As. J. Food Ag-Ind. **2009**, 2(04), 882-890

OPEN ACCESS

Asian Journal of Food and Agro-Industry
ISSN 1906-3040
Available online at www.ajofai.info

Research Article

Effect of vapourized fermented vinegar on *Salmonella enteritidis* on eggshell surface

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This paper was originally presented at Food Innovation Asia, Bangkok, Thailand, August 2009.
Recieved 1 July 2009, Revised 10 February 2010, Accepted 21 February 2010

Abstract

The presence of *Salmonella enteritidis* (SE) on eggshell has serious public health implications. Several treatments have been developed to control SE on eggshell. The inhibition of SE by fermented vinegar (FV) on surface of chicken eggshell was investigated. Preliminary study by Agar Overlay Disc Diffusion Method on Mueller Hinton Agar media using FV containing different acetic acid concentration (AAc) (0% to 5%v/v) showed that more acetic concentration used, more significant inhibition of SE was found. Therefore, the confirmation of SE inhibition by FV containing different AAc (1%-3%v/v with 0.1%v/v incretion) at 5 min of contact time was further studied *in vitro* in Tryptic Soy Broth (TSB). The 7.56 log cfu/ml SE cells were completely inhibited in TSB containing FV with 2%v/v AAc. The effect of vapourized FV at 2%v/v compared with 10%v/v AAc were investigated on SE on eggshell surface. Eggshells externally inoculated with SE were treated with FV vapour generated by pumping sterile air to FV solution. In this study, the level of SE consisting of 6 log cfu/g on eggshell were originally prepared. In order to reduce 1 log cycle, the 90 min vapourizing time of 2%v/v AAc or 30 min of 10%v/v AAc vapourizing time was required. The results suggested that both suitable AAc and vapourizing time should be considered for effective egg sanitization treatment. The microbial quality on eggs treated by vapourizing 2%v/v and 10%v/v FV was considered. The results showed no SE was detected both on eggshell and in egg content when kept at 4°C and room temperature (31± 1°C) within 28 days. The advantage of FV vapourizing procedure is not only to successfully control eggshell microbial quality but also to protect the environmental safety.

Keywords: Vapourized fermented vinegar, acetic acid concentration, microbial inhibition, *Salmonella enteritidis*, poultry, eggshell, food safety, Thailand.

Introduction

Egg-associated infections are mainly caused by *Salmonella enteritidis* (SE), and eggshells are considered as the predominant source of human salmonellosis [1]. Eggshell contamination can occur with any organism that is excreted by the laying hen and by contact with nesting material, dust, and feed [2]. In theory, the risk of contamination with *Salmonella* and particularly with SE might be higher when eggs are produced in some noncage systems, because of the greater exposure of layers and their eggs to environmental contamination [3]. As a result of 9.4% *Salmonella* contamination on eggshells were found in local markets of middle and east Thailand [4]. The SE was 11.4% of the 25 most common serovars isolates from humans reported in 1993-2002 in Thailand. Similar trends were detected from human infections, were relative incidence of SE contaminated in chickens and eggs [5]. Moreover, in the United States, *Salmonella* transmission through contaminated eggshell or egg products results in 700,000 cases of salmonellosis and costs $ 1.1 billion annually [6]. Presence of SE on eggshell constitutes a public health hazard and poses a considerable economic impact on the poultry and egg industry.

Generally, boiling water [7], chlorine and iodine [8], hydrogen peroxide [9] and washing disinfectants [7] were used for Salmonellae decontamination procedures. Most of them are chemical treatment except in case of boiling water. However consumers are increasingly avoiding consumption of food treated with chemicals and so natural alternatives are needed to achieve high-degree safety with respect to foodborne pathogens [10]. The natural sanitizers, such as organic acids have been investigated because of their bactericidal activity [11]. They are also generally as safe (GRAS) and therefore are utilized for preservation in many food. In a study where 13 acids were evaluated, acetic and propionic acids were found to have the most inhibitiory effect against salmonellae [12]. Among the natural products, vinegar has been known as the acetic acid which contains sanitizing properties. Vinegar is naturally found in variety of fruits and fermented foods, has been carried out by several researchers. People commonly soak vegetables in vinegar solution as a sanitizing treatment [13]. The objective of this study is to investigate the effect of vaporized fermented vinegar (FV) activity on reduction of SE which primarily inoculated on eggshell.

