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Combination Impact of Turmeric Extract and Fermented Vinegar on Reduction of Inoculated *Salmonella* Typhimurium on Fresh Lettuce

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Abstract

The impact of turmeric extract (TE), fermented vinegar (FV) and the mixture (TE-FV) on reduction of *Salmonella* Typhimurium (ST) on lettuce was investigated. The TE-FV solution consisting of 0.05 mg/ml TE and 1.7% v/v FV, with pH 3.8 provided more impact of ST reduction on fresh lettuce than individual use of TE or FV ($p \leq 0.05$). The results showed that the ST was gradually decreased for 3 log cycles from 7.11±0.1 log CFU/g to 3.60±0.1 log CFU/g after treatment of high inoculated ST (7.11±0.1 log CFU/g) by TE-FV for 20 min. Simultaneously, the complete inhibition of low level ST which initially inoculated for 3.14±0.1 log CFU/g was found. By the use of scanning electron microscope, it could be observed that the limit of ST inhibition was due to the irregularities of ST attachment on lettuce leaves. Moreover, the appearance change of un-inoculated lettuce leaves treated with TE-FV for 20 min was investigated. The slight changes in crispiness of lettuce leaves was generally noticed after storing at 4°C for 4 days. Therefore, it indicated that TE-FV solution has potential applicability as a natural sanitizer for improvements of microbiological quality and safety of fresh and fresh-cut fruits as well as vegetables in retail-catering industrial.

Keywords: turmeric extract, fermented vinegar, *Salmonella* Typhimurium, lettuce

1. Introduction

Consumption of fresh vegetables has increased over the past decade due to the awareness of their health benefits among consumers. A large number of minimally processed products are commercially available in supermarket as well as in catering service. With an increase consumption, the outbreaks of illness associated with raw vegetable were increased. Most of the reported outbreaks were also caused by pathogenic bacteria, especially *Salmonella* spp. [1]. The most frequently reported was *Salmonella* Typhimurium (ST) which commonly found in a wide

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variety of raw meats, dairy products, vegetables and water. Cross contamination with ST may still occur during production cycle originated from soil, water, equipments, animals and humans. Therefore, the efficient impact of sanitizing agents to control and reduce the population of ST is normally studied depending on treatment conditions such as concentration and contact time [2]. However, higher concentrations of chemical sanitizers may cause residues and result in potential health risks. This is an important issue to search for novel antimicrobial compounds from natural sources instead of chemical substances. In our study, natural compound of turmeric (*Curcuma longa* Linn) was focused. It is recognized as a rich source of essential oil, especially from rhizome and leaves. Moreover, it also contains antibacterial activity. The minimum concentration (MICs) of 15.62 and 125 μl/ml was found in rhizome and leaves, respectively [3]. In addition, the antibacterial potential of turmeric rhizome extracts has been evaluated and found that it contains a potential substance against pathogenic strains of Gram-positive (*Staphylococcus aureus, Staph. epidermidis*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella* Typhimurium) bacteria [4]. Moreover, a quantity of 0.3 ml. of turmeric juice could affect *E. coli, S.* Paratyphi, *S.* Typhimurium and *Staph. aureus* [5]. However, turmeric extract is limited in its application because of coloring compound of curcuminoids. Turmeric contains a major yellow pigment with the amounts of about 3-6%. When it is dissolved in a base solution, it changes to a brownish-red color, while it has a light yellow color when it reacts with acid [6]. Additionally, the fermented vinegar (FV) is an alternative natural compound which can be applied for adjusting pH condition in acid solution. Several studies have reported that it could inhibit the growth of microorganisms in various products such as fungi on dried yellow strip trevally fish [7], *S.* Anatum of fresh pork loin [8], *S.* Enteritidis on surface of eggshell [9]. While FV was recommended to extend shelf life of fresh strawberries [10], the report of Sengun and Karapinar [11] showed that the reduction of ST on carrot was 0.79-3.95 log CFU/g by using lemon juice-vinegar mixture (1:1). The efficacy cleaning method in reducing bacterial contamination of fresh produce such as apples, tomatoes and lettuce by using 5% vinegar solution as well as 13% lemon solution followed by rinsing the fruits and vegetables under tap water were reported by Kilonzo-Nthenge *et al.* [12]. Consequently, the purpose of this study was to investigate the reduction of ST by a mixture solution of turmeric extract (TE) and FV as a natural sanitizer in substitution of chemical substance.

