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Effect of the Surface Charge of Stainless Steel on Adsorption Behavior of Pectin

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Received 23 March 2001/Accepted 21 July 2001

The effect of the surface charge of stainless steel particles on the adsorption behavior of pectin was studied over the pH range of 2.5 to 5.0. Stainless steel particles having different surface charge properties were prepared by chemical passivation in nitric acid, ozone oxidation, and heat treatment. The degree of the ionization of pectin increased with increasing pH, inducing a more extended structure. The saturation amount of pectin adsorbed (Γ_s) on all the surface-treated stainless steel particles increased with decreasing pH, showing the largest values at pH 2.5. The Γ_s also depended on the apparent density of the positive surface charge of stainless steel particles. It was found that the relative binding strength of unionized pectin on positively charged stainless steel surfaces was lower than that of ionized molecules and it also depended on the apparent density of the positive surface charge of stainless steel particles. These results showed that the surface charge of stainless steel, as well as the degree of the ionization of pectin, was an important factor determining the adsorption behavior of pectin at stainless steel-water interfaces.

Key words : Stainless steel/ Surface charge/ Pectin/ Adsorption behavior/ Binding strength.

INTRODUCTION

The adsorption behavior of acidic polysaccharides at solid-water interfaces, with reference to fouling and biofilm problems, is a subject of great importance in the food industry. Pectins are naturally occurring acidic polysaccharides and major cell wall components in plants (Olson et al., 1969). Pectins, mainly extracted from citrus peel and apple pomace, are widely used as thickening and gelling agents for various kinds of processed foods and beverages. Approximately 70 to 80% of pectins is composed of α -1,4 linked D-galacturonic acid units with varying degrees of methylated carboxyl groups, and the remainder is composed of several neutral sugars (Baig and Burgin, 1982; Rees and Wight, 1971). Monovalent cation salts of pectins are highly ionized in aqueous solution and they have random coil conformation with flexibility (Chou et al., 1991). The gels and aggre-

gates of pectins are formed and stabilized through cross-linkage between carboxyl groups by divalent cations, hydrogen bonds, and hydrophobic interaction (Oakenfull and Scott, 1984; Rees, 1969). Thus, pectins have a strong tendency to adsorb onto various equipment surfaces because of a diversity of functional groups and their amphipathic nature. On the other hand, the accumulation of large quantities of extracellular polymeric substances, called glycocalyx, is a characteristic of the formation of biofilms or adsorbed microbial communities (Costerton and Irvin, 1981; Harris and Mitchell, 1973; Mattila-Sandholm and Wirtanen, 1992). A glycocalyx matrix usually contains an acidic polysaccharide such as uronic acid, and it plays mediating roles both in the firmer adhesion of microbes to the surface and in the protection of microbes from hostile environments (Carpentier and Cerf, 1993).

In avoiding and minimizing fouling and biofilm formation, the smoothness and physicochemical properties of equipment surfaces are very important factors (European Hygienic Equipment Design Group, 1993a

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and 1993b; McGuire and Krisdhasima, 1991). Stainless steel has been the most widely used material in the construction of food machinery because of its excellent corrosion resistance. The food-contact surfaces of stainless steel can be treated by mechanical polishing and electropolishing to obtain smooth surfaces on which it is easier to remove microbes and repress biofilm formation (Mattila-Sandholm and Wirtanen, 1992; Timperley, 1981). However, the properties of passive film on stainless steel depend strongly on conditions of passivation and pretreatment (Rossi and Elsener, 1995; Virtanen and Bohni, 1995), even if the smoothness of surfaces is of the same order of magnitude. Apparently, the electrostatic nature of stainless steel would also vary by surface treatment, which in turn may affect the adsorption affinity of acidic polysaccharides such as pectin for stainless steel surfaces. However, little is known about the effect of the surface charge of stainless steel on pectin adsorption.

The purpose of this study is to determine the effect of the surface charge of stainless steel on the adsorption behavior of low methoxyl pectin at stainless steel-water interfaces. The authors used surface-treated 316L stainless steel particles that have different surface charge properties. This paper describes how the adsorption affinity of pectin for stainless steel depends both on the degree of ionization of pectin and on the apparent density of the positive surface charge of stainless steel particles.

MATERIALS AND METHODS

Chemicals and materials

A citrus pectin with a degree of esterification of approximately 35% was purchased from Wako Pure Chemical Industries, Ltd. (Osaka) and it was used as received. All other chemicals were of analytical grade and were purchased from commercial sources. Nonporous stainless steel particles (SUS 316L) with the specific surface area of $0.34\text{m}^2/\text{g}$ were obtained from The Nilaco Corp. (Tokyo). Before use, stainless steel particles were washed with acetone and ethanol with sonication, and then dried at 150°C (non-surface-treated stainless steel particles).

Surface treatment of stainless steel particles

Each 20-g aliquot of stainless steel particles was (i) chemically passivated with 200 ml of 30% HNO_3 for 1 h at 60°C , (ii) heated in a furnace at 300°C for 4 h, or (iii) exposed to 0.2% (v/v) gaseous ozone generated by a pure oxygen-fed silent discharge ozonizer (Model 961; OHNIT Co. Ltd., Okayama) for 1 h. A 10-g aliquot of the HNO_3 -passivated particles obtained

was (iv) further exposed 0.2% gaseous ozone as described above. After these treatments, the surface-treated stainless steel particles were rinsed with 200 ml of deionized water in 500-ml centrifugation tube 10 times by centrifugation ($1700\times g$ for 5 min), and then dried at 150°C . Specific surface areas of the particles treated with 30% HNO_3 , 30% HNO_3 + 0.2% ozone, 0.2% ozone, and heating at 300°C , as determined by the BET method, were 0.41, 0.42, 0.42, and $0.39\text{m}^2/\text{g}$, respectively.

