# A Study of Asparagus Preservation Capacity of Chitosan-Alginate and Chitosan-Carrageenan Biofilms

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**Abstract:** Fresh postharvest green asparagus rapidly deteriorate due to its senescence process and high transpiration rate. This thesis aims to utilize the available sources of carrageenan and alginate for combining with pure chitosan coating solution, thus create a new multi-component coating that can overcome the limitations of pure chitosan coating, and show more effective in maintaining the quality of postharvest green asparagus. The gel solution ratio 2:1 (v/v) of chitosan 1% (w/v) and alginate 0.2% (w/v), had ability to prolong shelf-life of asparagus for 3 days (from weight loss point of view) and 7 days (from visual quality point of view) compared to control sample. The weight loss was less than about 12% and also ensured lower firmness change, maintained higher chlorophyll content, exhibiting better quality of asparagus compared to control and chitosan coated sample. The chitosan-alginate (2:1 v/v) coated asparagus achieved highest sensory score in day 7 and 14 of storage and lowest total aerobic growth in 14 days of storage at 4 °C. The coating biofilm of chitosan-alginate could be considered as the new multi-component edible coating which showed high effectiveness in quality preservation and shelf-life extension of asparagus.

Keywords: Asparagus preservation; Chitosan-alginate biofilm; Chitosan-carrageenan biofilm.

# 1. Introduction

Currently, due to increasing consumer demand, ensuring quality, safety as well as increasing the storage time for agricultural products after harvest are urgent needs. Therefore, the research on preserving perishable agricultural products such as fresh fruits and vegetables by simple methods or using eco-friendly materials is being focused.

Among perishable fruits and vegetables such as strawberries, dragon fruit, avocado, etc., asparagus is one of the most easily spoiled type with relatively short shelf-life (about 4 - 6 days when handled and stored at room temperature; 6 - 8 days when stored at 2 - 4oC) [1]. Moreover, growing of asparagus is relatively difficult. Farmers have to harvest each shoot by hand, and the cost of planting is high compared to others. Planting and harvesting asparagus require advanced caring techniques but it is riskier than the other fruits and vegetables due to short shelf-life [1].

Recently, the development and application of edible films and coatings made from bio-polymers including polysaccharides, proteins, lipid in fruits and vegetables preservation is interested in research due to their sustainability, environmentally friendly property and safety for human health [2].

Chitosan has been received lots of attention in coating-forming treatment for fruits and vegetables due to its good biological compatibility, biodegradability, low toxicity and excellent coating property compared to other polysaccharide as well as approved by USFDA as a Generally Recognized as Safe (GRAS) food additive (USFDA 2013) [3]. However, the mechanical resistance of chitosan films and coatings is relatively low [4]. In order to cover these drawbacks, chitosan-based polyelectrolyte complexes have been developed via ionic interactions between oppositely charged polymers. Since chitosan is positively charged polysaccharide, thus a natural polymer such as alginate, carrageenan are potential materials for such interactions due to their opposite electrostatic properties to chitosan, bioactive and non-toxic properties, and their abundant source with low cost [5].

Utilizing the available sources of carrageenan and alginate to combine with pure chitosan coating solution aiming to create a new multi-component coating that can overcomes the limitations of pure chitosan coating, and thus shows more effective in maintaining the quality of postharvest green asparagus (*Asparagus officinalis* L.).

# 2. Materials and methods

#### 2.1 Materials

Asparagus were harvested from an orchard located in Ninh Thuan province, Phan Rang city, Vietnam, in early morning, and immediately transported to the lab within 5 - 7 hours. The fresh, same size and good quality asparagus spears were used for research immediately. Chitosan was supplied by Tin Cay joint stock company, Vietnam. Carrageenan and alginate are purchased HiMedia Laboratories Pvt. Ltd., India. Others analysis chemical is analytical grade.

