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The antimicrobial, antibrowning, and shelf-life study of fruit and vegetable sanitizer from herbal extracts

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Abstract

In the past 3 years, The Centers for Disease Control and Prevention (CDC) reported that there were at least 7 outbreaks in fresh produces, for example; *Escherichia coli* O121 in raw clover sprouts (2014), *E. coli* O157:H7 in ready-to-eat salads (2013), and *Salmonella* Saintpaul in cucumbers (2013). The cleaning of fresh produces is the first step in order to prevent foodborne outbreak. Therefore, this research was aimed to develop and study the shelf life of fruit and vegetable sanitizer from Thai herb crude extracts including Chili (*Capsicum annuum*), Lemongrass (*Cymbopogon citratus*), Kaffir lime (*Citrus hystrix*),. Chrysanthemum (*Chrysanthemum indicum* L) and Som-poi (*Acacia concinna* (Willd.) D.C). The fruit and vegetable sanitizer was prepared by adding 1.5% of each 0.2 mg/ml crude extracts into Som-poi's pod water and was sterilized at 121 °C for 15 min. The percentage of antimicrobial efficiency was used for evaluating the antibacterial activity and studying shelf-life comparing with 2 commercial brands (Jirada and Veggie). Lettuce (*Lactuca sativa*) as vegetable model was soaked in 1 % sanitizer for 20 min and was done for total plate count every month for 3 months. The results showed that the fruit and vegetable sanitizer had the highest percentage of antimicrobial efficiency in 1st and 2nd month; 87.00±13.58, 78.70±25.01, respectively. The antimicrobial efficiency was significantly decreased after 2nd month. This indicated that the fruit and vegetable sanitizer from herb extracts showed antibacterial activity and its shelf-life was only 2 months. For antibrowning activity, apple (*Malus domestica*), as fruit model, was soaked in 0.1 % fruit and vegetable sanitizer for 2 min, and then color was measured by using colorimeter every 10 min for 50 min. The total color difference (ΔE^*) was used to evaluate the antibrowning activity. The results showed that fruit and vegetable sanitizer had no antibrowning activity.

Keywords: Antimicrobial, Antibrowning, Herb Extract, Fruit and Vegetable Sanitizer.

1. Introduction

Nowadays, many people are interested on their health so the fresh vegetable is the new food trend. In the past 3 years, The Centers for Disease Control and Prevention (CDC) has been reported at least 7 outbreaks about food safety in fresh produce such as fresh vegetables and fruits, for example, *Escherichia coli* O121 in raw clover sprouts (2014) [1], *E. coli* O157:H7 in ready-to-eat salads (2013) [2], *Salmonella* Saintpaul in cucumbers (2013) [3], *Salmonella* Typhimurium and Newport in cantaloupe (2012) [4], and *E. coli* O157:H7 in spinach and spring mix salad (2012) [5].

Foodborne disease is an increasingly serious public health problem. So, cleaning of fresh produce is the first step that can prevent these foodborne outbreaks. Therefore, this research aimed to develop and study the shelf life of the fruit and vegetable sanitizer from Thai herb extracts by using crude extracts including, *Capsicum annuum*(Chili), *Cymbopogoncitratus* (Lemongrass), *Citrus hystrix* (Kaffir lime), *Chrysanthemum indicum* L. (Chrysanthemum) and *Acacia concinna* (Willd.) D.C (Som-poi). Its antibrowning activity was also determined. These herbs have been reported for their antibacterial activity [6] [7] [8] [9] [10][13].

2. Materials and Methods

2.1 Preparation of herbs

Thai herb samples: *A. concinna* (Willd.) D.C (Som –poi pod), *C. annuum* (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime peel), and *C. indicum* L. (Chrysanthemum) were bought from local fresh market in Ramkamhaeng, Bangkok, Thailand. The herbs were cut into small pieces and dried in air oven (Memmert, UM500) at 45°C for 20 hours. Then, dried herbs were blended by using blender to reduce

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the size of herbs. The herb powder was packed in polyethylene bag and refrigerator at 4°C until use.

2.2 Preparation of crude extracts

The 20 g of herbs powder was mixed with 100 ml of 95 % Ethanol was added and soaked for 48 hours under shaking at 120 rpm at room temperature. After that, the liquid part was filtered out. Then, crude extract was evaporated by rotary evaporator at 120 rpm, 45°C for 15 minutes until slurry crude extract was obtained. The crude extract was diluted to 0.2 g/ml. It was kept in freezer at -20°C until use.

2.3 Preparation of fruit and vegetable sanitizer

100g *A. concinna* (Willd.) D.C (Som poi pod) was mixed with 500 ml water and then, 0.2g Disodium ethylene diaminetetra acetate (2NaEDTA) and 10 ml Polysorbate Tween20 were added. Thereafter 1.5% of each 0.2 g/ml crude extracts using 95% ethanol; *C. annuum* (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum) were added. The vegetable sanitizer was sterilized at 121 °C for 15 min. and kept at room temperature.

