

## Binding Capacity of Chitin and Chitosan to Anthocyanin Pigment Isolated from Purple Perilla Leaves

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### Abstract

The binding capacity of chitin and chitosan to anthocyanin pigments isolated from purple perilla leaves was investigated. The pigment binding capacity increased with increasing pigment concentrations and decreasing pH without being affected by reaction temperatures and particle sizes. Regression analysis revealed significantly high correlations between pigment binding capacity of chitin and chitosan and pigment concentration at ranges of 25-100 mg of pigment/g of sample. After 1 hr of settling, release of pigment from pigmented chitin and chitosan increased with increasing pH, up to 24.9% and 17.4%, respectively, at pH 9. In general, pigment binding capacity of chitosan was higher than that of chitin. These results suggest that chitosan may be useful as a potential adsorbent capable of stabilizing anthocyanin pigment.

**Key words:** anthocyanin pigment, purple perilla leaf, chitin, chitosan, adsorbents

### INTRODUCTION

Recently, there has been increased demand for natural food colorants with increased consumer palatability for natural products and heightened consumer awareness of the risks associated with synthetic food colorants. Anthocyanin pigments, one of the most widespread water-soluble pigments in the plant kingdom, have considerable potential use as natural food colorants in the food industry due to their apparent harmlessness to health (1). In addition, anthocyanin pigments are receiving renewed interest as food coloring agents with many biochemical and pharmacological effects such as antiinflammation (2,3), anticonvulsion (4), and antioxidation (5-7).

Purple perilla (*Perilla ocimoides* L. var. *crispa* Benth) leaves have been widely used as a source of anthocyanin pigment for coloring traditional Japanese pickles such as umeboshi (pickled Japanese plum) and benishoga (pickled ginger). However, this anthocyanin pigment is unstable towards a variety of chemical and physical factors, together with high cost and low tinctorial strength (1,8). Recently, many efforts toward the stabilization of anthocyanin pigments using various adsorbents such as cyclodextrin, polystyrene, and celite have been made without satisfactory success (9-11). Therefore, the development of novel technology capable of stabilizing anthocyanin pigments is needed.

Chitin, poly- $\beta$ -(1 $\rightarrow$ 4)-N-acetyl-D-glucosamine, is a cellulose-like biopolymer distributed throughout nature, especially in marine invertebrates, insects, fungi, and yeasts (12). Chitin and its deacetylated form, chitosan, have attracted significant interest in view of varied proposed novel applications in biomedical, food, and various chemical industries (13-15). In

studies on functional properties of chitinous polymers, chitin and chitosan have been documented to possess a distinctive property for use in dye binding (16,17).

Knorr (16) studied dye binding properties of chitin and chitosan using FD&C Red No. 40. Ahn and Lee (18) found dissimilarities in dye (Red No. 40) binding capacities of various chitins, chitosans, and microcrystalline chitins with products and sources. Byun et al. (19) reported that acetylchitin, N-acetylchitosan, chitosansulfate, and chitosan are suitable for use as dye (Blue R-250 and Red No. 2) adsorbents. More recently, No et al. (20) demonstrated the potential of chitin as a synthetic dye carrier. Thus, it is realized that chitin and chitosan can be used as an adsorbent for anthocyanin pigments. In addition, we previously found that the acylated anthocyanins in perilla leaf had stronger binding capacity to chitin and chitosan than simple anthocyanins in black rice (21). However, no information is so far available on adsorption of anthocyanin pigments of perilla leaf by chitin and chitosan.

The objective of the present study was to evaluate the binding capacity of chitin and chitosan to anthocyanin pigment isolated from purple perilla leaves. The release of anthocyanin pigment from treated chitin and chitosan also was examined at various pH levels.

### MATERIALS AND METHODS

#### Materials

Commercial chitin and chitosan (from crab shell, practical grade) used in this experiment were obtained from Keumho Chemical Co. (Seoul, Korea). The degree of deacetylation of

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chitin and chitosan was 17.1% and 87.6%, respectively.

