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Pretreatment with citric acid or a mixture of nitric acid and citric acid to suppress egg white protein deposit formation on stainless steel surfaces and to ease its removal during cleaning

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ABSTRACT

Fouling, adhesion of protein onto a food contact surface, is an important difficulty hindering the pasteurization processing of egg products. To explore a strategy for efficient cleaning of a food contact surface fouled by adherent egg protein, this study investigated the effects of stainless steel surface pretreatment with citric acid or a mixture of nitric acid and citric acid on the adhesion and removability of egg white protein. The 1.05% citric acid pretreatment for 120 min was effective to suppress egg white protein adhesion to a stainless steel surface at 30–80 °C. Pretreatment with nitric acid (1.05% or 4.55%) containing 1.05% citric acid was also effective at 60 °C, which is relevant as a practical pasteurization temperature of egg products. Reducing the pretreatment time from 120 to 15 min was still effective to suppress egg white protein adhesion significantly. Pretreatment with 1.05% nitric acid containing 1.05% citric acid caused higher removability of adhered protein during the cleaning process, especially at higher temperatures. These results demonstrate that pretreatment with nitric acid containing citric acid might be an excellent choice for promoting the efficient cleaning of food manufacturing equipment that has been fouled with egg products.

1. Introduction

Chicken eggs have been used worldwide not only for meals at home but also as food ingredients in the food manufacturing industry. Food ingredients produced from chicken eggs include liquid whole eggs, liquid egg whites, fortified whole eggs or egg yolks, seasoned whole eggs or egg yolks, and various blends of egg products (Chmielewski, Beck, Juneja, & Swayne, 2013). Chicken eggs are frequently contaminated by microorganisms such as *Salmonella* bacteria. Therefore, food ingredients produced with chicken eggs must be pasteurized. For instance, in the United States, heating at 55.6 °C for a minimum holding time of 6.2 min, or 56.7 °C for 3.5 min, is necessary for liquid egg whites (Code of Federal Regulations, 2010; Geveke & Torres, 2013).

An important problem that occurs during this pasteurization process is fouling (Li et al., 2013; Ling & Lund, 1978; Pelegrine & Gasparetto, 2006), which is the result of protein adhesion onto a food contact surface (Nakanihi, Sakiyama, & Imamura, 2001). The fouling layer potentially acts as a nutrient for microorganisms, which can lower the quality of products or cause food poisoning. It also damages the pasteurizer heat exchanger, impairing its performance. To prevent these problems, the pasteurization equipment must be cleaned regularly. Cleaning equipment fouled with protein deposits requires much water, chemicals, energy, and time. Therefore, methods to suppress the adhesion of protein to food contact surfaces are necessary for efficient cleaning.

Our previous study examined the effect of citric acid pretreatment of stainless steel surface on the adhesion of two major egg white proteins, ovalbumin and ovomucoid, onto a stainless steel surface under various conditions (Sakiyama, Sato, Tsuda, Sugiyama, & Hagiwara, 2013). Stainless steel is a commonly used material in the construction of food manufacturing equipment. Results from ovalbumin adhesion at 30 °C and pH 7.4 demonstrated that citric acid pretreatment greatly reduced the adhesion amount of ovalbumin to the stainless steel surface. Even at higher temperature of 80 °C, this reduction remained apparent. The adhesion of

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ovomucoid was also suppressed at both 30 and 80 °C by citric acid pretreatment. These results suggest that the citric acid pretreatment is a promising method for the suppression of egg white protein adhesion to the stainless steel surface.