2.4 Antimicrobial efficiency

100 g of *Lactuca sataiva* (Lettuce) was used as a vegetable model. The lettuce was soaked in 1 % of fruit and vegetable sanitizer for 20 min [11]. Then *L. Sataiva* was swapped. The plate count agar (PCA) was used for total plate count (CFU/ml). The antimicrobial efficiency of fruit and vegetable sanitizer was evaluated as the antimicrobial efficiency percentage using the following equation:

$$\text{Antimicrobial efficiency percentage} = \frac{\text{CFU/ml unwash} - \text{CFU/ml wash}}{\text{CFU/ml unwash}} \times 100$$

The experiments were done duplicate and 3 times independently.

2.5 Antibrowning efficiency

Malus domestica (apple) was used as fruit model. 6 pieces of *M. domestica* was soaked in 0.1 % of fruit and vegetable sanitizer for 2 min, then was air dried at room temperature. The color was measured by color meter (HunterLab, model MSEZ) every 10 min for 1 hr. The total color difference (ΔE^*) was used to evaluate the antibrowning activity. The color parameters, L, a* and b* were used to calculate total color difference using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Three readings were made in each replicate by changing the position of the apple pieces. The experiments were done duplicate and 3 times independently

2.5 The shelf-life study

The fruit and vegetable sanitizer was kept at room temperature in clear glass bottle. The antimicrobial and antibrowning efficiency of fruit and vegetable sanitizer was evaluated every 4 weeks for 12 weeks. The results were compared with 2 commercial brands; Veggie and Jitrada.

2.6 Statistical Analysis

The data were analyzed using SAS (Statistical Analysis System for 158 Windows, Version 9.3, 2015, SAS Institute Inc., Cary, NC).

3. Results and Discussion

The antimicrobial efficiency of fruit and vegetable sanitizer using *L. sataiva* as vegetable model in different week is shown in table 1. The results showed that the highest antibacterial efficiency of vegetable sanitizer was found in week 4 and 8 significantly. The antibacterial efficiency of vegetable sanitizer was significantly decreased after week 8 when compared with Veggie and Jirada. This indicated that shelf-life of vegetable sanitizer is only 2 months (8 weeks) for the best antibacterial efficiency.

Table 1: The antimicrobial efficiency percentage of fruit and vegetable sanitizer at different week using *L. sataiva* as vegetable model

Treatment	Time (Week)			
	0	4	8	12
Unwash	0.00^{Ba} ±0.00	0.00^{Ca} ±0.00	0.00^{Ba} ± 0.00	0.00^{Ba} ±0.00
Wash	0.00^{Bb} ±0.00	42.18^{Ba} ± 46.31	19.27^{Bab} ± 21.03	18.24^{Bab} ± 20.49
Veggie	12.67^{Bc} ± 31.04	58.88^{ABab} ± 42.20	78.70^{Aa} ± 25.01	22.03^{Bbc} ± 20.92
Jirada	4.38^{Bb} ± 8.29	44.95^{Ba} ± 49.36	55.05^{Aa} ± 45.96	63.90^{Aa} ± 28.68
Sanitizer	38.09^{Ab} ± 23.52	87.00^{Aa} ± 13.58	78.70^{Aa} ± 25.01	8.57^{Bc} ± 15.05

*Product differences within the same week (As shown in each row) - Same capital letter in each row means no significant difference.
*Shelf-life within the same product (As shown in each column) - Same small letter in each column means no significant difference.

The antibrowning efficiency, as the total color difference (ΔE^*), of fruit and vegetable sanitizer in different week was shown in table 2. The *M. domestica* (apple) was used as fruit model. The results showed that the total color difference (ΔE^*) kept increasing. This indicated that fruit and vegetable sanitizer did not show antibrowning activity.

Table 2: The total color difference (ΔE^*) of *M. domestica* (apple) slices

Week	Time (min)					
	0	10	20	30	40	50
0th	3.04^{BCa} ± 1.52	2.66^{Ca} ± 0.59	3.54^{BCa} ± 1.57	3.94^{ABa} ± 1.54	2.82^{BCb} ± 0.78	4.90^{Aab} ± 1.27
4th	3.27^{Ca} ± 2.04	3.10^{Ca} ± 0.81	3.20^{Ca} ± 0.50	3.91^{BCa} ± 0.99	5.35^{ABa} ± 2.20	6.26^{Aa} ± 3.46
8th	2.84^{Aa} ± 1.73	2.87^{Aa} ± 1.23	2.83^{Aa} ± 1.52	2.82^{Aa} ± 1.30	3.71^{Ab} ± 2.57	2.92^{Ab} ± 1.47
12th	2.21^{Ba} ± 1.33	3.19^{ABa} ± 1.38	3.78^{Aa} ± 1.10	3.14^{ABa} ± 1.56	3.46^{Ab} ± 1.79	3.99^{Ab} ± 1.51

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*Timing differences within the same week (As shown in each row) - Same capital letter in each row means no significant difference.
*Week within the same time (As shown in each column) - Same small letter in each column means no significant difference.