To obtain a uniform size product, chitin and chitosan samples were ground separately through a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ); sifted with 40- (0.425 mm), 60- (0.250 mm), 80- (0.180 mm), and 100-mesh (0.150 mm) sieves; placed in opaque plastic bottles; and stored at ambient temperature. Ground chitin and chitosan of 0.180~0.150 mm size were used throughout this research to obtain reproducible and consistent results, except for the study evaluating the binding capacity of three different particle sizes of chitin and chitosan to anthocyanin pigment. Prior to binding studies, these samples were dried at 105°C for 2 hr.

Purple perilla (*Perilla ocimoides* L. var. *crispa* Benth) leaves used as a source of anthocyanin pigment were obtained from a farm of the Catholic University of Taegu-Hyosung.

#### Isolation and purification of anthocyanin pigment

Purple perilla leaves (100 g) were extracted with 50% aqueous methanol containing 0.1% trifluoroacetic acid (TFA) at room temperature overnight. The extract was evaporated to a small volume *in vacuo* and then applied to the Amberlite XAD-7 (Organo Chemical, Japan) column (5 cm i.d. × 30 cm) with 0.5% TFA aqueous solution. After washing the column with 1.0 L of deionized water followed by 1.0 L of 20% aqueous methanol, anthocyanin pigment was eluted with 2.0 L of 0.5% TFA in 80% aqueous methanol solution. The eluted pigment was evaporated, dissolved in a small volume of 1.0% HCl in methanol, and then precipitated by the addition of diethyl ether. The precipitated pigment was collected by centrifugation at 2,500 rpm for 20 min and dried (1.5 g) in a vacuum desiccator.

#### Preparation of pigment solution

Anthocyanin pigment solution was prepared by dissolving pigment in deionized water at concentrations of 250, 500, 750, and 1000 mg/L.

#### Determination of reaction time

To determine the optimum reaction time for sufficient pigmentation of chitin and chitosan, 0.2 g of chitin or chitosan and 20 mL of aqueous pigment solution (containing 10 mg of pigment) in a conical flask were shaken at ambient temperature for 0.5, 1, 2, or 3 hr using a shaking incubator (Sangwoo Scientific Co., Korea) at 150 rpm. The resulting pigment-chitin or chitosan was filtered through a glass filtering Gooch crucible (1G-3) using a glass microfiber filter paper (Whatman, 47 mm) and washed thoroughly with deionized water until the filtrate was clear. The pigment concentration of the combined filtrate was determined spectrophotometrically at 520~535 nm. The amount of pigment bound to chitin or chitosan was determined by calculating differences in concentrations between the initial pigment solution and the combined filtrate.

#### Pigment binding study

The binding of anthocyanin pigment to chitin and chitosan

was basically achieved by shaking 0.2 g of chitin or chitosan (0.180~0.150 mm) and 20 ml of aqueous pigment solution (containing 10 mg of pigment) in a conical flask at 20°C for 1 hr using a shaking incubator (150 rpm). The resulting pigment-chitin or chitosan was filtered and washed, and the amount of pigment bound to chitin or chitosan was quantitatively determined as above. Parameters and conditions evaluated for the pigment binding study were as follows: (a) the effect of pigment concentration (25, 50, 75, and 100 mg of pigment/g of sample); (b) the effect of temperature (20, 40, 60, 80, and 100°C); (c) the effect of particle size (0.425~0.250, 0.250~0.180, and 0.180~0.150 mm); and (d) the effect of pH (3, 4, 5, 6, 7, 8, and 9). The pH of the aqueous pigment solution containing 10 mg of pigment was adjusted either with 0.1 N HCl or with 0.1 N NaOH solution.