This study was undertaken to investigate the effectiveness of citric acid pretreatment in a more realistic situation. First egg white powder was used as a sample instead of ovalbumin or ovomucoid. Secondly, a mixture of citric acid and nitric acid was used as a pretreatment solution for the stainless steel surface. In typical cleaning procedures for food manufacturing equipment, alkali cleaning using basic detergent is conducted first to remove proteinous or organic deposit (Etienne, 2006; Inoue & Nishino, 2006). Then acid cleaning using acidic detergent is done to remove inorganic scales (Etienne, 2006; Inoue & Nishino, 2006). Nitric acid is used frequently for acid cleaning (Etienne, 2006; Inoue & Nishino, 2006). Therefore, when using nitric acid containing citric acid as an acid cleaning agent, the citric acid pretreatment for suppressing protein adhesion might be conducted simultaneously during acid cleaning. Finally, cleaning experiments of a stainless steel surface fouled by egg white protein were conducted to assess the effects of the pretreatment on the removal of adhered egg white protein.

2. Materials and methods

2.1. Egg white protein sample

Dialyzed and freeze-dried chicken egg white powder was supplied by Kewpie Corp., Japan. They were used without further purification for experiments.

2.2. Stainless steel powder

Fine 316L stainless steel powder PF-5F (Epson Atmix Corp., Hachinohe, Aomori, Japan) was used as the substrate surface for the adhesion experiments. Its specific surface area was 0.57 m^2/g. This large specific surface area is suitable for precise adhesion measurement. The stainless steel powder was washed before adhesion experiments as done in our previous work (Sakiyama et al., 2013; Thammathongchat, Hagiwara, & Sakiyama, 2010). It was washed with 0.1 N NaOH at 60 °C for 2 h first. Then, it was rinsed thoroughly with distilled water so that the pH of the rinse water became 7. After being dried in an oven at 50 °C, the powder was stored at room temperature until experiments.

2.3. Pretreatment of stainless steel powder

Pretreatment solutions used were the following.

- 1.05% citric acid
- 4.55% nitric acid containing 1.05% citric acid
- 1.05% nitric acid containing 1.05% citric acid
- 4.55% nitric acid

The procedure of pretreatment of stainless steel powder was conducted in the same way as in a previous study (Sakiyama et al., 2013). First, 10 g of washed stainless steel powder was mixed with 20 ml pretreatment solution in a glass vial. After being closed tightly with a cap, the glass vial was incubated at 30 °C for 120 min with vigorous shaking (BW101 shaking incubator; Yamato Scientific Co., Ltd., Tokyo, Japan). Then, the stainless steel powder was collected by filtration on an Omnipore membrane filter (Millipore, Billerica, MA, USA). After being rinsed repeatedly with distilled water until the pH of rinsed water became 7, it was dried at 50 °C and stored at room temperature until adsorption experiments.

To investigate the effect of pretreatment time, stainless powders with 15, 30, and 60 min incubation time were also prepared when 1.05 wt% nitric acid containing 1.05% citric acid was used as the pretreatment solution.

2.4. Adhesion experiments

A method similar to that used in previous research was used (Sakiyama et al., 2013). Egg white protein was dissolved at 2 mg/ml in 50 mM HEPES buffer. The buffer pH was set to 7.4. One milliliter of the protein solution was added to a glass vial containing 2 g of the stainless steel powder with or without pretreatment. The vial was closed tightly with a cap and was then incubated at 60 °C for 120 min with vigorous shaking (BW101 shaking incubator; Yamato Scientific Co., Ltd., Tokyo, Japan). After incubation, 0.2 ml of the supernatant was taken to measure the protein concentration by the BCA method (Pierce, Rockford, IL, USA). The amount of protein adhered onto the stainless steel surface, q (mg/m^2), was evaluated using the following equation, as in a previous study (Sakiyama et al., 2013).

$$q = \frac{\Delta C \times V}{W \times S}$$

Therein, ΔC stands for the difference between the protein concentrations before and after incubation (mg/ml), V denotes the volume of egg white protein solution in vial (ml), W is the weight of stainless steel powder in vial (g), and S represents the specific surface area of stainless steel powders (m^2/g). From the values of the amount of adhered protein with and without pretreatment, the reduction ratio (%), which is defined as the ratio of reduction of adhered protein by pretreatment to the adhered protein amount without pretreatment, was also calculated.

In some experiments, incubation temperature was altered to 30, 40, 50, 70, and 80 °C, respectively, to check the effect of temperature on adhesion.

