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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