#### Effect of pH on pigment release

The pigmented chitin and chitosan, previously prepared by shaking 0.2 g of chitin or chitosan for 1 hr in 20 ml of aqueous pigment solution (containing 10 mg of pigment) and washed thoroughly with deionized water, were separately added to 20 ml of deionized water adjusted to the pH levels of 3~9 and kept for 1 hr at ambient temperature without shaking. The resulting pigment-chitin or chitosan was then filtered and washed, and the pigment concentration of the filtrate was determined spectrophotometrically. The percentage of pigment released from the pigmented chitin or chitosan was calculated as follows: pigment released (%)=(amount of pigment released/amount of pigment bound to chitin or chitosan) × 100.

#### Statistical analysis

All experiments were carried out in duplicate, and means and standard deviations were reported. Mean separation and regression analysis were analyzed using the SPSS (Statistical Package for Social Sciences, SPSS Inc., USA) software package.

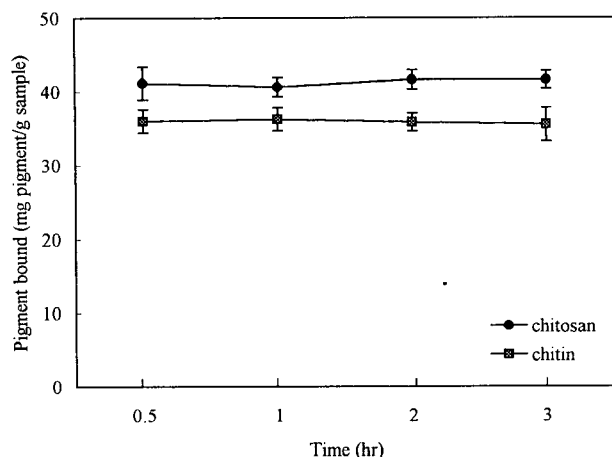
## RESULTS AND DISCUSSION

#### Determination of optimum reaction time

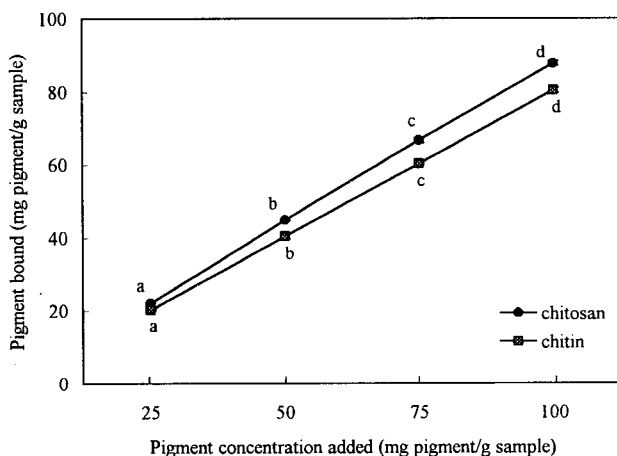
The anthocyanin pigment binding capacity of chitin and chitosan with reaction times was evaluated to determine the optimum reaction time. Results (Fig. 1) showed no significant differences ( $p>0.05$ ) in the amounts of pigment bound to chitin and chitosan for four reaction times, as observed by No et al. (20) with a synthetic dye. To ensure sufficient binding of pigment to chitin and chitosan, a reaction time of 1 hr was applied to subsequent binding studies.

#### Effect of pigment concentration

The effects of pigment concentrations on the binding capacity of chitin and chitosan were evaluated, and the results are given in Fig. 2. The binding capacity of chitin and chitosan linearly increased with increasing pigment concentrations. However, a significant difference ( $p<0.05$ ) was found



**Fig. 1.** Effect of reaction times on anthocyanin pigment binding capacity of chitin and chitosan.



**Fig. 2.** Effect of anthocyanin pigment concentrations on pigment binding capacity of chitin and chitosan. Means with different superscripts on a line indicate significant differences ( $p < 0.05$ ).

between chitin and chitosan, with the latter having a higher binding capacity than the former. Chitin and chitosan revealed a 80~81% and a 88~90% pigment binding capacity, respectively, at all pigment concentrations tested.