2.5. Cleaning experiments

The pretreatment was conducted using 1.05% nitric acid containing 1.05% citric acid. Two grams of the stainless steel powder with or without the pretreatment was incubated with 1 ml of 2 mg/l egg white protein at 60 °C for 120 min with vigorous shaking, as described in Section 2.4. The amount of protein adhered onto the stainless steel surface was calculated as explained in Section 2.4. Three hundred microliters of the supernatant was additionally removed and 0.5 ml of 0.1 NaOH was added to the vial. The vial was closed tightly with a cap and then incubated at constant temperature for 120 min to clean the stainless steel powder at a constant shaking rate (120 rpm; BW101 shaking incubator; Yamato Scientific Co., Ltd., Tokyo, Japan). To check the effect of temperature, the cleaning temperature was varied to 30, 40, 50, 60, and 70 °C. The supernatant was recovered to measure the amount of desorbed protein from the stainless steel surface using the BCA method. From the values of the amount of adhered protein before cleaning and desorbed protein, the removal ratio (%), which is defined as the ratio of desorbed protein amount to the adhered protein amount before cleaning, was calculated.

2.6. Statistical analysis

Every experimental run was replicated triplicate. The Student's t-test (to examine two samples assuming an equal variance for each) or analysis of variance (ANOVA) followed by Tukey's multiple

comparison test (to examine more than two samples) was carries out. Statistical significance value was set at p-value less than 0.05.

3. Results

3.1. Adhesion at different temperatures onto stainless steel pretreated with 1.05% citric acid

Fig. 1(A) portrays results of the amount of adhered egg white protein onto the stainless steel surface with and without pretreatment of 1.05% citric acid at different adhesion temperatures. The reduction ratio of adhered egg white protein by the pretreatment is also shown in Fig. 1(B). At all temperatures examined, egg white protein adhesion to the stainless steel surface decreased because of the pretreatment. Results show that citric acid pretreatment was effective in suppressing egg white protein adhesion at these temperatures. The reduction ratio by the pretreatment decreased as temperature increased.

3.2. Suppression of adhesion of egg white protein onto stainless steel pretreated with nitric acid containing citric acid

Fig. 2(A) presents the suppression behavior of egg white protein adhesion onto the stainless steel surface pretreated with nitric acid containing citric acid. For comparison, results for pretreatment with 1.05% citric acid or 4.55% nitric containing no citric acid are also shown. The adhesion procedure was conducted at 60 °C. The

