Combination of Chitosan Coating and Ultraviolet-C Irradiation for Reducing Escherichia Coli and Salmonella Sp. on Asparagus Spears

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***Abstract*—*Escherichia coli* and *Salmonella* sp. are bacterial pathogen that causes foodborne diseases in asparagus. Chitosan coating and ultraviolet C (UV-C) irradiation may reduce foodborne pathogen. Asparagus were coated with chitosan and inoculated with bacterial pathogen, then irradiated with UV-C. They were packed in foam tray, wrapped with 14 μm polyvinyl chloride film and stored at 10°C. *Escherichia coli, Salmonella* sp., total microbial and fungi counts were enumerated during storage. UV-C irradiation alone reduced *Escherichia coli*. Chitosan coating, UV-C irradiation and the combination of chitosan and UV-C were significantly reduced total microbial count. The lowest total microbial count was found in sample coated with chitosan and irradiated with UV-C. Fungi count was not detectable or below to the detection level, but there after the count were increased and higher to the detection level. The lowest water soaking was found in asparagus coated with chitosan combined with UV-C irradiation. The shelf life of asparagus spear was about 6 days.**

***Index Terms*—*escherichia coli, salmonella* sp., asparagus, UV-C, chitosan**

I. Introduction

Nakhon Pathom province, Thailand is a famous place to produce and export fresh asparagus spears (*Asparagus officinalis* L.). Asparagus is a highly perish vegetable because of it has a high respiration rate. It is rapidly deteriorate and senescence just after harvesting. Asparagus is a vegetable that grow on the soil, therefore it is easy to contaminate with various microorganisms especially foodborne pathogenic bacteria. *Escherichia coli* and *Salmonella* sp. are gram-negative foodborne pathogenic bacteria that have been reported to contaminate in many kind of fresh, fresh-cut produce, unpasteurized fruit and vegetable juices. These bacteria can cause foodborne disease outbreaks as reported in many countries [1], [2].

Chitosan is a natural heteropolysaccharide that obtain by deacetylation of chitin [3]. It applies to use as an edible coating material on the surface of fresh produce. There are a lot of research report that chitosan can maintain the quality and safety of fruit and vegetable such as guava fruit [4], whole cantaloupe [5], shiitake mushroom [6], fresh-cut broccoli [7], white asparagus [8] and green asparagus [9], [10]. The beneficial of chitosan coating on fruit and vegetables are delay chlorophyll degradation in guava fruit [4], maintain firmness and inhibit an increase of respiration rate of shiitake mushroom [6], maintain ascorbic acid content of green asparagus [9], retarding moisture loss of white asparagus [8] and green asparagus [10]. Moreover, it can inhibit the growth of microorganisms such as pseudomonads, yeasts and moulds of shiitake mushroom [6], inhibit total coliform of fresh-cut broccoli [7], reduce native bacteria and *Salmonella* on whole cantaloupe [5]. There are a few researches report on the effect of chitosan on the reduction of foodborne pathogen.

Ultraviolet-C (UV-C) has been use to act as an antimicrobial agent to destroy microorganism in fresh produce. It can cause DNA damage on bacterial cell and can induce resistance mechanisms against pathogens in fruit and vegetables [11]. Recently, it have been reported that UV-C irradiation reduce microbial contamination in

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fresh-cut carrot [12], asparagus [13], buckwheat sprouts [14], alfalfa and clover sprouts [15]. Martinez-Hernandez *et al*. [16] reported that UV-C irradiation can inactivated *Escherichia coli*, *Salmonella* Enteritidis and *Listeria monocytogenes* in fresh-cut kalian-hybrid broccoli. Poubol *et al*. [13] found that UV-C irradiation can reduced *Salmonella* sp. and *Escherichia coli* contaminated on spearmint. In the year 2014, Jahid *et al.* [17] reported that UV-C irradiation can inactivated *Salmonella typhimurium* in lettuce, whereas it can inactivated *Salmonella* enteric and *Escherichia coli* O157:H7 on grape tomato [18]. In this research, we have focusing on the effect of chitosan and UV-C irradiation on the reduction of *E. coli* and *Salmonella* sp. contaminated on asparagus spears.