Regression analysis (Table 1) revealed significantly ( $p < 0.05$ ) high correlations between the pigment binding capacity of chitin and chitosan and the pigment concentration at ranges of 25~100 mg of pigment/g of sample. Such high correlations also were observed by previous workers (16,20,22) who studied the binding capacity of chitin with various synthetic dyes at dye concentrations ranging from 0.2 to 20 mg of dye/g of chitin.

Chitosan with a higher amino group content was reported to be more effective for binding dyes than chitin (18,23). However, Cho et al. (24) demonstrated that the dye binding capacity of chitinous polymers differed considerably depending on the products. In the present study, chitosan showed a higher pigment binding capacity than chitin.

**Table 1.** Regression analysis of pigment binding capacity of chitin and chitosan on anthocyanin pigment concentration

Sample	n	Regression equation <sup>1)</sup>	R <sup>2</sup>
Chitin	4	$Y=0.801X+0.267$	1.000
Chitosan	4	$Y=0.875X+0.692$	1.000

<sup>1)</sup>Y=Pigment binding capacity of chitin and chitosan (mg of pigment/g of sample).

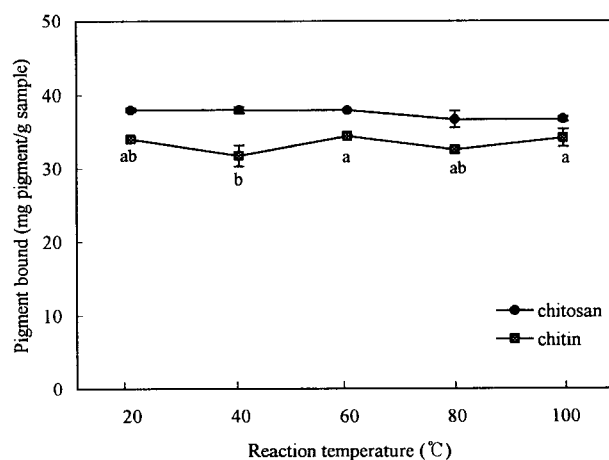
X=Pigment concentration (mg of pigment/g of sample).

### Effects of temperature

The effects of temperature on the pigment binding capacity of chitin and chitosan were evaluated using 50 mg of pigment concentration/g of sample. Results (Fig. 3) showed no noticeable differences in the amounts of pigment bound to chitin and chitosan for five reaction temperatures except for chitin at 40°C, which exhibited a slightly lower binding capacity than did chitin at 60 and 100°C. Chitosan revealed a higher binding capacity than chitin at all reaction temperatures except for 100°C.

It was reported that the effect of temperature on the adsorption of dyes to chitin or chitosan differs with dyes used (25,26). The decrease in adsorption capacity of dye with increasing temperature is due to the enhanced magnitude of the reverse (or desorption) step in the mechanism as the temperature increases. On the other hand, the increase in the adsorption capacity of dye with increasing temperature is due to an increase in dye mobility and a temperature-induced swelling effect within the internal structure of the chitin or chitosan, allowing the large dye ions to penetrate into the particles. However, a certain dye was found to be unaffected by change in temperature.

The present data indicate that the anthocyanin pigment binding capacity of chitin and chitosan may not be affected by change in temperature. Nevertheless, further studies are needed with other chitin and chitosan products since the effect of temperature on the adsorption of dye to chitin and chitosan was reported to differ depending on the products (24).



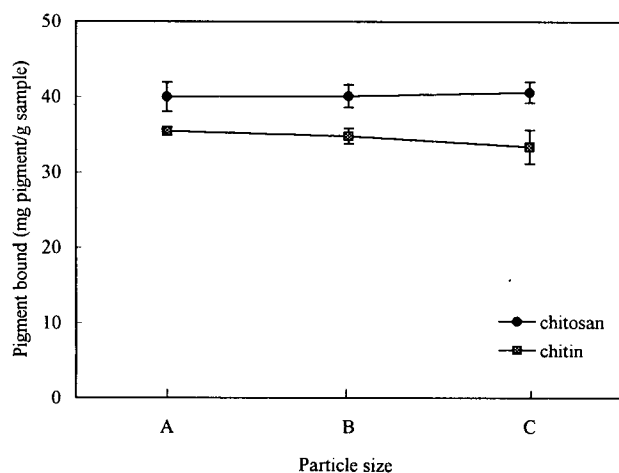
**Fig. 3.** Effect of temperatures on anthocyanin pigment binding capacity of chitin and chitosan. Means with different superscripts on a line indicate significant differences ( $p < 0.05$ ).

