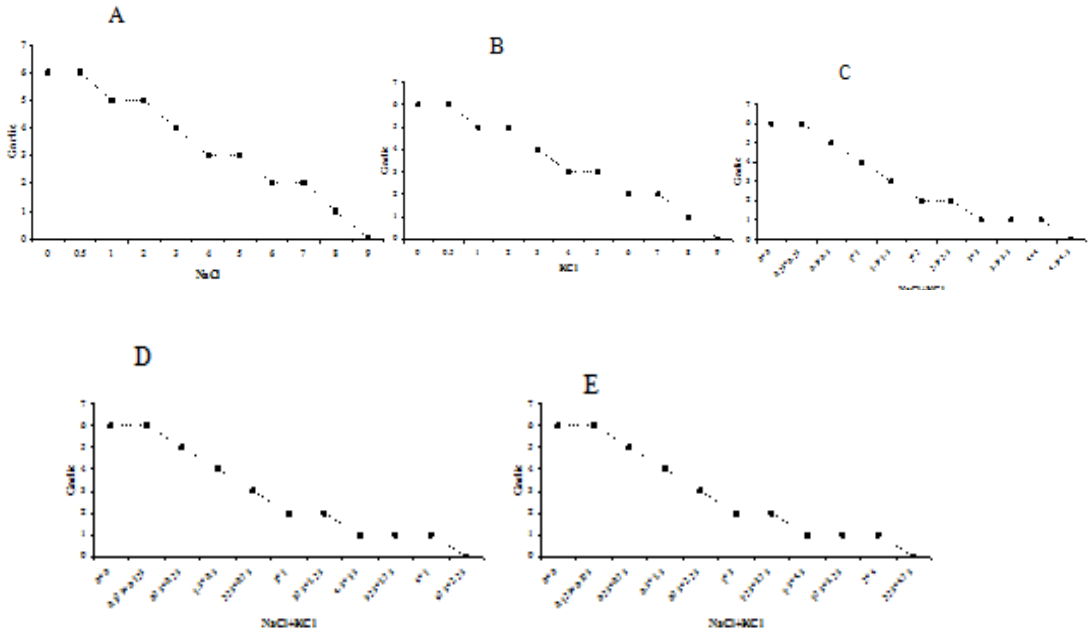

Fig. 1: MICs in combinations garlic extract with NaCl and KCl salts against *Escherichia coli* O157:H7
A=NaCl + garlic B=KCl+ garlic C=NaCl 50%+KCl 50%+garlic
D=NaCl 75%+KCl 25%+garlic E=KCl 75%+NaCl 25%+garlic

T3, T4 & T5 are partially replacement (50%, 25% & 75%) of NaCl by KCl in combination with garlic. According to the results have been obtained, NaCl or KCl at 0.5 % and 1 % not effect on MIC value of garlic extract for *Escherichia coli* O157:H7 in totally or partially replaced NaCl. On the other hand garlic concentration decreased at different treatments starting from addition of 2% of salts (partially replacement). *Salmonella typhimurium* able to tolerate NaCl up to 10% and KCl up to 9% (Table 2, Fig. 2).

Table 2: MICS of garlic extract in combination with sodium chloride and/ or potassium chloride (Totally or partially replacement) of *Salmonella typhimurium*

MIC in Combination %												
T1		T2		T3			T4			T5		
NaCl	G	KCl	G	NaCl	KCl	G	NaCl	KCl	G	NaCl	KCl	G
0	8	0	8	0	0	8	0	0	8	0	0	8
0.5	8	0.5	7	0.25	0.25	7	0.375	0.125	7	0.125	0.375	7
1	8	1	7	0.5	0.5	7	0.٧٥	0.25	7	0.25	0.75	7
2	8	2	6	1	1	6	1.5	0.5	6	0.5	1.5	6
3	7	3	5	1.5	1.5	6	2.25	0.75	5	0.75	2.25	6
4	5	4	4	2	2	5	3	1	4	1	3	5
5	4	5	3	2.5	2.5	3	3.75	1.25	3	1.25	3.75	4
6	3	6	3	3	3	2	4.5	1.5	2	1.5	4.5	3
7	2	7	2	3.5	3.5	2	5.25	1.75	1	1.75	5.25	2
8	1	8	1	4	4	1	6	2	1	2	6	1
9	1	9	1	4.5	4.5	0	6.75	2.25	0	2.25	6.75	0
10	0	10	0	-	-	-	-	-	-	-	-	-