II. Meterials and Methods

A. Plant Material, Chitosan Coating and UV-C Irradiation

Asparagus (*Asparagus officinalis* L.) spears were harvested from a farm in Nakhon Pathom province, Thailand. A 10 mm diameter spear was selected for uniformity of size and color, and then cut into 12cm. of height. The cut surfaces were washed thoroughly with tap water. Asparagus spear were dipped for 1 min in a 0.5% low molecular weight chitosan (deacetylation degree (DD) =75%) solution (Sigma-Aldrich, China), which was prepared by dissolving it in 0.1M acetic acid solution. The asparagus spears were allowed to dry in a biohazard laminar flow for 5min. Then, they were randomly inoculated with an equal mixed volume cocktail suspension of *Escherichia coli* and *Salmonella* sp. (final microbial population of 10^5 CFU/ml). All inoculated asparagus spears were left for about 5min in a biohazard laminar flow. Fifteen asparagus spears were aseptically placed in foam tray (144×203×16mm) and wrapped with 13μm thickness of polyvinylchloride (PVC) film. The CO_2 permeability of the film was 292,862ml/m^2·d at 25℃ and 95% RH.

To determine the effect of UV-C irradiation on microbial contamination of asparagus, fresh asparagus spear were inoculated with foodborne pathogenic bacteria as previous described. Fifteen asparagus spears were aseptically placed in foam tray (144×203×16mm) and then irradiated with 254 nm light energy UV-C (Sylvania, G15T8, Philips, the Netherlands) for 1 min at a dose of 1.2kJ/m^2 [13]. The radiation intensity of respective exposure time was measured by a digital Ultraviolet Radiometer (8.0 UVC, Solarmeter, USA). The trays were wrapped with 14 μm thickness of PVC film. To determine the combination effect of chitosan coating and UV-C irradiation on microbial contamination of asparagus, fresh asparagus were coated with chitosan solution and inoculated with foodborne pathogenic bacteria as previously described. Fifteen asparagus spears were aseptically placed in foam tray (144×203×16mm) and irradiated with UV-C for 1 min. All packages were stored at 10℃ for 6 days. All asparagus spears were compared with fresh asparagus spear (control).

B. Microbial Growth Conditions

A stock culture of *Escherichia coli* and *Salmonella* sp. were obtained from postharvest technology laboratory. *Escherichia coli* and *Salmonella* sp. were grown on Eosin Methylene Blue Agar (EMB Agar, Himedia Laboratories Pvt. Ltd., India) and Xylose Lysine Deoxycholate Agar (XLD Agar, Scharlau Chemie S. A. Barcelona, Spain), respectively. The plates were incubated at 35+2℃ for 48$\pm$3h. Media were prepared according to the manufacturer's instructions.

C. Microbial Enumeration and Quality Evaluation

Escherichia coli, *Salmonella* sp., total microbial and fungi counts were determine using standard enumeration methods. EMB, XLD, Plate count agar (Himedia Laboratories Pvt. Ltd., India) and Potato Dextrose Agar (Himedia Laboratories Pvt. Ltd., India) were used for enumeration of *Escherichia coli*, *Salmonella* sp., total microbial and fungi, respectively. Microbial population were analyzed duplicate plating on the surface of media by spread plating method at an initial day of storage, 3 and 6 days of storage. A 10 g of asparagus spear was mixed with 90 ml of 0.1% peptone water in a sterile stomacher bag and macerated with a stomacher (Masticator Nr2557/400, IUL instruments; Barcelona, Spain) for 1 min at room temperature [13], [19]. Tenfold serial dilution was made in 0.1% peptone water. Microbial counts were enumerated and expressed as log CFU/g. The disorder of asparagus spears (water soaking and wilting) was assessed by 10 trained panelists. Sample were evaluated using hedonic scale (1-5 scores), where 1 = excellent (no disorder), 2 = very good (<20% disorder), 3 = good (20-40% disorder, limit of marketability), 4 = fairly good (40-60% disorder), and 5 = poor (>60% disorder).