Material and Methods

Preparation of Salmonella Enteritidis (*SE*) *culture and growth conditions*

SE was provided from Department of Medical Sciences, Ministry of Health, Thailand. The culture originally isolated from infected chicken's eggs was resuscitated by aseptically transferring into Tryptic Soy Broth (TSB; Merck, Germany) for two successive cycles of 18 ± 2 h of incubation at 37°C. The culture were centrifuged at 3,600 × g for 15 min, washed twice, and re-suspended in 10 ml of sterile 0.1% Buffered Peptone Water (BPW; Merck, Germany). Finally, a density of approximately 10^8 cfu/ml of cell suspension was obtained. The bacterial count of SE was confirmed by plating 0.1 ml portions of appropriate dilutions on Tryptic Soy Agar (TSA; Merck, Germany) plates with incubation at 37°C for 18 ± 2 h.

SE inhibition by FV using agar overlay disc diffusion testing

Susceptibility testing was based on the Agar Overlay Disc Diffusion Method [14]. From SE suspension, 20 µl of adjusted inoculum was mixed with 5 ml sloppy Mueller Hinton Agar (MHA; Merck, Germany). The medium was cooled to 45°C before spreading SE. Petri dishes containing 10 ml of MHA were overlaid with sloppy MHA containing SE cells and allowed to solidify for 20 min at room temperature. The fermented vinegar (FV) used in the experiments were supplied by My Garden Co., Ltd., Suphanburi province, Thailand. The

manufacturer's stated content of 10%v/v acetic acid concentration (AAc) was confirmed by titration with 0.1 N NaOH. The adjusting FV containing 0-5%v/v AAc with 1.0%v/v incretion were dispensed onto paper disc. The following discs were tested onto the overlay plates and all plates were incubated at 37°C for 18 ± 2 h. The inhibition zones were measured by vernier calipers.

Effect of FV on SE viability in vitro

The viable SE was determined by their ability to survive in acidified TSB with FV. This experiment was conducted to confirm the interactive AAc *in vitro*. The TSB was acidified with AAc range as resulted from previous Agar Overlay Disc Diffusion test. The 1 ml SE suspension was added into 9 ml TSB which varied concentration with 0.1%v/v AAc incretion. To allow for contact 5 min at room temperature (31 ± 2°C), total viability of SE in each acidified TSB with AAc was enumerated on TSA. Additionally, the SE cells were determined from the absorbance at 625 nm with GENESYS 10 VIS spectrophotometer [15].

Inoculation of SE on eggshell

Before inoculation of SE on eggshell, the eggshell was prior sanitized by dipping the egg into 70% ethanol for 5 sec. and, then, dried under aseptic condition. The inoculation of SE on sterilized eggshell was carried out by dipping into 10^8 cfu/ml of SE suspension for 5 min. To allow for complete attachment of SE, the eggs were dried for 3h at 37°C and 30% relative humidity for protecting the SE penetration into egg content [7].

Vapourized FV treatment

The SE inoculated eggs were treated with FV vapour generated by pumping sterile air to FV solution. The AAc used in this study was composed of the resulted AAc from *in vitro* study and the commercial AAc 10%v/v.

Shelf-life study

The SE inoculated eggs from vapourized FV treatment were stored at 4°C and room temperature (31 ± 2°C) for 28 days. The egg samples were taken at 0, 7, 14, 21 and 28 days. Then, the SE on eggshell and in egg content was enumerated by ISO 6579: 2002.