T1=NaCl +Garlic extract (G), T2=KCl+Garlic extract, T3=NaCl/KCl (1:1) + Garlic extract,
T4= NaCl/KCl (3:1) + Garlic extract, T5=NaCl/KCl (1:3) + Garlic extract,
NaCl (w/v), KCl (w/v), Garlic extract (v/v)

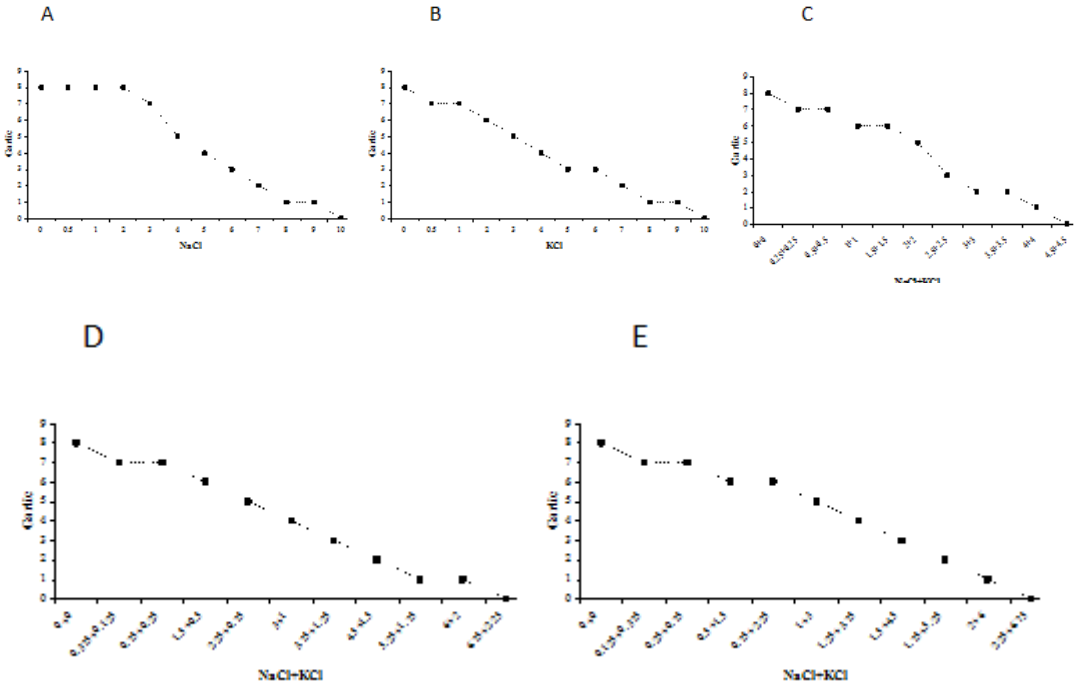

Fig. 2: MICs in combinations garlic extract with NaCl and KCl salts against *Salmonella typhimurium*
A=NaCl + garlic extract B=KCl+ garlic extract C=NaCl 50%+KCl 50%+garlic extract
D=NaCl 75%+KCl 25%+garlic extract E=KCl 75%+NaCl 25%+garlic extract.

Effect of different treatments on Escherichia coli O157:H7 *(killing time)*

At 37°C incubation temperature, 5% garlic reduced number of *Escherichia coli* O157.H7 gradually until 20 h of incubation time, but changed back, so the number increased again at 24 h by 0.2 log CFU/ml in BHI and 0.6 log CFU/ml in ground beef respectively. At 24 h of the incubation, the change was 1.9 log CFU/ml in BHI (24%) and 1.3 log CFU/ml in ground beef (16%). NaCl 5% reduced *Escherichia coli* O157.H7 number reduced by 2.2 log CFU/ml in BHI (27.8%) and 1.8 log CFU/ml in ground beef (22.8 %) (Tables 3 & 5).