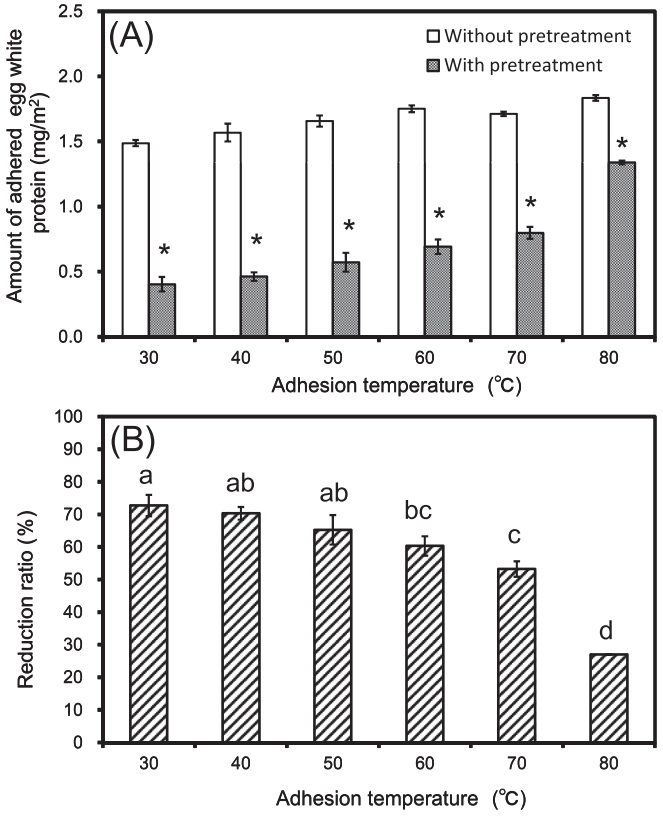

Fig. 1. (A) Amounts of adhered egg white protein onto the stainless steel surface with and without pretreatment of 1.05% citric acid at different adhesion temperatures. An asterisk indicates that the value is significantly different from that for the untreated surface ($p < 0.05$, Student's t-test). (B) Reduction ratio by the pretreatment at different adhesion temperature. The data having the same grouping letter indicate that the differences from changing adhesion temperature were not statically significant ($p < 0.05$, Tukey's multiple comparison test).

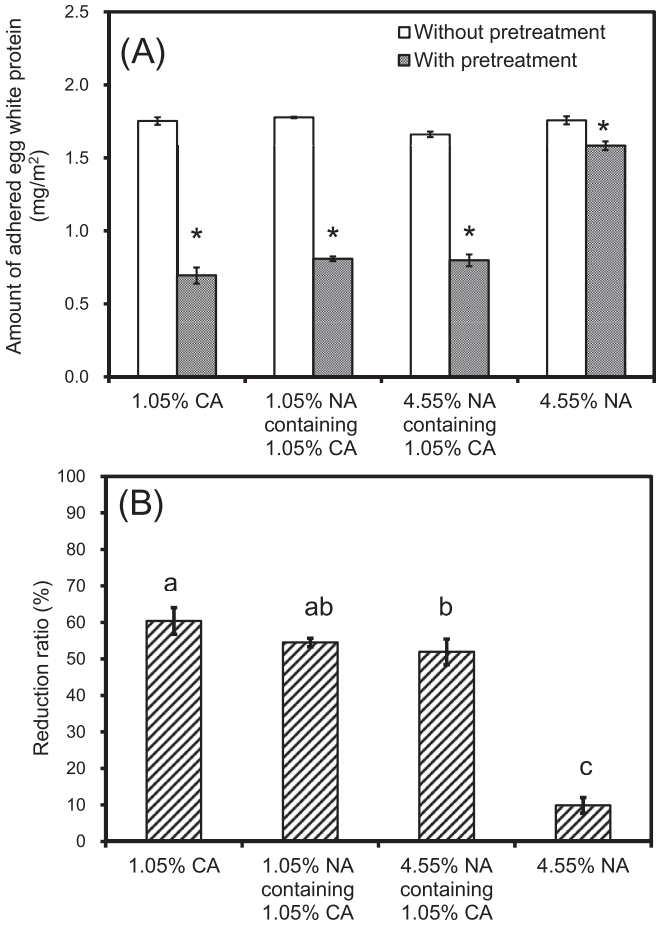

Fig. 2. (A) Suppression of egg white protein adhesion onto a stainless steel surface pretreated with nitric acid containing citric acid. The adhesion procedure was conducted at 60 °C. CA and NA respectively designate citric acid and nitric acid. An asterisk indicates that the value is significantly different from that for the untreated surface ($p < 0.05$, Student's t-test). (B) Reduction ratio by the pretreatment with different solutions. The data having the same grouping letter indicate that the differences from changing pretreatment solution were not statically significant ($p < 0.05$, Tukey's multiple comparison test).

reduction ratio of adhered egg white protein by the pretreatment is also shown in Fig. 2(B). Pretreatment using nitric acid without citric acid yielded only about 10% decrease in egg white protein adhesion, although pretreatment by nitric acid containing citric acid, by contrast, achieved about 50% reduction of adhesion as the same level as the pretreatment of citric acid, which shows that the reduction of adhered amount of protein by the pretreatment with nitric acid containing citric acid was caused mainly by the citric acid, not by nitric acid.