SE enumerating and detecting method

The viability of SE on eggshells was enumerated by modified method of Himathongkham *et al.* [7]. The egg content was removed before grinding shell and membrane in sterile mortar followed by rinsing 10 ml TSB. The concentration of SE on shell and membrane were determined by making 10-fold dilutions of each sample in 0.1% BPW and spreading 0.1 ml undilution and each dilution onto plates of TSA and Xylose-Lysine-Deoxycholate (XLD) Agar (Merck, Germany). The agar plates were incubated at 37°C for 18 ± 2 h. Then, the 5% of colonies grown on TSA were taken and transferred onto XLD and Brilliant-green Phenol-red Lactose Agar (BPL; Merck, Germany) supplemented with 0.02 mg/ml novobiocin (Sigma Chemical Co., St. Louis, Mo.) for confirmation. Finally, SE colonies were confirmed by biochemical and serological tests according to ISO 6579.

The detection of SE on eggshells and in egg content was determined under ISO 6579: 2002 procedure. Shell and membrane were weighed under aseptic condition and mixed with BPW in ratio 1:10. To determine the possible presence of SE in egg content, 25 ml of mixture of egg white and yolk was transferred to 225 ml BPW in plastic bag and homogenized in stomacher for 2 min at medium speed. Homogenates of samples in BPW were incubated at 37°C for 18±2 h and then 1 ml was transferred to 10 ml of Muller-Kauffmann Tetrathionate Novobiocin Broth (MKTTn; Merck, Germany) and 0.1 ml to Rappaport-Vassiliadis medium

with soya (RVS; Merck, Germany). After incubating tubes at 37°C (MKTTn) and 41.5°C for 24±3 h, cultures were streaked on XLD and BPL. Plates were incubated at 37°C for 24±3 h and then examined for suspect colonies. Finally colonies were confirmed by biochemical and serological tests.

Statistical analysis

Three replicate trials were done for each experiment. Data were subjected to analysis of variance and Duncan's New Multiple Range Tests (DMRT) (SPSS 10.0 for Windows pocket program) to determine if significant difference ($P \leq 0.05$) in population of SE existed between mean values.

Results and Discussion

SE inhibition by FV using agar overlay disc diffusion testing

In principle of disc diffusion, the FV of various AAc diffused into MHA. As the inhibition zone increased, there was a logarithmic reduction of SE in more AAc of FV used. The extent of FV diffusion was affected by the depth of the MHA [16]. As a result (Figure 1) showed that higher AAc (5%v/v) of FV used, the large broadened inhibition zone was observed. Considering mean of inhibition zone shown in Table 1, the largest inhibition zone was 17.71 mm at the highest AAc (5% v/v) of FV used. At the lowest AAc (0%v/v) of FV used, it could not observe the inhibition zone. Additionally, the inhibition zone of highest AAc of FV used was significant difference ($P \leq 0.05$) compared to inhibition zone at lower AAc of FV (0-4%v/v). However, there was non significant difference ($P > 0.05$) of inhibition zone of SE between 2 and 3%v/v AAc applied. The inhibition efficiency of SE was 55.2% and 58.6% as shown in Table 1. As mentioned in the report of Bell and Kyriakides [16], the inhibition efficiency of acetic acid was performed due to its pKa and pH. The bacteriocidal property of acetic acid and the others have been attributed to a lower pH below that needed for optimal growth. The broad range pH of acetic acid which affected various microorganisms was demonstrated by Branen *et al.* [17]. *Staphylococcus aureus* was inhibited by acetic acid with pH 5.0, while the pH of acetic acid affected

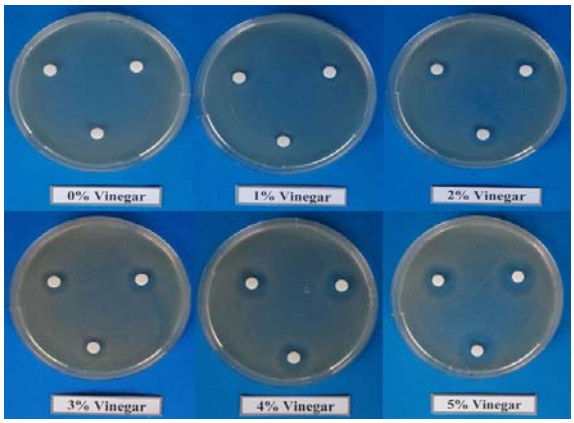

Figure 1. The inhibition zone on MHA by FV containing various AAc.