### Effect of particle size

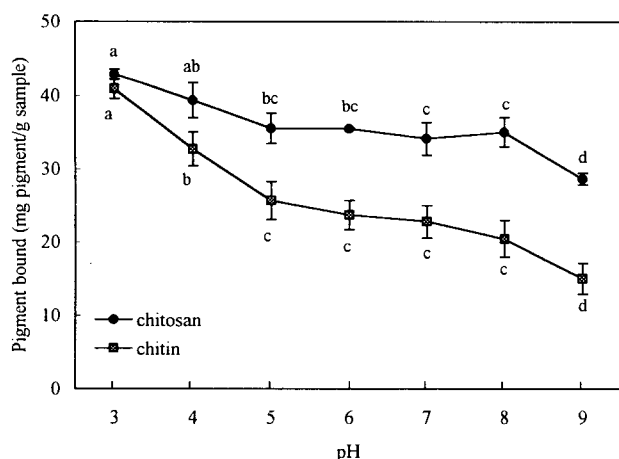
Three different particle size ranges of chitin and chitosan were investigated to compare their pigment binding capacity using 50 mg of pigment concentration/g of sample (Fig. 4). Chitin showed a slightly decreased pigment binding capacity (35.4, 34.8, and 33.3 mg of pigment/g of chitin), whereas chitosan revealed a slightly increased binding capacity (40.0, 40.1, and 40.6 mg of pigment/g of chitosan) with decreased particle sizes. However, these small differences were not statistically significant ( $p>0.05$ ), indicating the possible use of various sizes of chitin and chitosan particle as a pigment adsorbent. A similar trend also was found by No et al. (20) who studied dye binding capacity with four different particle size ranges of chitins.

### Effect of pH

The effect of pH on pigment binding capacity of chitin and chitosan using 50 mg of pigment concentration/g of sample is shown in Fig. 5. Binding capacity of chitin and chitosan



**Fig. 4.** Effect of particle size ranges on anthocyanin pigment binding capacity of chitin and chitosan. A, 0.425 ~ 0.250 mm; B, 0.250 ~ 0.180 mm; C, 0.180 ~ 0.150 mm.



**Fig. 5.** Effect of pH on anthocyanin pigment binding capacity of chitin and chitosan. Means with different superscripts on a line indicate significant differences ( $p<0.05$ ).

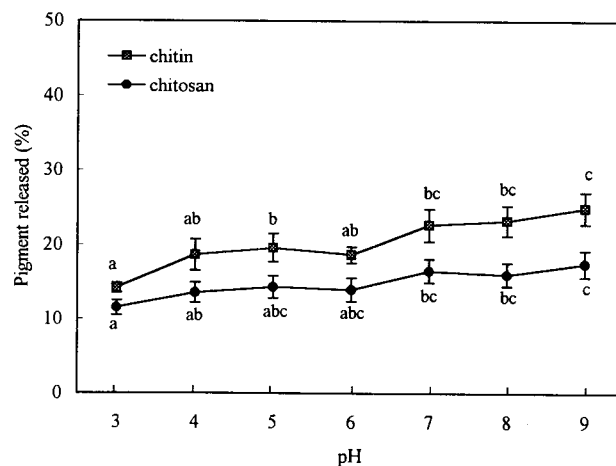
decreased with increasing pH. Within a pH range of 6~9, chitosan showed higher ( $p<0.05$ ) binding capacity than chitin.