D. Experimental Design and Statistical Analysis

The experiment was conducted in a completely randomized design (CRD) with three replications per treatment per evaluation period. Results were analyzed by performing analysis of variance and mean comparison Duncan's multiple range test (DMRT) at $p\leq0.05$. Standard error (SE) of the mean was also calculated.

III. Results and Discussions

A. Escherichia Coli and Salmonella Sp. Counts of Asparagus Spears

Fresh asparagus spears (control) had an initial *Escherichia coli* count of 2.3 log CFU/g, whereas asparagus spears irradiated with UV-C was not detectable (Fig. 1). This result indicated that UV-C irradiation exhibited the beneficial effect on the reduction of *Escherichia coli*. The effectiveness of UV-C irradiation on microbial safety of foods has been reported to use for surface disinfestations. The UV-C light energy at 254nm could reduce pathogenic bacteria and its spores contaminated in vegetables [12], [20]. It has been reported that UV-C treatments decreased the activity of catalase enzyme so this enzyme could not played as a

protective agent against active oxygen species [21] in microbial cell. Asparagus spear coated with chitosan had an *Escherichia coli* counts with the count was higher than the detection level (2.4 log CFU/g) regardless of UV-C irradiation. The *Escherichia coli* counts were about 3.2-3.3 log CFU/g).

During storage at 10 ℃, the control sample was not detected for *Escherichia coli* count, whereas those of sample trended to constant or slightly decreased on day 3. Asparagus spear coated with chitosan had a lower *Escherichia coli* count (2.5 log CFU/g) than that of sample coated with chitosan and irradiated with UV-C (3.3 log CFU/g) as well as found in asparagus spear irradiated with UV-C alone (3.2 log CFU/g). At the end of storage (6 days), *Escherichia coli* counts of control sample and sample that irradiated with UV-C were not detectable. The *Escherichia coli* counts of the sample coated with chitosan and irradiated or un-irradiated with UV-C were slightly decreased to about 2 and 2.5 log CFU/g, respectively.

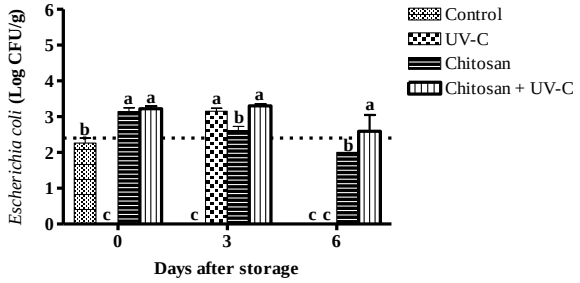

Figure 1. *Escherichia coli* count of asparagus under chitosan coating and UV-C irradiation. Dotted line represented the detection level.

At an initial day of storage, fresh asparagus spear had *Salmonella* sp. count of 1.1 log CFU/g which the count was below to the detection level, whereas *Salmonella* sp. counts of the other treatments were not detectable (Fig. 2). During storage at 10°C, *Salmonella* sp. counts of all treatments were not detectable or below to the detection level on day 3. At the end of storage (6 day), *Salmonella* sp. counts of control sample increased to about 2.3 log CFU/g, whereas those of sample coated with chitosan and/or irradiated with UV-C were slightly increase to about 1.4-1.6 log CFU/g. Chitosan coating, UV-C irradiation, and the combination of chitosan coating and UV-C irradiation could delayed *Salmonella* sp. growth of asparagus spear during storage at 10 ℃. However, the count of all asparagus spears were not significant different and the counts were below to the detection level.