2. Materials and Methods

2.1 Raw material preparation

Fresh lettuces were purchased from supermarket at Ladkrabang district, Bangkok. They were pretreated by the following steps. Firstly, they were selected in the same size. Then, the outer 4-5 leaves and core were removed, cleaned by tap water for 5 min and let them dry in laminar air flow cabinet for 30 min. They were shredded approximately 1 inch from their roots and selected the leaves which had similar weight (8-12g per leaf). Sample was taken to analyze for contaminated ST on XLD agar. The remaining fresh leaves were packed in plastic bag, stored at 5°C and used in the experiments.

2.2 Microorganism

Salmonalla Typhimurium (ST) ATCC 13311 was provided by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nontaburi province, Thailand. One loop full of ST was transferred to 20 ml of trypticase soy broth (TSB) and incubated at 37ºC for 18-20 h. Subsequently, the suspension was diluted with 80 ml of sterile (0.1%) peptone solution in order to achieve the ratio of suspension 1:5. The density was adjusted to 0.5 McFarland Standard

to achieve concentration of high population of 7 log CFU/ml, then bacterial culture was tenfold serially diluted by sterile (0.1%) peptone water to 3 log CFU/ml for low population.

2.3 Preparation of treatment solution containing turmeric extract and fermented vinegar

Based on our previous study in investigation of the survival of ST *in vitro* from the inhibitory impact of turmeric extract (TE), fermented vinegar (FV) and the mixture called "TE-FV" [13], three treatment solutions were applied as follows: 0.05% TE, 1.7% acetic acid concentration of FV and their mixture "TE-FV".

2.4 Treatment of turmeric extract and fermented vinegar on ST-inoculated lettuces

The pre-treated fresh lettuce samples were inoculated with ST suspension before placing in 9x12 inch polyethylene bags. Two levels of ST containing high and low populations were individually inoculated. After inoculation, the lettuce sample, called ST-inoculated lettuce was gently shaken for 5 min and let it dry on sterilized rack in laminar air flow cabinet for 10 min and stored at 20$\pm$2^{o}C for 24 hrs before tested with treatment solutions. Each ST-inoculated lettuce sample was soaked in the prepared treatment solution at the ratio of 1:20 (W/V). Each sample was exposed into the solution for 0, 5, 10, 20, 25 and 30 min, respectively. It was subsequently rinsed by sterile distilled water at the same ratio for 10 sec for residual removal. Then, the treated sample was let stand in laminar air flow cabinet at 30$\pm$2^{o}C for 30 min. By using stomach homogenous technique with medium speed for 2 min, 25g of ST-inoculated lettuce sample was placed in a sterile bag and 225 ml of sterile (0.1%) peptone water was then added in order to mix and rinse off the survival ST on surface of the lettuce. The ST survival in sample was enumerated. Three replicates were conducted.

2.5 Study on release of attached ST cells from ST-inoculated lettuces during treatment

After treated with TE, FV and the mixture TE-FV solution as above mentioned procedure (2.4), the release of attached ST cells from surface of ST-inoculated lettuces in each solution were simultaneously enumerated by spread plate technique on TSA and XLD. The experiments were done in three replicates.

2.6 Effect of TE-FV on appearance of lettuce after storage at 4^{o}C

Fresh lettuce samples were treated with TE, FV and the mixture TE-FV as the same as above experiment at the most effective inhibition/contact time on ST cells of ST- inoculated lettuce. The RO water was used as control. All samples were placed in stainless steel shallow tray and stored at 4^{o}C for 0-7 days. On each day, the appearance of lettuce, especially crispiness, was observed.

2.7 Analytical procedure

The ST cells were enumerated by spread plate technique on TSA and XLD. The scanning electron microscope (SEM) of microstructure of ST on the surface of lettuce after treatment was examined by S-150 scanning electron microscope, HITACHI, JAPAN.

2.8 Statistical analysis

Data was interpreted by SPSS Version11.5. Analysis of variance (ANOVA) was determined. Additionally, Duncan's new multiple range test (DMRT) at $p \leq 0.05$ was used to determine the differences between treatments.