Figures 1 & 2 show *E. coli* O157:H7 more sensitive than *Salmonella typhimurium* to different concentrations of NaCl with garlic combinations but opposite in combination of KCl with garlic. As well as partial replacing by 50 %,

25 % and 75 % of NaCl by KCl exhibit *Escherichia coli* O157:H7 more susceptible than *Salmonella typhimurium.* KCl reduced *Escherichia coli* O157.H7 number by 2.2 log CFU/ml in BHI (30.4%) and 1.8. log CFU/ml in ground beef (22,8%), T1 reduced *Escherichia coli* O157.H7 number by 4. log CFU/ml in BHI (50.6%) and 4.1 log CFU/ml in ground beef (51.9%), T2 reduced *Escherichia coli* O157.H7 number by 4.1 log CFU/ml in BHI (51.9%) and 4.4 log CFU/ml in ground beef (55.7%), T3 reduced *Escherichia coli* O157.H7 number by 4.3 log CFU/ml in BHI (54.4%) and 4.7 log CFU/ml in ground beef (59.5%), T4 reduced *Escherichia coli* O157.H7 number by 4.1 log CFU/ml in BHI (51.9%) and 4.3 log CFU/ml in ground beef (54.4%) while T5 reduced *Escherichia coli* O157.H7 number by 4.3 log CFU/ml in BHI (54.4%) and 4.4 log CFU/ml in ground beef (55.7%) at 24 h of incubation period. (Table 3 & Table 5).

Table 3: Inhibition curve of *Escherichia coli* O157:H7 (log CFU/ml) by garlic extract, NaCl & KCl alone and in combination in BHI broth

Time	G	S	P	T1	T2	T3	T4	T5	F-test	L.S.D.
0	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	n.s	-
6	7.9	7.8	7.8	7.7	7.8	7.7	7.8	7.7	*	0.046
12	6.8	6.6	6.6	6.8	6.7	6.7	6.7	6.7	*	0.032
16	6.4	6.3	6.2	5.6	5.5	5.3	5.5	5.4	**	0.041
20	6.1	5.9	5.8	5.5	4.6	4.5	4.6	4.6	**	0.034
24	6	5.7	5.5	3.9	3.8	3.6	3.8	3.6	**	0.045
% Change	**-24.0**	**-27.8**	**-30.4**	**-50.6**	**-51.9**	**-54.4**	**-51.9**	**-54.4**		

G=Garlic (6%) S=sodium chloride (5%) P=potassium chloride 5% T1=Garlic (3%) + sodium chloride (5%), T2 = garlic (2%) potassium chloride (5%), T3=Garlic (٣%)-sodium chloride (2.5%) & potassium chloride (2.5%) T4= Garlic (3%)-sodium chloride (3.75 %), potassium chloride (1.25%), T5=Garlic (3%), Sodium chloride (1.25 %) & Potassium chloride (3.75 %), *=significant ** =highly significant, ns=no significant

Table 4: Inhibition curve of *Salmonella typhimurium (log CFU/ml)* by garlic extract, NaCl & KCl alone and in combination in BHI broth

Time	G	S	P	T1	T2	T3	T4	T5	F-test	L.S.D.
0	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	n.s	-
6	5.8	5.8	5.8	5.7	5.7	5.7	5.7	5.7	*	0.039
12	5.7	5.8	5.8	5.6	5.6	5.6	5.6	5.6	*	0.032
16	5.5	5.4	5.3	5.5	5.5	5.4	5.5	5.4	*	0.021
20	4.9	5.3	5.1	5.3	4.6	4.5	4.6	4.6	*	0.031
24	4.6	5.1	4.8	3.9	3.8	3.6	3.8	3.7	**	0.065
% Change	**-20.7**	**-12.1**	**-17.2**	**-32.8**	**-34.5**	**-37.9**	**-34.5**	**-36.2**		