3.3. Effect of pretreatment time of nitric acid containing citric acid on adhesion

In Fig. 3 the adhesion amount of egg white protein onto the stainless steel surface pretreated for various times (15–120 min) (A) and the reduction ration of adhered egg white protein by the pretreatment (B) are shown, respectively. 1.05% nitric acid containing 1.05% citric acid was used as the pretreatment solution. The adhesion procedure was conducted at 60 °C. Even 15 min pretreatment reduced the adhesion amount of egg white protein significantly. To reduce about half amount of adhered egg white

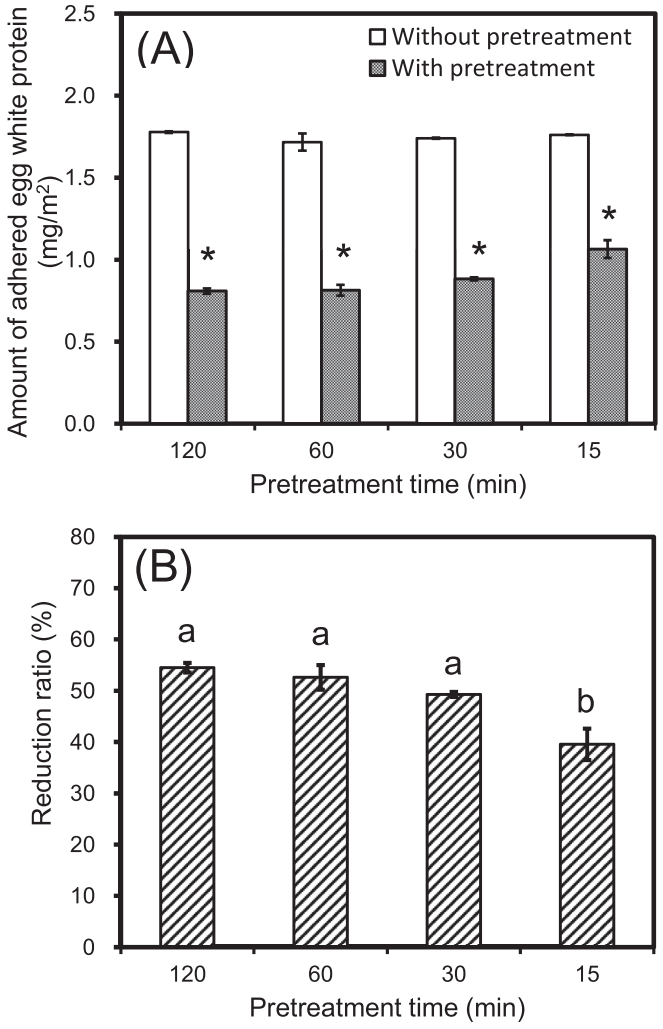

Fig. 3. (A) Adhesion amounts of egg white protein onto the stainless steel surface pretreated for different times. The pretreatment solution was 1.05% nitric acid containing 1.05% citric acid. The adhesion procedure was conducted at 60 °C. An asterisk indicates that the value is significantly different from that for the untreated surface ($p < 0.05$, Student's *t*-test). (B) Reduction ratio by the pretreatment with different pretreatment time. The data having the same grouping letter indicate that the differences from changing pretreatment time were not statically significant ($p < 0.05$, Tukey's multiple comparison test).

protein to the same degree as 120 min pretreatment, 30 min pretreatment was sufficient.

3.4. *Effect of pretreatment on removability of adhered protein during cleaning at different temperatures*

Regarding the results shown in Section 3.3, the pretreatment time was set to 30 min for the experiments in this section. Fig. 4 presents a comparison of the removal ratio (%) between non-pretreated and pretreated stainless steel surface at different cleaning temperatures. At 30 °C, it was about 40%, exhibiting no clear difference between the non-pretreated and pretreated stainless steel powder. At temperatures higher than 40 °C, the removal ratio with pretreatment was larger than without pretreatment. At 70 °C, more than 98% of adhered protein was removed from the pretreated stainless steel surface, whereas 20% of adhered protein remained on the non-pretreated stainless steel surface after cleaning. Consequently, the pretreatment affected the desorption behavior of the adherent protein.