#### 2.2 Biofilm coating asparagus procedure

The solution of chitosan 1% [6], alginate 0.2% [7] and Carrageenan 1% [8] were separately prepared and mixed to form two different gel solution of chitosan – alginate (CS-Ag) and chitosan – carrageenan (CS-CR) with the ratio 2:1 (v/v) [9] and 1:1 (v/v) [10], respectively. About 200 g sample scale of spears asparagus were totally immersed into gel solution for 1 minute then held upside down for drain and allowed to air dry upright for 15 minutes. Sample were stored at 4 °C. The analysis experiments of all samples were conducted in 0, 7 and 14 days, after the coating process completed. Two control samples were simultaneously prepared, no coating sample and coating in chitosan 1% solution.

#### 2.3 Analysis methods

The quality of asparagus sample was evaluated by physical analysis, including weight loss [11] and firmness [6], chlorophyll content [6] sensory analysis [12] and total aerobic bacteria count using Petrifilm Aerobic Count Plates (Microbiology Products, 3MCenter, Bldg 275-5W-05, St. Paul, MN 55144, USA) [13]. Weight loss of all samples were determined daily from day 0 to day 14 of storage. Others analysis were conducted in day 0, 7 and 14 of storage.

The firmness of asparagus was determined by TA.XT Express-v3.1 texture analyzer - Stable Micro Systems, Godalming, UK. The probe used in this method is Warner Bratzler Blade "V" which is a 1 mm thick metal blade with a triangular hole into which is inserted a cylindrical sample.

#### 2.4 Statistical analysis

All experiments were carried out by triplicates. The data are expressed as means  $\pm$  standard deviation (SD). All statistical analyses were performed with SPSS version 13.0. One – way analysis of variance (ANOVA) and the least significant difference test for mean comparisons were used to determine the difference among treatments and storage time.

### 3. Results and discussion

#### 3.1 Weight loss

The changes of weight loss in the control, chitosan – alginate (2:1 v/v) and chitosan – carrageenan (1:1 v/v) coated samples stored at 4 °C are shown in Fig. 1. Over the 14 days of storage, the weight losses were reduced to 24.18% in chitosan – alginate (2:1 v/v) and 28.83% in chitosan – carrageenan (1:1 v/v). These data showed that the chitosan – alginate (2:1 v/v) coating significantly reduced the loss of water to the environment over the storage period compared to the chitosan – carrageenan (1:1 v/v) coating (P < 0.05).

This result was probably explained by the lower water vapor permeability of alginate compared to carrageenan. Moraes et al. (2012) [14] evaluated the effect of the alginate and carrageenan on the weight loss of Williams pears and found that the pears treated with 2% w/v alginate was greater sufficient to minimize the loss of water than the 0.5% w/v of carrageenan, the result above most likely accordant with this research.

Another explanation for the better performance in weight loss of chitosan – alginate (2:1 v/v) was that the primary mechanism of moisture loss from asparagus is vapor – phase diffusion, which was impelled by the water vapor pressure difference between the asparagus and the surrounding air [15] and the alginate films or coatings have a resistance to being dissolved in water and, therefore, have the potential for coating high moisture fresh food like asparagus [7]. This was shown as after samples were taken out of the refrigerator, during weighing process, some parts of the chitosan – carrageenan coated samples surface showed some signs of a solid-to-liquid phase transition of coating. In contrast, chitosan – alginate coating layer was relatively stable. The surface of sample coated with this coating was dry and there were no gels appears.

Moreover, higher concentration of chitosan reduced the water vapor permeability of coating, so the resulted, chitosan – alginate (2:1 v/v) showed good performance of water loss prevention [16]. Probably this could be due to the improvement and cohesion within the film matrix, in consequence the interaction between polymer chain and structure [17].



**Figure 1.** Effect of chitosan – alginate (CS – AG) (2:1 v/v) and chitosan – carrageenan (CS – CR) (1:1 v/v) coatings on weight loss of asparagus spears during storage at 4 °C for 14 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

### 3.2 Firmness

The influence of chitosan – alginate (2:1 v/v) and chitosan - carrageenan (1:1 v/v) coatings on firmness of asparagus is presented in Fig. 2. The shear force values of the control samples still increased significantly (P < 0.05) compared to the coated spears from the 1<sup>st</sup> to the 14<sup>th</sup> day. The firmness value of samples coated with chitosan – alginate (2:1 v/v) was significantly (P < 0.05) different to that of chitosan - carrageenan (1:1 v/v) coated sample, and much closer to initial value in day 0 which suggested a better firmness maintenance.