3. Results and Discussion

3.1 Inhibitory impact of turmeric extract (TE), fermented vinegar (FV) and the mixture of turmeric extract and fermented vinegar (TE-FV) on ST inoculated on lettuces

The inhibitory impact of 0.05 mg/ml TE, 1.7% (v/v) acetic acid concentration of FV called as "1.7% (v/v) FV", and the mixture TE-FV on inoculated ST on surface lettuces were investigated. High level (7.11±0.1 log CFU/g) and low level (3.14±0.1 log CFU/g) of ST were separately inoculated on lettuce samples. Results in reduction of high or low population of inoculated ST on lettuces by 0.05mg/ml TE, 1.7% v/v FV and the mixture "TE-FV" were shown in Table 1. The significant reduction of inoculated ST was found after 5 to 10 min of contact time among all treatments. It could be noticed that the TE-FV provided the most impact at reduction of inoculated and attached ST cells on lettuces compared with only TE or FV, individually. In case of high level inoculation, the 73.3% ST reduction by TE-FV was found after 30 min contact time while low level of attached ST cells were completely inhibited after 20 min contact time ($p \leq 0.05$). Results of inhibitory impact of TE-FV on ST belongs to the integration of antibacterial property of both acetic acid in FV and major components of TE which generated in the mixture TF-FV solution. In case of acetic acid in FV, it is attributed to direct pH reduction by ionization of the un-dissociated acid molecule, or disruption of substrate transport into metabolism of ST cell membrane [14,15]. Furthermore, the turmerone, an antibacterial agent, from TE causes effective damage of ST cells in both inner and outer cell membrane. All substances in cytoplasm of bacterial cells are continually released externally and finally caused the death of ST cells [16].

Table 1 Survival of inoculated high or low population of ST on lettuce after treatment with 0.05 mg/ml TE, 1.7% (v/v) FV and the mixture TE-FV solution

	Survival (log CFU/g)*					
Contact time (min)**	**High population of ST**			**Low population of ST**		
	TE	**FV**	**TE-FV**	**TE**	**FV**	**TE-FV**
0	$7.11^{aA}\pm0.1$	$7.11^{aA}\pm0.1$	$7.11^{aA}\pm0.1$	$3.14^{aA}\pm0.1$	$3.14^{aA}\pm0.1$	$3.14^{aA}\pm0.1$
5	$6.50^{bA}\pm0.1$	$6.30^{bA}\pm0.1$	$5.80^{bB}\pm0.1$	$2.70^{bA}\pm0.1$	$2.40^{bA}\pm0.1$	$2.20^{bA}\pm0.1$
10	$5.20^{cA}\pm0.1$	$4.80^{cA}\pm0.1$	$4.00^{cB}\pm0.1$	$2.00^{cA}\pm0.1$	$1.70^{cAB}\pm0.1$	$1.40^{cB}\pm0.1$
15	$4.80^{cdA}\pm0.1$	$4.50^{cA}\pm0.1$	$3.80^{cdB}\pm0.1$	$1.70^{cA}\pm0.1$	$1.30^{cAB}\pm0.1$	$1.00^{cB}\pm0.1$
20	$4.40^{dA}\pm0.1$	$3.80^{dB}\pm0.1$	$3.60^{dB}\pm0.1$	$1.10^{dA}\pm0.1$	$0.80^{dA}\pm0.1$	$0.10^{dB}\pm0.1$
25	$3.40^{eA}\pm0.1$	$3.00^{eA}\pm0.1$	$2.50^{eB}\pm0.1$	$1.00^{dA}\pm0.1$	$0.70^{dA}\pm0.1$	$0.00^{dB}\pm0.1$
30	$2.80^{fA}\pm0.1$	$2.60^{eA}\pm0.1$	$2.00^{fB}\pm0.1$	$0.90^{dA}\pm0.1$	$0.50^{dA}\pm0.1$	$0.00^{dB}\pm0.1$

Mean and standard deviation for n=3; *Means with different letters within the same column were statistically significant ($p \leq 0.05$) according to DMRT. ** Means with different capital letters within the same row of high population of ST or low population of ST were statistically significant ($p \leq 0.05$) according to DMRT.