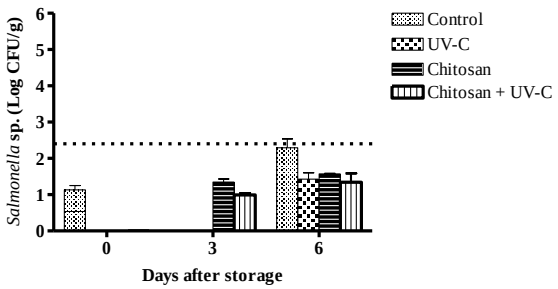

Figure 2. *Salmonella* sp. count of asparagus under chitosan coating and UV-C irradiation. Dotted line represented the detection level.

B. Total Microbial and Fungi Counts of Asparagus Spears

At an initial day of storage, total microbial count of all asparagus spears showed not significant different, which the counts were in a range of 3.7-3.8 log CFU/g (Fig. 3). During storage at 10 ℃, total microbial count of control sample was significantly increased (4.6 log CFU/g), whereas those of sample were constant or slightly decreased to about 3.4-3.6 log CFU/g on day 3. At the end of storage, the counts of all treatment except for the sample coated with chitosan and irradiated with UV-C were increased to about 3.8-4.6 log CFU/g. The lowest total microbial count was found in sample coated with chitosan and combined with UV-C irradiation. These results revealed that the combination of chitosan coating and UV-C irradiation exhibited the beneficial effect on the reduction of total microbial count of asparagus spears more than chitosan coating alone. However, UV-C irradiation alone did not exhibit the beneficial effect on the reduction of total microbial count at the end of storage as compared to the third day of storage. This result may due to UV-C alone be able to cause damage on the surface tissue of asparagus spear which could support total microbial growth on the spear. Therefore, the chitosan coating could be used to combine with UV-C irradiation to maintain the surface of asparagus spear before irradiated with UV-C.

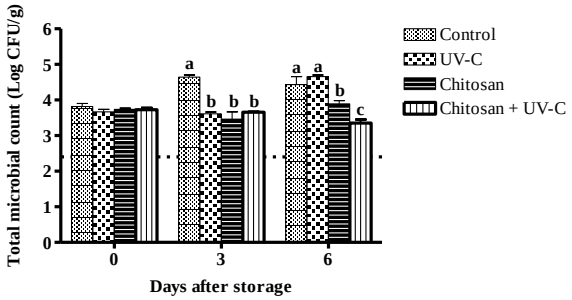

Figure 3. Total microbial count of asparagus under chitosan coating and UV-C irradiation. Dotted line represented the detection level.

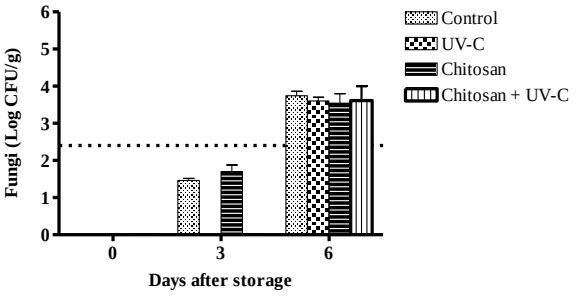

Figure 4. Fungi count of asparagus under chitosan coating and UV-C irradiation. Dotted line represented the detection level.

Fungi counts of all asparagus spears were not detectable at an initial day of storage (Fig. 4). During storage at 10 ℃, fungi counts were not detectable or slightly increased on day 3. However, the counts were below to the detection level. At the end of storage, fungi counts of all treatments were not significant different increased to about 3.4-3.6 log CFU/g which was higher to the detection level. These result indicated that chitosan

coating and/or UV-C irradiation had no effected on the reduction of fungi count of asparagus spears. The antimicrobial activity of chitosan is influenced by type of chitosan, the degree of chitosan polymerization, the host, the natural nutrient constituency, the chemical or nutrient composition of the substrates and environmental conditions such as water activity and moisture content [22]. Moreover, the fungicidal effect of chitosan against fungi may due to the minimum inhibitory concentrations (MIC), pH and interfering substances such as lipids and proteins [23].