G = Garlic (8%) S=sodium chloride 5%, P=potassium chloride 5% T1=Garlic (4%) +sodium chloride (5%), T2 = garlic (3%) potassium chloride (5%), T3=Garlic (4%)-sodium chloride (2.5%) & potassium chloride (2.5%) T4= Garlic (3%), sodium chloride (3.75 %), & potassium chloride (1.25%), T5=Garlic (4%), sodium chloride (1.25 %) & potassium chloride (3.75 %), *=significant ** =highly significant, ns=no significant

Table 5: Inhibition curve of *Escherichia coli* O157:H7 (log CFU/ml by garlic extract, NaCl & KCl alone and in combination in ground beef

Time	G	S	P	T1	T2	T3	T4	T5	F-test	L.S.D.
0	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	n.s	-
6	7.7	7.8	7.8	7.7	7.8	7.7	7.7	7.7	*	0.047
12	6.8	6.8	7.8	6.6	6.4	6.6	6.6	6.6	*	0.042
16	6.8	6.7	7.8	5.2	5.4	5.1	5.4	5.2	**	0.044
20	6.9	6.3	6.7	5.4	4.4	4.4	4.4	4.3	**	0.054
24	6.6	6.1	6.1	3.8	3.5	3.2	3.6	3.5	**	0.051
% Change	**-16.5**	**-22.8**	**-22.8**	**-51.9**	**-55.7**	**-59.5**	**-54.4**	**-55.7**		

G=Garlic (6%) S=Sodium chloride (5%) P=Potassium chloride 5% T1=Garlic (3%) +sodium chloride (5%), T2 = Garlic (2%) potassium chloride (5%), T3=Garlic (3%)-sodium chloride (2.5%) & potassium chloride (2.5%) T4= Garlic (3%)-sodium chloride (3.75 %), potassium chloride (1.25%), T5=Garlic (3%), Sodium chloride (1.25 %) & Potassium chloride (3.75 %), *=significant ** =highly significant, ns=no significant.

***Effect of different treatments on Salmonella typhimurium (killing time*)**

At 37°C incubation temperature, 5% garlic reduced number of *Salmonella typhimurium* gradually until 20 h of incubation time, but changed back, so the number increased again at 24 hr of the incubation time by 0.4 log CFU/ml in BHI and 0.5 log CFU/ml in ground beef. At 24 h of the incubation, the change was 1.2 log CFU/ml in BHI (20.7%) and 1.5 log CFU/ml in ground beef (25.9%), NaCl 5% reduced *Salmonella typhimurium* number reduced by 0.7 log CFU/ml in BHI (12.1%) and 1.1log CFU/ml in ground beef (15.5 %) (Tables 4 & 6).

Table 6: Inhibition curve of *Salmonella typhimurium (log CFU/ml)* by garlic extract, NaCl & KCl alone and in combination in ground beef

Time	G	S	P	T1	T2	T3	T4	T5	F-test	L.S.D.
0	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	n.s	-
6	5.8	5.8	5.8	5.7	5.7	5.7	5.7	5.7	*	0.021
12	5.7	5.8	5.8	5.6	5.6	5.6	5.5	5.5	**	0.034
16	5.4	5.3	5.3	5.4	5.5	5.4	5.4	5.4	**	0.037
20	4.6	5.2	4.9	5.2	4.5	4.5	4.4	4.5	**	0.021
24	4.3	4.9	4.7	3.8	3.7	3.6	3.5	3.6	**	0.071
%Change	**-25.9**	**-15.5**	**-19**	**-34.5**	**-36.2**	**-37.9**	**-39.7**	**-37.9**		