3.2 Release of attached ST cells from ST-inoculated lettuces during treatment

According to the soaking step of ST-inoculated lettuce sample in the treatment solution, it was necessary to monitor the release of ST into the solution which may cause in reduction of ST on the lettuce sample. Result as shown in Table 2 indicates that the release of attached ST cells from ST-inoculated lettuces occurred during treatment with 0.05 mg/ml TE, 1.7% (v/v) FV and the mixture. The high amount of released ST cells was found during the prior 5 min of contact time in both high and low populations of ST. The released ST cells in each solution was significantly inhibited

by the antibacterial agents of TE, FV or TE-FV. Additionally, the highest inhibitory impact on released ST was also found. According to the study of Dansai and Krusong [13], the complete inhibition of ST *in vitro* by TE-FV occurred after 10 min of contact time. However, the longer contact time was noticed when TE-FV was applied to lettuce leaves. This result belonged to the effect of irregularities of the surface of lettuce leaves. To support this finding, the scanning electron microscope (SEM) of attached ST on surface of lettuce leaves was studied. The results as shown in Figure 1a and 1b, revealed that ST cells located in the irregularities on the intact surface and the inner regions of the trichome. It seemed that the surface of lettuce acted as a shield for ST cells. Therefore, attached ST cells on lettuce leaves were resistant to antibacterial agent.

3.3 Effect of TE-FV on appearance of lettuce during storage at 4ºC

The effect of TE, FV and the mixture TE-FV on appearance of fresh lettuces after treatment and storage for 7 days at 4ºC was investigated. Results were shown in Figure 2a-2d. Based on sensory evaluation by visual observation, it could be observed that crispiness of lettuce leaves was slightly changed within 3 days of storage among treatments. However, after 4 days of storage, the browning leaves and decay of crispiness appearance of lettuces were found in sample treated with 1.7% (v/v) FV as noticed in Figure 2b. At the same period, both of 0.05 mg/ml TE (Figure 2a) and RO water (Figure 2d) slightly affected with crispiness and browning at the edge of lettuce leaves. While lettuce leaves treated by TE-FV (Figure 2c) showed minor change in translucency and crispiness appearance of a fresh lettuce. Additionally, Fillion and Kilcast [17] reported that crispy and crunchy textures are a desirable quality and particularly important in fruits and vegetables, where consumers associate them with freshness. Therefore, it could be summarized that the treatment of TE-FV could extend shelf-life of fresh lettuce leaves during storage at 4ºC for 4 days.

Table 2 Release of inoculated high or low population of ST on lettuce during treatment with 0.05 mg/ml TE, 1.7% (v/v) FV and the mixture TE-FV solution

	Survival (log CFU/g)*					
Contact time** (min)	High population of ST			Low population of ST		
	TE	FV	TE-FV	TE	FV	TE-FV
0	$0.00^{gA}\pm0.1$	$0.00^{fA}\pm0.1$	$0.00^{dA}\pm0.1$	$0.00^{gA}\pm0.1$	$0.00^{eA}\pm0.1$	$0.00^{cA}\pm0.1$
5	$0.60^{aA}\pm0.1$	$0.55^{aB}\pm0.1$	$0.50^{aC}\pm0.1$	$0.40^{aA}\pm0.1$	$0.32^{aB}\pm0.1$	$0.26^{aC}\pm0.1$
10	$0.44^{bA}\pm0.1$	$0.42^{bA}\pm0.1$	$0.35^{bB}\pm0.1$	$0.25^{bA}\pm0.1$	$0.22^{bAB}\pm0.1$	$0.20^{bB}\pm0.1$
15	$0.35^{cA}\pm0.1$	$0.30^{cB}\pm0.1$	$0.24^{cC}\pm0.1$	$0.20^{cA}\pm0.1$	$0.14^{cB}\pm0.1$	$0.02^{cC}\pm0.1$
20	$0.30^{dA}\pm0.1$	$0.25^{dB}\pm0.1$	$0.02^{dC}\pm0.1$	$0.15^{dA}\pm0.1$	$0.10^{cdB}\pm0.1$	$0.00^{cC}\pm0.1$
25	$0.25^{eA}\pm0.1$	$0.10^{eB}\pm0.1$	$0.00^{dC}\pm0.1$	$0.10^{eA}\pm0.1$	$0.06^{deA}\pm0.1$	$0.00^{cB}\pm0.1$
30	$0.15^{fA}\pm0.1$	$0.08^{eB}\pm0.1$	$0.00^{dC}\pm0.1$	$0.08^{fA}\pm0.1$	$0.02^{eB}\pm0.1$	$0.00^{cB}\pm0.1$

Mean and standard deviation for n=3;*Means with different letters within the same column were statistically significant ($p \leq 0.05$) according to DMRT.** Means with different capital letters within the same row of high population of ST or low population of ST were statistically significant ($p \leq 0.05$) according to DMRT.