Salmonella spp. and *Bacillus cereus* was at 4.9. As a result from our study was similar to the previous study, the pH of 1- 3%v/v AAc of FV were in range of inhibition (pH 3.5-4.9). Therefore, the confirmation of SE inhibition by FV containing different AAc (1-3%v/v) was further studied *in vitro*.

Table 1. Average inhibition zone and inhibition efficiency of SE on MHA medium using different AAc of FV and the pH of FV at different AAc.

FV concentration (%v/v)	pH	Inhibition zone (mm)*	Inhibition efficiency** (%)
0	7.0	$\leq 6.00^{e} \pm 0.00$	0.0
1	4.9	$7.64^{d} \pm 0.29$	14.0
2	4.4	$12.46^{c} \pm 0.52$	55.2
3	3.5	$12.86^{c} \pm 0.62$	58.6
4	3.0	$15.34^{b} \pm 0.33$	79.8
5	2.3	$17.71^{a} \pm 0.54$	100.0

* Mean and standard deviation for n=3; Means with different letters within the same column are statistically significant ($P \leq 0.05$) according to DMRT.

** Inhibition efficiency of SE was calculated based on the size of inhibition zone at 0-4%v/v AAc compared with the highest inhibition zone at 5%v/v AAc.

Effect of FV on SE viability in vitro

Figure 2 shows the growth of SE as indicated by $OD_{625\,nm}$ and the viability of SE in acidified TSB with FV containing different AAc between 1-3%v/v with 0.1%v/v incretion compared to 0%v/v as control. An initial SE cell suspension (7.56 log cfu/ml) was declined one log cycle with 1.1%v/v AAc after 5 min. Our result was similar to Medina *et. al* [18] mentioned that numbers of *Salmonella* was decreased about 1 log cycle by 1.0%v/v acetic acid treatment. Moreover, results showed that strange effect in reducing 2 log cycle SE *in vitro* was found at 1.5-1.6%v/v AAc. The SE was completely inactivated by FV containing 2.0%v/v AAc after 5 min contacted time. Similar results were observed in the growth of SE in acidified TSB which was indicated by optical density ($OD_{625\,nm}$). In addition, our results supported the result of many studies. Pornpukdee-watana *et al.* [19] suggested that SE were inhibited by 2.0%v/v AAc of FV treated at pH 4.3-4.6. Kob-charoentham *et al.* [20] demonstrated that 2.0%v/v AAc of FV could destroy other Enterobacteriaceae, *Escherichia coli*. However, the result in inhibition of microorganism by acetic acid is explained by two hypothesis. The first one is due to the effect of acetic acid on microbial cell wall by changing protein structure. Additionally, acetic acid can easily penetrate lipid bilayer and changes the cell membrane structure which interferes with the membrane permeability. The others hypothesis involves the effect of acetic acid on microbial cytoplasmic membrane. The short chain acetic acid interferes with energy metabolism by altering the structure of cytoplasmic membrane proteins and then, causes to reduce ATP regeneration of membrane by uncoupling the electron transport side [21].

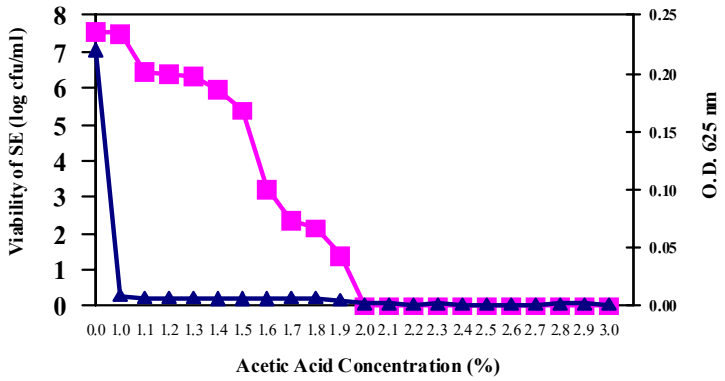

Figure 2. Viability (■) and growth as indicated by $OD_{625\,nm}$ (▲) of SE in acidified TSB containing 1-3%v/v AAc of FV for 5 min contact time at room temperature (31 ± 2°C).