A. concinna's pod or Som -poi s pod is the main herb that used in this fruit and vegetable sanitizer. The different parts (pod, leave, and bark) of *A. Concinna* have been reported for their antimicrobial activity. It was reported that the crude extracted of *A. concinna* pod can inhibit the growth of *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumonia* [12]. It have been reported that the terpenoids, saponins, tannin, alkaloids, and flavonoids can be isolated from *A. concinna* with the chloroform, benzene, methanol, and aqueous extraction condition [12]. The *A. concinna*'s leaves have been in development of natural vegetable sanitizer with the antibacterial activity as well [11]. The 95% ethanol crude extract of *A. concinna* shown the highest antibacterial activity against *S. enterica* and *S. enterica* Typhimurium [13]. These reports supported the result in this experiment that *A. concinna* exhibited antibacterial activity.

The *C. citrates* or lemongrass ethanol extracts showed appreciable antimicrobial activity [14,15]. The *C. citratus* essential oil can exhibited antibacterial activity against *L. monocytogenes* ATCC 19117 [14]. Previous study showed that 95 % ethanolic crude extracts of *C. Citrates* gave the highest antibacterial activity against *L. monocytogenes 10403S* [10,16]. These results confirm that *C. citrates* extarct is one of important ingredient, which has antibacterial activity, in vegetable sanitizer

The peel of *C. hystrix* or kaffir lime was used in this experiment. In general, the inhibitory activity of essential oils was greater than that of ethanolic extract, especially in kaffir lime peels [17]. The ethyl acetate extract of kaffir peel showed broad spectrum of inhibition against all gram-positive bacteria, yeast and molds including *S. aureus, B. cereus, L. monocytogenes, S. cerevisiae* var. sake and *A. fumigatus* TISTR 3180 [18]. This indicated that peel of *C. hystrix* extract has antibacterial activity

The dry chili in Thai seasoning Tom-Yum cannot inhibit *L. monocytogenes* [19]. It showed that by adding 1% w/v of dried chili in BHI can slightly inhibit the growth of *L. monocytogenes* [20]. The plain and heated extracts were found to exhibit varying degrees of inhibition against *B. cereus, B. subtilis, C. sporogenes, C. tetani, and S. Pyogenes* [21]. The capsaicin is a major antimicrobial compound of chili that might be antibacterial agent of chili in this experiment.

It was found that the essential oil of *C. indicum* or chrysanthemum exhibited moderate activities against most of tested streptococci species and also the essential oil showed the strong antimicrobial activity against obligate anaerobic bacteria[22]. The crude 95% ethanolic extract of *C. indicum* had antibacterial activity under normal and osmotic stress against both *B. cereus* and *L. monocytogenes* 10403S [8]. In other study, the crude 95% ethanolic extract of *C. indicum* had antibacterial activity under low pH against both *B. cereus* and *L. monocytogenes* 10403S [6]. This confirmed that *C .indicum* had the antibacterial activity in the vegetable sanitizer.

The crude extract of *C. annuum* (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum) and the *A. Concinna*'s pod or Som –poi's pod water showed the combined effect of antimicrobial efficiency. However, the result in table 1 showed that the antimicrobial efficiency significantly decreased after week 8. This indicated that the shelf-life of the fruit and vegetable sanitizer was 8 weeks. The fruit and vegetable sanitizer was stored in clear glass bottle. So the light oxidation of active compound in the fruit and vegetable sanitizer might be possible cause that the antimicrobial activity decreased. However this hypothesis need to further investigation.

The result in table 2 showed that the total color difference (ΔE^*) of *M. domestica* (apple) slices continuously increased after 50 min of storage. This showed that the fruit and vegetable sanitizer did not have the antibrowning efficiency. However, the polyphenol oxidase (PPO) assay will be done for further investigation in order to confirm the antibrowning efficiency of that the fruit and vegetable sanitizer .

4. Conclusions

The fruit and vegetable sanitizer from herb extracts showed antimicrobial efficiency which can reduce microbial load in fresh vegetable. The shelf-life study found that the antimicrobial efficiency decreased significantly after week 8. This indicated that the vegetable sanitizer had shelf-life 8 weeks for the best antimicrobial efficiency.

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