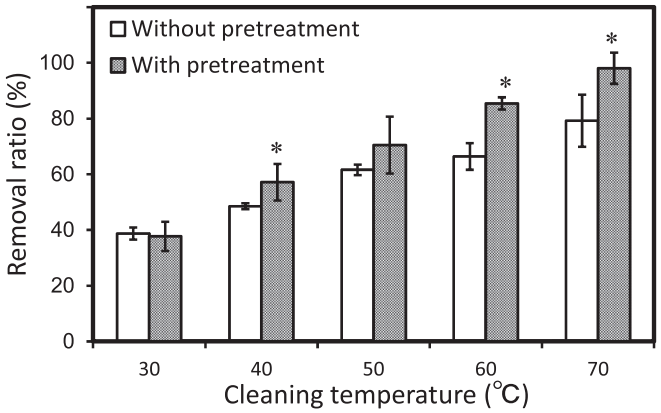

Fig. 4. Comparison of removal ratio between non-pretreated and pretreated stainless steel surface at different cleaning temperatures. The pretreatment solution was 1.05% nitric acid containing 1.05% citric acid. An asterisk indicates that the value is significantly different from that for the untreated surface ($p < 0.05$, Student's *t*-test).

4. Discussion

For not only ovalbumin or ovomucoid but also for egg white protein, the citric acid pretreatment was effective to suppress adhesion to the stainless steel surface. In addition, it remained effective at the higher temperatures used for the practical pasteurization temperature of egg products. In a previous study, we proposed the mechanism of the suppression effect by citric acid pretreatment as follows (Sakiyama et al., 2013): at pH 7.4, the bare surface of stainless steel is charged positively (Takahashi & Fukuzaki, 2008). By the pretreatment, citric acid molecules attach to the stainless steel surface. Citric acid has three carboxyl groups (-COOH) (Fig. 5(A)), with pK, respectively, of 3.13, 4.76, and 6.40 (Lide, 2009). Therefore, at pH 7.4, the carboxyl groups of citrate acid are deprotonated (Fig. 5(B)) and make the effective surface charged of stainless steel negative. Because ovalbumin (pI = 4.5; Fennema, 1985) and ovomucoid (pI = 4.8; Fennema, 1985) are charged negatively at pH 7.4, electric repulsive interaction occurs between these proteins and the pretreated stainless steel surface, resulting in suppression of the adhesion of these proteins. According to this mechanism, even adhesion from a mixture of various proteins can be suppressed if they are negatively charged. The compositions of

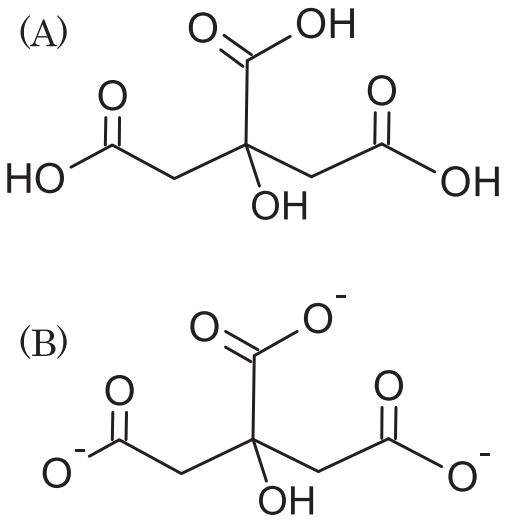

Fig. 5. Chemical structures of citric acid (A) and their ion (B).

Table 1
Composition of hen egg white proteins.