Inactivation of SE by vapourized FV

The initial 6.14 log cfu/g inoculated SE on eggshell was prepared. The effect of vapourized treatment with 2%v/v and 10%v/v AAc of FV on inoculated SE on eggshell is shown in Table 2. Population of SE on inoculum eggshell was reduced 1.21 log cfu/g by vapourized 2%v/v AAc of FV after 1.0 h and achieved complete inhibition within 6.0 h. In case of 10%v/v AAc of FV, the viability of SE was reduced 1.11 log cfu/g within 1 h. The SE was completely inhibited after 5.0 h. It means that decrease of SE viability was found when the increase of AAc of FV was used. Moreover, more AAc was used, the vapourizing time was shortened.

Table 2. Viability of SE on eggshell after vaporizing by 2%v/v and 10%v/v AAc of FV at room temperature (31 ± 2°C).

Time (h)	2%v/v FV treatment		10%v/v FV treatment	
	Viability (log cfu/g)[a]	(%) Survival[b]	Viability (log cfu/g)[a]	(%) Survival[b]
0.0[c]	6.14 ± 0.05	100	6.14 ± 0.07	100
0.5	5.73 ± 0.07	93	5.01 ± 0.11	82
1.0	4.93 ± 0.08	80	4.15 ± 0.03	68
1.5	4.85 ± 0.06	79	3.55 ± 0.08	58
2.0	3.98 ± 0.10	65	3.17 ± 0.06	52
2.5	3.65 ± 0.12	59	2.76 ± 0.04	45
3.0	3.35 ± 0.07	55	2.38 ± 0.04	39
3.5	3.09 ± 0.03	50	2.09 ± 0.06	34
4.0	2.58 ± 0.08	42	1.71 ± 0.08	28
4.5	1.94 ± 0.04	32	1.48 ± 0.01	24
5.0	1.50 ± 0.01	24	ND	< 0.01
5.5	0.37 ± 0.10	6	ND	< 0.01
6.0	ND[d]	< 0.01	ND	< 0.01

[a] Population of SE detected on eggshell after treatment with vaporized FV. Means of three replications ± standard errors.
[b] Percentage of survival SE cell after FV vaporizing treatment.
[c] Initial number of SE (6.14 cfu/g) on eggshell after dipping egg in culture suspension containing 8.73 log cfu/ml cells.
[d] ND: not detected.

Shelf-life study

Initial 6.09 log cfu/g of SE-inoculated eggshell was treated with vaporizing 2%v/v and 10%v/v AAc of FV for 6.0 h and 5.0 h, respectively, compared with 0%v/v AAc of FV as control. During 28 days of storage at 4°C, egg sample was taken every 7 days for detected SE both on eggshell surface and in egg content. As a result showed in Table 3, there was no detected SE on eggshell and in egg content at the whole period of storage. It was similar result when eggs were kept at room temperature (31 ± 2°C). In this study revealed that no SE was detected in contents of any inoculated eggs. In fact the penetration of SE into egg content was possible, but it could not survive due to the integrity of vitelline membrane and the antimicrobial activity of white egg. This is a natural protection of egg content on microbial contamination as mentioned by many studies [7, 22].

Table 3. Effect of vapourized FV at 2%v/v and 10%v/v AAc on SE inoculated on eggshell surface during storage at 4°C and room temperature (31 ± 2°C) for 28 days.

AAc of FV treatment (%v/v)	Storage time (days)	SE (positive/total)*			
		4°C		Room temperature	
		Eggshell	Egg content	Eggshell	Egg content
0	0	3/15	3/15	3/15	3/15
	7	3/15	3/15	3/15	3/15
	14	3/15	3/15	3/15	3/15
	21	3/15	3/15	3/15	3/15
	28	3/15	3/15	3/15	3/15
2	0	0/15	0/15	0/15	0/15
	7	0/15	0/15	0/15	0/15
	14	0/15	0/15	0/15	0/15
	21	0/15	0/15	0/15	0/15
	28	0/15	0/15	0/15	0/15
10	0	0/15	0/15	0/15	0/15
	7	0/15	0/15	0/15	0/15
	14	0/15	0/15	0/15	0/15
	21	0/15	0/15	0/15	0/15
	28	0/15	0/15	0/15	0/15

* SE (positive/total); positive meant number of egg sample that SE was detected; total meant total number of egg samples used in the experiment.