As mentioned above, the texture of asparagus spears related to water loss during the storage period. As a result, the reason for the better water loss retaining of chitosan – alginate (2:1 v/v) than chitosan - carrageenan (1:1 v/v) was that the alginate coating showed higher tensile strength, elongation, and elastic modulus compared to the carrageenan coating. Moreover, the alginate coating had lower water vapor permeability and showed a significant difference from carrageenan coating [14]. This is probable due to ionic crosslinking in alginate films reducing the segmental mobility of the polymer [18]. In addition, the chitosan – alginate resistant to dissolution in water leading to enhance stability of coating during storage [19], and thus the gas and water vapor barrier property of coating become more stable and effective than that of chitosan – carrageenan coating.



**Figure 2.** Effect of chitosan – alginate (CS – AG) (2:1 v/v) and chitosan – carrageenan (CS – CR) (1:1 v/v) coatings on texture of asparagus spears during storage at 4°C for 14 days. Each data point is the mean of three replication. Means in groups of four columns with different letters are significantly different (P < 0.05). Vertical bars represent standard deviation of means.

### 3.3 Chlorophyll content

The effect of chitosan – alginate (2:1 v/v) and chitosan – carrageenan (1:1 v/v) coatings on quality of asparagus were shown in Fig. 3. As shown in this figure, in 14th day of storage, the lowest remained chlorophyll content was observed in control sample (with 31.8871 mg/100g FW), followed by that of chitosan – carrageenan (1:1 v/v) and

chitosan – alginate (2:1 v/v) with 39.8386 (mg/100g FW) and 40.4118 (mg/100g FW), respectively. Although the content of chlorophyll in chitosan – carrageenan (1:1 v/v) and chitosan – alginate (2:1 v/v) coated sample were considerably (P < 0.05) higher that of control sample, there was no significant difference between these two coatings.



**Figure 3.** Effect of chitosan – alginate (CS – AG) (2:1 v/v) and chitosan – carrageenan (CS – CR) (1:1 v/v) coatings on chlorophyll content of asparagus spears during storage at 4°C for 14 days. Each data point is the mean of three replication. Means in groups of four columns with different letters are significantly different (P < 0.05). Vertical bars represent standard deviation of means.

# 3.4 Sensory analysis

Sensory evaluation of chitosan - alginate (2:1 v/v) and chitosan - carrageenan (1:1 v/v) coated and control asparagus spears at the end of the storage period revealed significant (P < 0.05) differences in appearance, off – odor, wilting and color (Table 1.). The chitosan - alginate (2:1 v/v) coated spears had the highest scores in all parameters after 14 days of storage, while those coated with chitosan - carrageenan (1:1 v/v) achieved lower scores. This result was in agreement with Moraes et al. (2012) who denoted that the coating with alginate showed a better result in maintaining the green color of the fruit than the carrageenan coating when they conducted an experiment on pears [14]. This was probably explained that the chitosan – alginate (2:1 v/v) provided a thick barrier against gas exchange between inner and outer environments, combined with its ability to resist to dissolve in water, thus delayed the water loss and color change as previously mentioned.