Protein name	Relative % (w/w)	Molecular weight (kDa)	Isoelectric point
Ovalbumin	54.0	45	4.5–4.8
Ovotransferrin	12.0	78–80	6
Ovomucoid	11.0	21	4.8
Ovomucin	3.5	25.4 (α) 400–700 (β)	45–5.0
Lysozyme	3.4	14.3	10–11
G2 globulin	4.0	49	5.5
G3 globulin	4.0	49	5.8
Ovoinhibitor	1.5	48	5.1–5.2
Ovoglycoprotein	1.0	24.4	3.9
Ovoflavoprotein	0.8	32	3.9–4.1
Ovomacroglobulin	0.5	780	4.9
Cystatin	0.05	13.1	5.1
Avidin	0.05	67	10.5

egg white protein in the literature are presented in Table 1 (Fennema, 1985; Kovacs-Nolan, Phillips, & Mine, 2005; Stadelman & Cotterill, 1995). Except for lysozyme and avidin, all other proteins have lower pI than 7.4. This fact is the reason for the effectiveness of the pretreatment on suppression of egg white protein. As for lysozyme and avidin, they are charged positively at pH 7.4 (pI = 10–11 for lysozyme; pI = 10.5 for avidin). Therefore, in principle, citric acid pretreatment might increase its adhesion amount through attractive electric interaction to the pretreated stainless steel surface. However, their fractions in egg white protein are so small (3.4% for lysozyme; 0.05% for avidin) that increased adhesion of lysozyme and avidin did not greatly influence surely the total amount of adherent proteins, resulting in decrease of the adhesion amount of total protein.

In typical cleaning procedures for food manufacturing equipment, alkali cleaning using basic detergent such as 1–2% NaOH (Etienne, 2006; Inoue & Nishino, 2006) is frequently conducted first to remove proteinous or organic deposits. Then acid cleaning using acidic detergent such as 1–2% nitric acid (Etienne, 2006; Inoue & Nishino, 2006) is done to remove inorganic scales. The results obtained using the nitric acid containing citric acid as a pretreatment solution indicate high feasibility of the pretreatment with nitric acid containing citric acid as a practical method for suppressing protein adhesion. It is expected that using nitric acid containing citric acid as an acid cleaning detergent for citric acid pretreatment can be conducted simultaneously during acid cleaning, although effects of citric acid addition to nitric acid on the performance of removing inorganic scales should be investigated. From Fig. 2, it is clear that the reduction of adhered amount of protein by the pretreatment with nitric acid containing citric acid was caused mainly by the citric acid. We hypothesize that this reduction was also caused by the same mechanism proposed by our previous study (Sakiyama et al., 2013). In order to prove the mechanism and versatility of citric pretreatment as a method for suppressing protein deposit formation and enhancing its removal during cleaning, the experiment by using other acid (e.c. hydrochloric acid, sulfuric acid, and phosphoric acid) containing citric acid as a pretreatment solution should be conducted further.

Raising the incubation temperature increased the adhesion amount of protein (Fig. 1), as reported also in a previous study about ovalbumin and ovomucoid (Sakiyama et al., 2013). The mechanism proposed in earlier studies is the promotion of the thermal aggregation of protein (Itoh et al., 1995; Sakiyama et al., 1998; Sakiyama et al., 2013). The denatured protein molecules aggregate through disulfide linkage or hydrophobic interaction to the protein molecules adhering directly to the surface. In this study, the same mechanism is expected to account for the increase of adhesion amount of protein. However, it must be pointed out that even at higher temperatures such as those related to practical pasteurization temperature, marked reduction of amount of adhered protein was achieved by the pretreatment with citric acid or nitric acid containing citric acid (Figs. 1 and 2). In addition, only 15 min pretreatment was sufficient to suppress adhesion of proteins (Fig. 3). Furthermore, the pretreatment improved the removability of adhered protein during the cleaning process, especially at higher cleaning temperatures (Fig. 4). These results demonstrate that the pretreatment with nitric acid containing citric acid might be an excellent choice for promoting efficient cleaning of food manufacturing equipment that is fouled with egg products.

