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Citric acid pretreatment for suppressing adhesion of major egg allergens to a stainless steel surface

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ABSTRACT

Adhesion of proteins to solid surfaces can cause a number of problems in food manufacturing. In particular, a tiny amount of adherent allergenic proteins may pose a cross-contamination risk. To explore a strategy for suppressing adhesion of major egg allergens to stainless steel surfaces, the effect of citric acid pretreatment of stainless steel surfaces on the adhesion of ovalbumin and ovomucoid was studied under various conditions. Stainless steel powder was subjected to adhesion experiments after treatment with 50 mM citric acid solution, rinsing with water, and drying. Results from ovalbumin adhesion at 30 °C and pH 7.4 demonstrate that citric acid was more effective against ovalbumin adhesion than any univalent or divalent organic acid tested. This supports the idea that the suppression of adhesion is ascribed to the acid anions attaching to the stainless steel surface and yielding an effective negative surface charge. Citric acid pretreatment also suppressed the adhesion of ovalbumin at higher pH of 9.0 and temperature of 80 °C. The adhesion of ovomucoid was also suppressed at both 30 and 80 °C by citric acid pretreatment. These results suggest that the citric acid pretreatment is effective against the adhesion of major egg allergens to stainless steel surfaces.

1. Introduction

Adhesion of proteins to solid surfaces can occur under various conditions and cause a number of problems in food processing and manufacturing. For example, adherent proteins can form a fouling layer on the surface of food processing equipment and interfere with heat transfer (de Jong, 1997; Wallhäußer, Hussein, & Becker, 2012). Proteins may provide a nutritional environment, and insufficient cleaning may result in bacterial growth on the equipment surface (Dat, Hamanaka, Tanaka, & Uchino, 2010). These bacteria can contaminate subsequent product manufacture. Moreover, if the adherent proteins are allergenic, they may pose a cross-contamination risk. Even a very small amount of cross-contamination may cause serious problems in respect of undeclared allergens. Food manufacturers have to minimize cross-contamination risk by sufficient cleaning. Allergen removal through cleaning has been recognized as one of the critical points for effective allergen control (Jackson et al., 2008). To lower the cross-contamination risk further, methods to suppress the adhesion of allergenic proteins to food contact surfaces are required.

Hen egg is one of the most frequently implicated causes of rapid allergic responses to food in children (Bush & Hefle, 1996). The estimated frequency of food allergy to egg in North America is 1.5% for infants and children, second after that to milk (Sicherer & Sampson, 2010). Egg has been reported as an undeclared allergen found frequently in processed foods. A survey of food recall actions because of the presence of undeclared allergens reported to the US Food and Drug Administration showed that egg accounted for the greatest number of food recalls among common food allergens (Vierk, Falci, Wolyniak, & Klontz, 2002). Although inadequate ingredient statements including omissions and errors were the most common cases, cross-contamination from manufacturing equipment accounted for 40% of all recalled products. A recent study on the pre-packaged food products available commercially in Thailand also showed that 17 out of 129 products contained egg as an undeclared allergen at levels greater than 10 ppm (Surojanametakul et al., 2012). Thus, information on the adhesion behavior of egg proteins to processing equipment surfaces is essential to the control of undeclared egg allergens in processed foods. However, the literature information on their adhesion is rather limited.

In our previous study (Sugiyama, Hagiwara, Watanabe, & Sakiyama, 2012), the adhesion behavior of three egg white

Abbrevations: ANOVA, analysis of valiance; CA, citric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; OVA, ovalbumin; OVM, ovomucoid; SS, stainless steel; Tris, 2-amino-2-hydroxymethyl-propane-1,3-diol.

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proteins, ovalbumin (OVA), ovomucoid (OVM), and lysozyme, to a stainless steel (SS) surface was studied at 30 °C and pH 7.4 with consideration of the effect of coexisting ionic species. OVA (45 kDa, pI 4.5) and OVM (28 kDa, pI 4.1) are major allergens and constitute 54 and 11% of total egg white protein, respectively (Mine & Yang, 2008). SS is one of the most common materials used in food processing equipment. Our results demonstrated that the presence of such multivalent anions as phosphate and citrate reduced the adhesion of OVA and OVM to the SS surface. A low level adhesion of OVA was also observed by pretreating the SS surface with a phosphate buffer. This suggests that the low level adhesion resulted from phosphate ions attaching to the SS surface even after water rinsing. Based on these results, we hypothesize that treating a SS surface with an appropriate anionic substance can reduce the adhesion of major egg allergens.

This study is focused on the effect of pretreatment of a SS surface with citric acid (CA) on the adhesion of OVA and OVM. The adhesion of OVA is studied more extensively because most undeclared egg-positive products are found to contain OVA (Surojanametakul et al., 2012). The adhesion to CA-treated surfaces is compared to untreated surfaces at different temperature and pH values. The effect of pretreatment conditions is also studied.