C. Product Quality

At an initial day of storage, water soaking was not observed on the tip of all asparagus spears (Fig. 5). Therefore, the quality of all asparagus spears were excellent (1 score). During storage at 10 ℃, water soaking of all asparagus spears was slightly increased to about 1.1-1.3 scores on day 3. However, the quality of asparagus spears was excellent as well as observed in the sample at an initial day of storage. At storage at 10°C for 6 days, water soaking of all asparagus spear was significantly increased to about 2-3 scores which the quality was good to very good. The lowest water soaking was found in asparagus spear coated with chitosan and irradiated with UV-C, whereas the highest water soaking was found in asparagus spear irradiated with UV-C alone. These results indicated that chitosan coating combined with UV-C radiation could delay water soaking development on asparagus spear stored at 10 ℃.

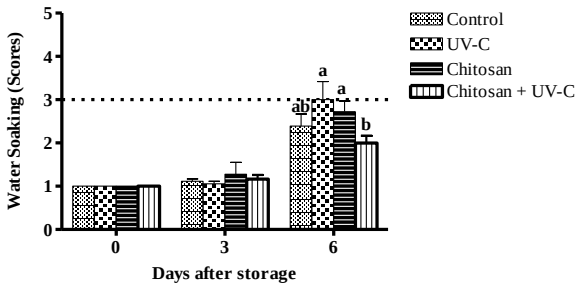

Figure 5. Water soaking of asparagus under chitosan coating and UV-C irradiation. Dotted line represented limit of marketability.

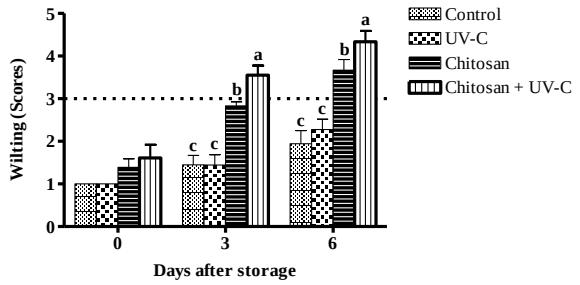

Figure 6. Wilting of asparagus under chitosan coating and UV-C irradiation. Dotted line represented limit of marketability.

At an initial day of storage, the wilting was observed on asparagus spears coated with chitosan (1.4-1.6 scores), which the highest wilting was found in sample coated with chitosan and irradiated with UV-C (Fig. 6). The change in wilting may related to the loss of tissue water and increase of lignin, which reflected on hardening texture as previously reported [8]. It has been reported that the use of carboxymethyl-cellulose and sucrose fatty acid esters as an edible coating on white asparagus could delayed weight loss as compared to uncoated sample [8]. However, in this research chitosan coating was used as an edible coating alone and combination with UV-C irradiation could not delay weight loss of asparagus as observed by increasing of wilting on asparagus spear during storage.

During storage at 10 ℃, wilting of all asparagus spears were slightly increased to about 1.5 scores in control sample and irradiated UV-C sample. Unexpected, rapidly wilting development were found in asparagus spears coated with chitosan, which the highest development of wilting was found in sample coated with chitosan and irradiated with UV-C. Asparagus spear treated with UV-C alone had a wilting score as same as in control sample. Thus, the severity of wilting that observed in sample coated with chitosan combined with UV-C may due to the great affected of chitosan coating. These results indicated that the combination of chitosan coating and UV-C irradiation showed disadvantage on asparagus spears, therefore the quality appearances of asparagus spears coated with chitosan were marketability limited only for 3 days. The shelf life of control and asparagus spear treated with UV-C was about 6 days.

IV. Conclusion

UV-C treatment alone was effective against *Escherichia coli* and total microbial contaminated on asparagus spears. UV-C irradiation, chitosan coating, and the combination of chitosan coating and UV-C irradiation on asparagus spear were effective against *Salmonella* sp. and total microbial. Fungi were not affected by UV-C irradiation and chitosan coating. Chitosan coating combined with UV-C irradiation could be used to maintain quality by delaying water soaking development of asparagus spears.

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