G=Garlic (8%), S=Sodium chloride 5%, P=Potassium chloride 5% T1=Garlic (4%) +sodium chloride (5%), T2 =Garlic (3%) potassium chloride (5%), T3=Garlic (4%)-sodium chloride (2.5%) & potassium chloride (2.5%) T4= Garlic (3%), sodium chloride (3.75 %), & potassium chloride (1.25%), T5=Garlic (4%), Sodium chloride (1.25 %)
& Potassium chloride (3.75 %), *=significant ** =highly significant, ns=no significant

KCl reduced *Salmonella typhimurium* number by 1 log CFU/ml in BHI (17.2%) and 1.1. log CFU/ml in ground beef (19%), T1 reduced *Salmonella typhimurium* number by 4.0 log CFU/ml in BHI (50.6%) and 4.1 log CFU/ml in ground beef (51.9%), T2 reduced *Salmonella typhimurium* number by 1.9 log CFU/ml in BHI (32%) and 2 log CFU/ml in ground beef (34.5%) , T3 reduced *Salmonella typhimurium* number by 2 log CFU/ml in BHI (34.5%) and 2.2 log CFU/ml in ground beef (37.9%), T4 reduced *Salmonella typhimurium* number by 2 log CFU/ml in BHI (34.5%) and 2.3 log CFU/ml number in ground beef (39.7%) while T5 reduced *Salmonella typhimurium* number by 2.2 log CFU/ml in BHI (36.2%) and 2.2 log CFU/ml in ground beef (37.9%) at 24 h of incubation period (Tables 4 & Table 6).

DISCUSSION

Providing healthy nutrition has become necessary especially for elderly people, that because most elderly patients have several different diseases. There are benefits and side effects of NaCl salt and garlic, so minimization of NaCl salt and garlic concentrations must be considered to avoid the side effects. Replacement of NaCl by KCl, furthermore mixing of salts with garlic are valuable additives for many reasons, so that replacement of NaCl and/or mixing with garlic able to minimize risk of hypertension by NaCl, able to control of food borne pathogens, and able to overcome effect of NaCl tolerance or garlic resistance by food borne pathogens. Garlic used as alternative medicine as possibility effective aid in controlling of high blood pressure (Capraz *et al*., 2006). Strains from *Salmonella typhimurium* and *Escherichia coli* exhibited NaCl tolerance (Garrity *et al*, 2005), also NaCl enhanced recovery and growth of acid stressed *Escherichia coli* O157:H7 (Jordan and Davies, 2001).

Concerning the minimal inhibitory concentration of garlic extract, 6% of garlic able to inhibit of *Escherichia coli* O157:H7 while 8% of garlic inhibits *Salmonella typhimurium* without incorporation with either NaCl or KCl. Other research study exhibit that *Salmonella typhimurium* was resistant to Alliin, which may show that this strain would be the survivor of acid pasteurized garlic paste **(**Koluman *et al*. 2013). In comparison with data recorded by other investigators, the MIC of garlic for *E. coli, Salm. typhi, Staph. aureus* and *L.*

monocytogenes was 3·95, 7·0, 5·0 and 8·8%, respectively (Kumar and Berwal,1998). Durairaj *et al*. (2009) found that the minimal inhibitory of concentration (MIC) was in the ranges of 6–11 mg/mL and 7–21 mg/mL, in Gram-positive and Gram-negative organisms respectively. From the observation of this research study, 6 % garlic reduced number of *E. coli* O157:H7 and 8% *Salmonella typhimurium* gradually until 20 h of incubation time, but the number increased back again at 24 h of the incubation time. Abdulraouf *et al*. (1993) stated that the time is important factor affecting on survival of *E. coli* O157:H7 in ground beef. Durairaj *et al*. (2009) found that the diameter of the inhibition zone of garlic against some food borne pathogens decreased by time. The reason may be due to the antimicrobial active substance, allicin in crushed garlic extracts breaking down within 16 h at 23°C (Hahn 1996, Unal *et al*. 2001).