Conclusion

According to the results of our experiment on the effect of FV on SE inhibition using Agar Overlay Disc Diffusion Method, the strongly significant effect of FV on SE was found when more AAc of FV was used. The SE inhibition was up to the concentration of acetic acid in the culture medium. More AAc used, more SE inhibition was occurred. However, the 1-3%v/v AAc of FV showed the acceptability of SE inhibition while the range of pH of these AAc was 3.5- 4.9 which normally recommended for microbial inhibition [17]. These concentration ranges, therefore, were used in the confirmation test of SE inhibition by using acidified TSB *in vitro*. The severe effect of FV in different AAc on SE was found. This was due to the acetic acid in TSB could directly contact and provided the severe effect to SE cells. The FV containing 2%v/v AAc caused the complete inhibition of SE after 5 min contact time at room temperature (31 ± 2°C). Then, the 2%v/v AAc of FV was used in the study of SE inhibition by vaporizing FV procedure. However, the result was comparable with 10%v/v AAc of FV, a commercial concentration produced in factory. The inoculated SE on eggshell was completely inhibited by 2%v/v AAc of FV vaporized with 6.0 h while 5.0 h vaporizing time in case of 10%v/v AAc of FV was treated. These results meant that only 2%v/v AAc of FV was adequate for preparing the FV vapor. It is simple procedure for protection of SE contamination in industry level. Additionally, results from shelf-life study of SE inoculated eggs after FV vaporizing treatment showed that no SE was detected both on eggshell and in egg content when egg samples were kept at 4°C and room temperature (31 ± 2°C). Based on our previous study in reduction of SE on surface of chicken eggshell with dipping and spraying treatment of FV [23], it could notice that both eggshell and cuticle layer were severe damaged. However, no physical damage of eggshell was

noticeably appeared in case of FV vaporizing treatment. So, it is strongly recommended that the FV vaporizing treatment is the effective procedure for controlling SE contamination on chicken eggshell. It is a natural treatment to achieve high-degree safety with respect to foodborne pathogens**.**

References

1. Messens, W., Grijspeerdt, K., Reu, K.D., Ketelaere, B.D., Mertens, K., Bamelis, F., Kemps, B., Baerdemaeker, J.D., Decuypere, E. and Herman, L. (2007). Eggshell penetration of various types of hens' eggs by *Salmonella enterica* serovar Enteritidis. **Journal of Food Protection**, 70, 623-628.

2. Messens, W., Grijspeerdt, K. and Herman, L. (2005). Eggshell penetration by *Salmonella*: a review. **Worlds Poultry Science Journal**, 61, 71-85.

3. EFSA. (2005). Opinion of the scientific panel on animal health and welfare on a request from the commission related to the welfare aspects of various systems of keeping laying hens. **EFSA Journal**, 197, 1-23.

4. National Food Institute. (2007). *Salmonella* spp. Available from http://www.techno.msu.ac.th/fn/ecenter/pathogens/salmonella_spp.htm. Accessed Date on 27 March 2005.

5. Bangtrakulnonth, A., Pornreongwang, S., Pulsrikarn, C., Sawangpanyalert, P., Hedriksen, R.S., Lo Fo Wong, D.M.A. and Aarestrop, F.M. (2004). *Salmonella* serovars from humans and other sources in Thailand, 1993-2002. **Emerging Infectious Diseases**, 10, 131-135.

6. Frenzen, P.D., Buzby, C. and Roberts, T. (1999). An updated estimate of the economic costs of human illness due to foodborne *Salmonella* in the United States, pp. 216-218. In P.B. Bahnson (ed.) Proceedings of the 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork, Washington, D.C. University of Illinois, Urbana-Champaign.