By particularly addressing structural differences of the adhered protein layer, the mechanism of larger removal ratio of adhered protein on a pretreated stainless steel surface (Fig. 4) might be proposed as follows. Fig. 6(A and B) shows the schematic illustration of possible modes of protein adhesion to stainless steel surfaces with and without pretreatment as proposed by our previous study (Sakiyama et al., 2013). As explained earlier, protein

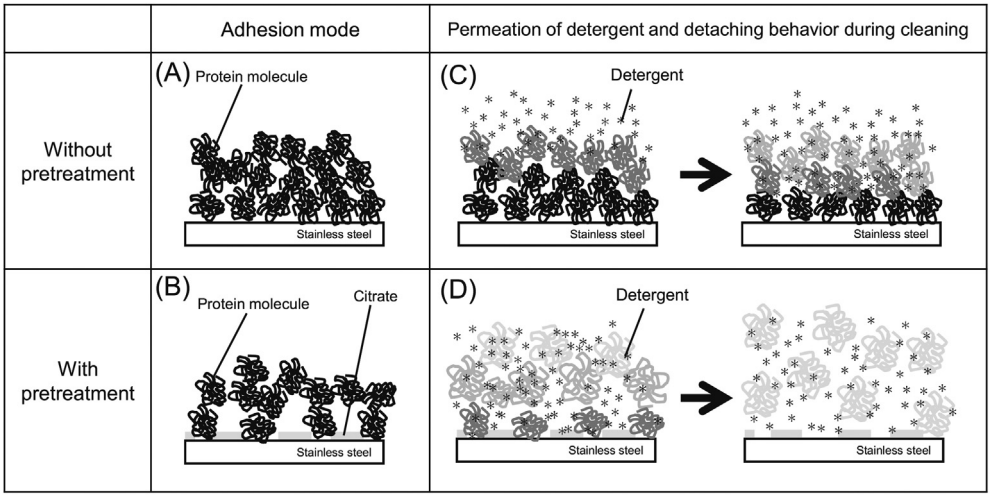

Fig. 6. Schematic illustration of possible modes of protein adhesion to stainless steel surfaces, permeations of detergent, and processes of detachment of adhered protein with and without pretreatment.

molecules aggregate through disulfide linkage or hydrophobic interaction to the protein molecules adhering directly to the surface. Although with the pretreatment the same formation of adhered protein layer occurs, the number of protein molecules adhering directly to the surface is smaller. Therefore, the resultant adhered protein layer is more porous than that without the pretreatment (Fig. 6(B)). Consequently, the detergent easily permeates into the protein layer (Fig. 6(C and D)). According to preceding studies (Gillham, Fryer, Hasting, & Wilson, 1999, 2000), the cleaning process of solid surface fouled by protein deposit with NaOH-based solution involves three stages after initial removal of loosely adhered proteins: (i) a swelling stage, where the protein reacts to form a high voidage matrix structure; (ii) a uniform stage, where the rate of cleaning is constant; and (iii) a decay stage, where the protein matrix breaks down and cleaning involves the erosion of islands of residual deposit (Gillham et al., 2000) The cleaning rate in a swelling stage is smaller than that of a uniform stage (Gillham et al., 1999, 2000). Considering these earlier results, the larger removal ratio observed with the pretreatment in this study might be from entering a uniform stage earlier because of the easy permeation of detergent (Fig. 6(C and D)). In addition, the porous structure of adhered protein layer might contribute to its rapid solubilization and breaking-down in a uniform stage. No clear improvement in removal ratio was observed at 30 °C, which might indicate that the cleaning temperature was so low that the cleaning rate was low after initial removal of loosely adhered proteins, and that no difference in desorbed amount of protein was detected between pretreatment and non-pretreatment.

5. Conclusions

Pretreatment with citric acid reduced the adhesion of egg white protein to a stainless steel surface. This reduction was also achieved with pretreatment with nitric acid containing citric acid. Reducing the pretreatment time from 120 min to 15 min still had marked ability to suppress the adhesion of egg white protein. Pretreatment with nitric acid containing citric acid showed higher removability of adhered protein during the cleaning process, especially at higher temperatures. These results demonstrate that pretreatment with nitric acid containing citric acid might be an excellent option for promoting the efficient cleaning of food manufacturing equipment fouled with egg products.

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