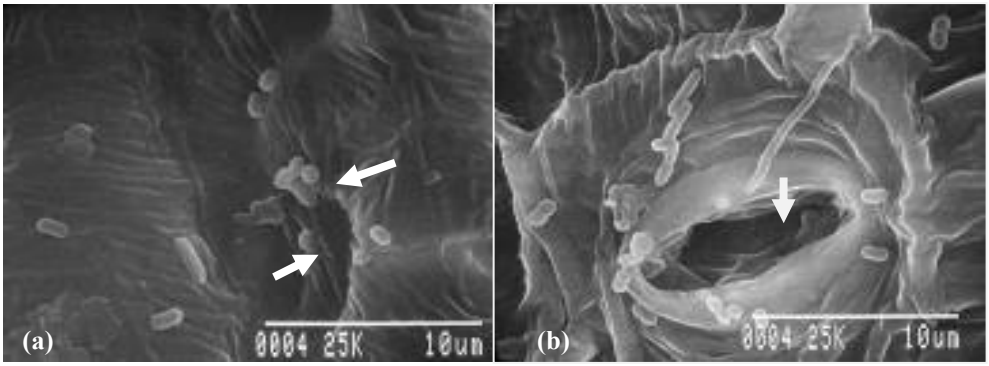

Figure 1 Scanning electron microscope of ST cells on the surface of lettuce after treated with TE-FV solution: (a) the attachment of ST cells on intact surface of lettuce leaves;
(b) the attachment sites on the inner regions.

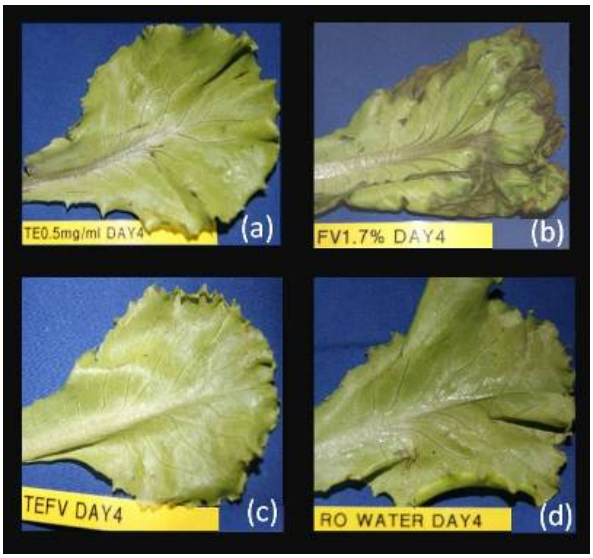

Figure 2 Appearance of lettuce leaves after treatment with 0.05 mg/ml TE, 1.7% (v/v) FV and the mixture TE-FV solution for 20 min and storing at 4° C for 4 days: a, 0.05 mg/ml TE; b, 1.7% (v/v) FV; c, the mixture TE-FV; d, RO water as control.

4. Conclusions

The mixture TE-FV solution containing 0.05mg/ml turmeric extract (TE) and 1.7% (v/v) acetic acid concentration of fermented vinegar (FV) provided significant inhibitory impact of inoculated-ST cells on lettuces higher than individual use of TE or FV ($p \leq 0.05$) At high ST population, the 73.3% reductions of ST was found after treatment with TE-FV for 30 min while 62.9% and 68.7% reductions were observed after TE or FV treatment at the same contact time, respectively. Moreover, the ST attached cells on lettuce leaves were completely inhibited by TE-FV within 20

min at low ST population. The TE-FV is recommended as a suitable natural sanitizer in decontamination of fresh vegetables and reduces the risk of chemical residue from chemical sanitizer and increases safety for food consumers. This application might improve the microbiological quality and safety of fresh cut produce in retail-catering industrial.

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