7. Himathongkham, S., Riemann, H. and Ernst, R. (1999). Efficacy of disinfection of shell eggs externally contaminated with *Salmonella* Enteritidis implications for egg testing. **International Journal of Food Microbiology**, 49, 161-167.

8. Knape, K.D., Carey, J.B., Burgess, R.P., Kwon, Y.M. and Ricke, S.C. (1999). Comparison of chlorine with an iodine-based compound on eggshell surface microbial populations in a commercial egg washer. **Journal of Food Safety**, 19, 185-194.

9. Padron, M. (1995). Egg dipping in hydrogen peroxide solution to eliminate *Salmonella* Typhimurium from eggshell membranes. **Avian Diseases Journal**, 39, 627-630.

10. Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., Pihlaja, K., Vuorela, H. and Vuorela, P. (2000). Antimicrobial effect of Finnish plant extracts containing flavonoids and other phenolic compounds. **International Journal of Food Microbiology**, 56, 3-12.

11. Uyttendaele, M., Neyts, K., Vanderswalmen, Y., Notebaert, E. and Debevere, J. (2004). Control of *Aeromonas* on minimally processed vegetables by decontamination with lactic acid, chlorinated water and thyme essential oil solution. **International Journal of Food Microbiology**, 90, 263-271.

12. Chung, K.C. and Geoepfert, J.M. (1970). Growth of Salmonella at low pH. **Journal of Food Science**, 35, 326-328.

13. Chang, J.M. and Fang, T.J. (2007). Survival of *Escherichia coli* O157:H7 and *Salmonella* enterica serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. **International Journal of Food Microbiology**, 24, 745-751.

14. Charteris, W.P., Kelly, P.M., Morelli, L. and Collins, J.K. (1998). Antibiotic susceptibility of potentially probiotic Lactobacillus species. **Journal of Food Protection**, 64, 1636-1643.

15. Alexander, M.C., Kim, Y.H., Tahk, S., Hong, P., Waring, A.J. and Calcitermin, T.G. (2001). A novel antimicrobial peptide isolated from human airway secretions. **FEBS Letters**, 504, 5-10.

16. Bell, C. and Kyriakides, A. (2002). *Salmonella* : A practical approach to the organism and its control in food. Blackwell Science. United Kingdom.

17. Branen, A.L., Davidson, P.M. and Salminen, S. (1989). **Food Additives**. Marcel Dekker, Inc.New York.

18. Medina, E., Romero, C., Brenes, M. and Castro, A.D. (2007). Antimicrobial activity of olive oil, vinegar and various Beverages against foodborne pathogen. **Journal of Food Protection**, 70, 1194-1199.

19. Pornpukde-watana, S., Krusong, W., Sweawiwat A., Wijitraka, A. and Tuntratian, S. (2006). Prolonged shelf-life of fresh chicken meat by using vinegar. The 8th Agro Industrial Conference: Food Innovation, 15-16 June 2006. BITEC, Bangna, Bangkok, Thailand.

20. Kob-charoentham, V., Adisaiparadee, A. and Krusong, W. (2004). Development of prolong shelf-life of dried yellowstrip trivally fish product by using vinegar. **King Mongkut's Institute of Technology Ladkrabang Journal**, 12, 43-49.

21. Sheu, C.W., Salomon D., Simmons J.L., Sreevalsan, T. and Fereese, E. (1975). Inhibitory effects of lipophilic acids and related compounds on bacteria and mammalian cells. **Antimicrobial Agents Chemother**, 7, 349-363.

22. Ernst, R., Fuqua, L., Riemann, H.P. and Himathongkham, S. (1998). Effect of sweating on shell penetration of *Salmonella enteritidis*. **Journal of Applied Poultry Research**, 7, 81-84.

23. Yeesibsan, J. and W. Krusong. (2009). Reduction of *Salmonella enteritidis* on surface of shell of chicken egg with dipping and spraying by fermented vinegar. **KMITL Agricultural Journal**, 27, 80-88.