The flavylium cation structure of anthocyanins, which is stable only at very low pH, is readily transformed into colorless carbinol at pH 4~5, purple quinoidal at pH 6~7, deep blue ionic anhydrobase at pH 7~8, and then chalcone at above pH 8.0, stepwise (27,28). In particular, major acylated anthocyanins, malonylshisonin, of perilla leaf was reported to show marked stability at pH 2.0 (29). Since the color of the aqueous pigment solution is changed with pH, the amount of pigment bound to chitin and chitosan after reacting for 1 hr at each pH level was determined by calculating differences in concentrations between the initial pigment solution at pH 3 and the combined filtrate adjusted to the pH 3 with 0.1 N HCl.

In the present study, pigment binding capacity of chitin and chitosan decreased with increasing pH. The highest binding capacity of chitin and chitosan at pH 3.0 may be caused by complexing the flavylium cation form of the anthocyanins with chitin and chitosan, and preventing their transformation into other less stable forms. However, the binding capacity of chitin or chitosan to synthetic dyes in terms of pH showed considerably different trends. According to No et al. (20), the pH did not noticeably influence the dye binding capacity of chitins within a pH range of 3~9. These differences are probably due to the difference in stability between pigment and dye with pH. Anthocyanin pigment is less stable at higher pH while dye is stable within a pH range of 3~9 studied. Knorr (16) also found that the dye binding capacity of chitin was stable within a pH range of 2~7, while chitosan formed gels below pH 5. In the present study, however, chitosan did not form gels even at pH 3 adjusted with 0.1 N HCl. Further studies are needed to clearly explain such a difference.

### Effect of pH on pigment release

The effect of pH on the pigment release from pigmented chitin and chitosan after 1 hr of settling was investigated, and the results are shown in Fig. 6.



**Fig. 6.** Effect of pH on the release of anthocyanin pigment from pigmented chitin and chitosan. Means with different superscripts on a line indicate significant differences ( $p<0.05$ ).

The release of pigment from pigmented chitin and chitosan increased with increasing pH. These results may be attributed to the decrease in binding capacity of chitin and chitosan with increasing pH as seen in Fig. 5. Under the pH levels tested, 14.2~24.9% and 11.5~17.4% of the bound pigment were released from pigmented chitin and chitosan, respectively. However, no significant differences ( $p>0.05$ ) in pigment release were observed between pigmented chitin and chitosan at all pH levels.

In the effect of pH on the dye release from dyed chitin after 1 hr of settling, No et al. (20) found that 0.1~0.3% of bound red dye and 0.2~1.7% of bound yellow dye were released within pH 5~9. No dye was released between pH 2 and 4. Earlier, Knorr (16) also reported no release of dye from dyed chitin between pH 2 and 6. Beyond this range, 2.9~5.7% of bound dye was released. These results suggest that the stability of anthocyanin pigmented chitin and chitosan to pH may be different from that of dyed chitin and chitosan.

## CONCLUSION

This investigation has provided information on the potential of chitin and chitosan, especially chitosan, as an anthocyanin pigment adsorbent. The binding capacity of chitin and chitosan to anthocyanin pigments isolated from purple perilla leaves increased with an increase in pigment concentrations and a decrease in pH without being affected by temperatures and particle sizes. Pigmented chitin and chitosan were less stable at higher pH with 24.9% and 17.4% release of pigment, respectively, at pH 9.

Recently, much attention has been paid to anthocyanin pigments as a potential source of natural food colorants. However, since these anthocyanin pigments are unstable towards a variety of chemical and physical factors, use of chitin or chitosan as a color stabilizer and a pigment adsorbent is justified. In the food industry, the pigmented chitin or chitosan possibly can be used as food colorants for bread, ice cream, soft drinks, alcoholic beverages, etc. Thermal degradation of anthocyanin pigments during processing may be reduced by use of these pigmented biopolymers. In projected food applications of chitin and chitosan as a functional ingredient, uniformity and purity are of particular importance. Further investigations with different molecular weights of chitins or chitosans are needed to more effectively utilize these chitinous polymers as a pigment adsorbent.

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