2. Materials and methods

2.1. Stainless steel powder

Fine 316L SS powder (PF-5F; specific surface area: 0.57 m^2/g) obtained from Epson Atmix Co. (Hachinohe, Aomori, Japan) was used as the substrate surface for the adsorption experiments. Its large specific surface area is favorable for the precision required in adsorption measurements. As in our previous work (Thammathongchat, Hagiwara, & Sakiyama, 2010), the powder was washed successively with 0.1 M NaOH, distilled water, and ethanol, before being dried at 50 °C and stored at room temperature until needed.

2.2. Egg allergens

OVA (Sigma A5503) purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) was used without further purification. OVM was purified from hen egg white as described in our previous work (Sugiyama et al., 2012), through precipitation by the addition of trichloroacetic acid/acetone and gel filtration chromatography on Sephadex G-100 (fractionation range for globular proteins: 4–150 kDa). The solution of purified OVM was dialyzed against distilled water, freeze-dried, and stored at −20 °C until needed.

2.3. Pretreatment of stainless steel powder

SS powder (10 g) was mixed with 20 ml of 50 mM CA solution in a glass vial. After being tightly sealed with an aluminum cap, the glass vial was incubated at 30 °C for 120 min with vigorous shaking. The SS powder was collected by filtration on a hydrophilic polytetrafluoroethylene membrane (Millipore, Billerica, MA, USA). The powder on the membrane filter was rinsed repeatedly with distilled water. After being dried at 50 °C, the powder was stored at room temperature until needed for the adsorption experiments.

For comparison of the pretreatment conditions, a univalent or divalent acid was used instead of CA (trivalent acid) without changing any other conditions. The acids used for the pretreatment are listed in Table 1 with their pKa values (Lide, 2009). In other experiments, CA concentration and pretreatment time were changed as described in later text.

Table 1
Organic acids tested for the pretreatment of stainless steel surface.

Name	Structure	pKa
Acetic acid	CH_3–COOH	4.76
Succinic acid	HOOC–CH_2–CH_2–COOH	4.21, 5.64
L-Malic acid	HOOC–CH_2–CHOH–COOH	3.40, 5.11
L-Tartaric acid	HOOC–CHOH–CHOH–COOH	2.98, 4.34
Citric acid	HOOC–CH_2–C(OH)(COOH)–CH_2–COOH	3.13, 4.76, 6.40

2.4. Adsorption experiments

OVA or OVM was dissolved at 2 mg/ml in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4). One milliliter of the protein solution was added to a glass vial containing 2 g of the SS powder with or without pretreatment. After being sealed tightly with an aluminum cap, the glass vial was incubated at 30 °C for 120 min with vigorous shaking. The supernatant was withdrawn to measure the protein concentration by a bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). The amount of adherent protein was calculated from the difference between the protein concentrations before and after incubation. In some adhesion experiments, pH or temperature was altered to check the effect of adhesion conditions. For the adhesion experiments at pH 8.5 and 9.0, 50 mM 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris) buffer was used instead of HEPES buffer. The adhesion experiment was repeated at least three times.

2.5. Statistical analysis

The significance in difference between the data sets was tested, if necessary, using Student's *t*-test or a one-way analysis of variance (ANOVA) in GraphPad Prism 5.04 (GraphPad Software, CA, USA). The statistical significance threshold was set to $p \leq 0.05$.

3. Results and discussion

3.1. Effect of organic acid pretreatment on OVA adhesion

Fig. 1 compares the effect of various organic acids used in the pretreatment of the SS surface on OVA adhesion at 30 °C in 50 mM HEPES buffer (pH 7.4). The mean value and standard deviation of the experimental data are shown for each organic acid. The

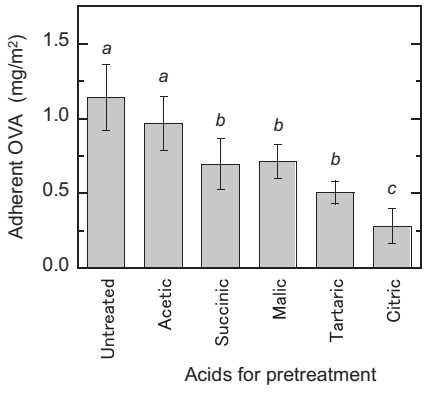

Fig. 1. Effect of pretreatment of SS surface with different types of organic acid on OVA adhesion. Adhesion experiments were performed in 50 mM HEPES buffer (pH 7.4) at 30 °C. Error bars represent standard deviations. Different italic letters indicate significant differences in adherent amount of OVA ($p < 0.05$, one-way ANOVA followed by Tukey's multiple comparison test).