Sensory quality attributes	Day of storage			
		0	7	14
Appearance	Control	$10.0 \pm 0.0$	$6.7\pm0.5^{\mathrm{a}}$	$5.8\pm0.4^{\rm a}$
	CS-AG (2:1 v/v)	$10.0\pm0.0$	$7.6\pm0.3^{\circ}$	$6.8\pm0.4^{\circ}$
	CS-CR (1:1 v/v)	$10.0\pm0.0$	$7.1 \pm 0.2^{b}$	$6.3\pm0.2^{b}$
Off – odor	Control	$0.0 \pm 0.0$	$2.5\pm0.5^{\circ}$	$3.4 \pm 0.2^{\circ}$
	CS-AG (2:1 v/v)	$0.0 \pm 0.0$	$1.4 \pm 0.2^{\mathrm{a}}$	$1.8\pm0.4^{\mathrm{a}}$
	CS-CR (1:1 v/v)	$0.0 \pm 0.0$	$1.9\pm0.2^{b}$	$2.3\pm0.4^{b}$
Wilting	Control	$0.0 \pm 0.0$	$2.4\pm0.2^{\circ}$	$3.6\pm0.3^{\circ}$
	CS-AG (2:1 v/v)	$0.0 \pm 0.0$	$1.4 \pm 0.2^{\mathrm{a}}$	$2.4\pm0.3^{\rm a}$
	CS-CR (1:1 v/v)	$0.0 \pm 0.0$	$1.9\pm0.3^{\rm b}$	$2.9\pm0.2^{\rm b}$
Browning	Control	$0.0 \pm 0.0$	$2.5\pm0.5^{\circ}$	$3.6\pm0.3^{b}$
	CS-AG (2:1 v/v)	$0.0 \pm 0.0$	$1.2\pm0.2^{\mathrm{a}}$	$1.7\pm0.2^{\mathrm{a}}$
	CS-CR (1:1 v/v)	$0.0 \pm 0.0$	$1.7\pm0.2^{b}$	$2.1\pm0.5^{\rm a}$
Color	Control	$10.0\pm0.0$	$7.3\pm0.4^{\rm a}$	$6.7\pm0.3^{\mathrm{a}}$
	CS-AG (2:1 v/v)	$10.0\pm0.0$	$8.4\pm0.3^{\rm b}$	$7.7\pm0.3^{\circ}$
	CS-CR (1:1 v/v)	$10.0\pm0.0$	$8.2\pm0.3^{b}$	$7.2\pm0.2^{b}$

**Table 1.** Effect of chitosan – alginate (CS – AG) (2:1 v/v) and chitosan – carrageenan (CS – CR) (1:1 v/v) coatings on the sensory evaluation of asparagus spears during storage at 4 °C for 14 days.

\* Mean of three replications  $\pm$  standard deviation. Means in the same row with different letters are significantly different (P < 0.05).

## 3.5 Total anaerobic microbial count

The anaerobic microbial count of control and sample coated with chitosan - alginate (2:1 v/v) was conducted to confirm more about the ability to inhibit microorganism growth of selected multi-component coating.

The changes in total aerobic count (LogN) of control and coated asparagus are presented in Fig. 4. From this figure, the total aerobic microorganisms count generally tend to increase during storage period, and that of coated sample were lower compared to control in both day 7 and 14 of storage. After 7 days of storage, the number of total aerobic microorganisms in the control sample was 16 times more than that of day 0 while the total aerobic count of coated sample was only 8 times more. The coating hindered the increase in total aerobic microorganism on day 14 of storage. Similar effect of coating was observed in reducing the growth of aerobic microorganism on day 14 of storage. The control sample had total aerobic count 40 times more compared with day 0, and that of sample coated with chitosan – alginate (2:1 v/v) was more about 14 times compared with day 0.

The data from these results suggest that the chitosan – alginate (2:1 v/v) coating was relatively effective at maintaining aerobic count in samples to a level much significantly less than that in the control during 14 days of storage, and is similar to research conducted by Vu et al.(2015) [20], which concluded that a 0.8% oligochitosan coating successfully inhibit growth of total aerobic microorganism in asparagus after a storage period of 15 days.



**Figure 4.** Total aerobic count of control and CS-AG (2:1 v/v) coated samples in day 0, 7, 14 of storage. Each data point is the mean of three replication. Means in groups of two columns with different letters are significantly different (P < 0.05). Vertical bars represent standard deviation of means.

#### 4. Conclusion

The asparagus coating by chitosan – alginate (2:1 v/v) gel solution was showed the highest effectiveness in maintenance quality of asparagus in terms of weight loss, firmness and sensory evaluation compared to asparagus coating by chitosan – carrageenan (1:1 v/v) gel solution.

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