Concerning minimal inhibitory concentrations of both NaCl and KCl, there is no inhibitory effect by NaCl or KCl at low concentrations on *E coli* O15:H7 or 0n *Salmonella typhimurium.*

According the result of this research study, *Escherichia* coli O157:H7 can survive up to 9% of NaCl and KCl while *Salmonella typhimurium* can live up to 10 % of both chloride salts. In comparison with data recorded by other investigators, Doudoroff (1940) concluded that *E. coli* can survive up to 7% NaCl concentration without any change in number of colonies. *Escherichia* coli O157:H7 founded that it is able to survive in marine water (Miyagi, 2001). Hrenovic and Ivankovic (2009), found that *Escherichia coli* was multiplied during 72 h at 5% NaCl concentration. Vera and Lazar, (2003) found that *Escherichia coli* O157:H7 able to survive at high concentration of NaCl. *Salmonella typhimurium* tolerate salt up to 9 % called osmotolerant (Garrity *et al*. 2003), in addition, other research results showed many pathogenic strains such as *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus aureus* not only tolerate sodium chloride but also growth dependent (Markus *et al*. 2003). Cayley *et al*. (1992) concluded that some isolated strains are salt tolerant and are able to survive at high salt concentrations due to its ability to produce proline to protect itself against salt stress.

In this study, specific acceptable concentrations of both NaCl and KCl with garlic extract were selected to imply that to evaluate killing time assay. The mixtures of garlic, NaCl and KCl showed that highly significant in reduction of *E. coli* O157:H7 or *Salmonella typhimurium* numbers.

Concerning killing time required assay of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in BHI and ground beef, the concentrations were 50%, 75% substitution of concentration 5% salt. 50% NaCl replacement by KCl with garlic are the most effective combinations by reduction 54.4% in BHI 59.5% of *Escherichia coli* O157: H7 number in ground beef, while 75% NaCl replacement by KCl with garlic extract is the most effective combination for reduction of *Salmonella typhimurium* by 36.2% in BHI and 37.9% in ground beef.

The products with 20% and 40% replacement of NaCl with KCl were acceptable, those with 60% were in the limit of acceptability and products with 80% KCl were unacceptable. There is the possibility of replacement sodium chloride by potassium chloride in meat products, but limited by its bitter taste (lilic *et al*. 2008). The level of substitution of NaCl with KCl can be at least 25% without risking the microbiological safety, but not as high as 50% (Zarei *et al*. 2012). 50% substitution of NaCl with KCl, at 2.5% total salt, could inhibit *E. coli* (Gandhi *et al*. 2014). Angienda and Hill (2011) concluded that the use of concentrations less than MIC in combination with low pH and or NaCl has the potential of being used as an alternative to "traditional food preservatives. KCl similar to NaCl used in controlling of microbial hazards and KCl is more effective than NaCl against *Clostridium perfringens* (Eva *et al*., 2008). In other research study, it was found that the replacement of NaCl with KCl did not affect the degree of inhibition

and or inactivation, but did alter the taste of the foodstuffs (Gimeno *et al*. 2001).

Abdulkarim *et al*. (2009) discussed that growth of *Escherichia coli* sharply decreased in the case of KCl increasing compared with using NaCl and concluded that higher salt concentrations of the medium increase its osmolarity, which is likely to have resulted in hyper osmotic shock to *E. coli* cells causing growth suppression.

Xu *et al*. (2014) resulted that the growth rates pathogenic bacterial cells were decreased with increasing NaCl concentration. José *et al*. (2015), found that the best choice treatment was 45% NaCl, 25% KCl, 20% $CaCl_2$ and 10% $MgCl_2$ on the physicochemical properties of food and microbial counts, and could be successfully used for sodium reduction.

CONCLUSIONS

To conclude, bacterial pathogens in the food controlled by many factors, linked together and challenged against food borne pathogens. NaCl is one of the important factors affecting on bacterial growth has been substituted by KCl partially to reduce health risks caused by NaCl. KCl-NaCl-garlic mixture more effect on bacterial pathogens to overcome NaCl adaptation by bacterial pathogen and at same time to overcome garlic allicin breaking down within time. Garlic NaCl and KCl in mix should be considered for controlling of foodborne pathogens in the food products.

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