Adsorption experiment

The experimental procedure was the same as that used for protein adsorption (Urano and Fukuzaki, 2000) without minor modifications. Pectin solution was freshly prepared before each adsorption experiment by dissolving 0.2 to 1.0 g of pectin in 100 ml of 10^{-3} M KNO_3 solution. The pH value of pectin solution was adjusted to 2.5 to 5.0 with HNO_3 or KOH (0.1 M solution). A 3-ml aliquot of the pectin solution was introduced to a 25-ml glass vial containing 2.0 g of stainless steel particles. Then, the vial was reciprocally shaken at 40°C (140 oscillations per min) for 2 h. The pectin-adsorbed particles were washed twice with 5 ml of 10^{-3} M KNO_3 solution by centrifugation ($2300\times g$ for 10 min). After being washed, the particles were dried at 40°C for 16 h. The dried pectin-adsorbed particles were used for the determination of the amount of pectin adsorbed (Γ) and the cleaning experiment. Each adsorption experiment was replicated three times at each pH.

Cleaning experiment

A 1-g portion of pectin-adsorbed stainless steel particles was put in a stainless steel column ($4\text{ mm } \phi \times 50\text{ mm}$), which was then installed vertically in a column oven at 40°C . First, 10^{-3} M KNO_3 solution (pH 5.9) was allowed to flow upward through the column at a flow rate of 0.3 ml/min, which corresponds to a space time (τ) of 1.6 min, for 120 min both to fill the column with the solution and to wet the particles. Second, NaOH solution (pH 12.1 to 13.1) was then fed from the bottom of the column at τ of 1.6 min. When the NaOH solution reached the column outlet after 7 min of feeding, the alkali eluent was fractionally collected at intervals of 2 min, and then at intervals of 5, 7, and 10 min, depending on the amount of desorbed pectin. The natural logarithm of the relative amount of residual pectin at time t (S) to that immediately before feeding NaOH ($S_0 = \Gamma$), $\ln(S/S_0)$, was plotted against t to obtain the curve of pectin desorption (Urano and Fukuzaki, 2001).

Surface charge measurements

Surface charges of stainless steel particles and pectin were determined by potentiometric titration as described previously (Fukuzaki et al., 2001a). A 1.5-g aliquot of stainless steel particles suspended or 0.05 g of pectin dissolved in 100 ml of 10^{-3} M KNO_3 solution (pH 2.5) was titrated with 0.1 M KOH under N_2 (99.999%, v/v) ambient. The uptake of H^+ and OH^- by stainless steel suspension or pectin solution was measured and compared with the uptake of these ions by a reference solution (10^{-3} M KNO_3 solution) of the same liquid volume as that of the suspension or the solution. Since stainless steel particles and pectin used in this study were not made isoionic, the apparent surface charge of stainless steel particles and the apparent net proton charge of pectin were expressed as the relative adsorption density of H^+ and OH^- ($[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]$) as a function of the dispersion pH, where Γ_{H^+} and Γ_{OH^-} are the adsorption densities of H^+ and OH^- , respectively.

Analyses

Pectin was determined by using a total organic carbon analyzer (TOC-5000A; Shimadzu Co., Kyoto) equipped with a solid sample module (SSM-5000A; Shimadzu Co., Kyoto). Most of the inorganic carbon, i.e. carbonate species, in pectin solution was removed by the addition of HNO_3 (adjusted to $\text{pH} < 1$) and bubbling of N_2 gas before measurement. A 250- μl aliquot of the solution and a 1.0-g aliquot of the pectin-adsorbed stainless steel particles were analyzed, respectively. The dry weight of pectin was calculated from the whole carbon content of 37% (w/w), as determined by the TOC analyzer with glucose as the standard. The specific surface area of stainless steel particles was determined from the amount of N_2 adsorbed (Monosorb; Yuasa Ionics Co., Osaka) using

the BET equation, assuming the cross-sectional area of $16.2 \text{ \AA}$ for an N_2 molecule. The chemical composition of stainless steel surfaces was analyzed by X-ray photoelectron spectroscopy (XPS) as described previously (Fukuzaki et al., 2001b). Measurement of the isotherm of water vapor adsorption onto stainless steel particles (25°C) and BET analysis of the isotherm were conducted as described previously (Fukuzaki et al., 2001a). Intrinsic viscosities ($[\eta]$) of pectin in 10^{-3} M KNO_3 solution were determined at 40°C in the pH range of 2.5 to 6.0 with an Ostwald viscometer by measuring specific viscosities (η_{sp}) at pectin concentrations (c) of 5×10^{-3} to 5×10^{-2} g/l and by extrapolating the graph of η_{sp}/c versus c to zero concentration.

RESULTS AND DISCUSSION

Surface properties of surface-treated stainless steel particles

Table 1 shows the chemical composition of the outermost surfaces of the surface-treated stainless steel particles and the results of BET analysis of water adsorption isotherms on the particles. HNO_3 treatment resulted in the enrichment of chromium content, whereas heat treatment led to the enrichment of iron content. Ozone treatment of non-surface-treated and HNO_3 -treated particles resulted in no significant changes in the chemical composition of their outermost surfaces. The monolayer saturation amount (V_m) is the amount of water physisorbed onto surface hydroxyl groups ($\text{M}-\text{OH}$) on stainless steel particles through hydrogen bonding, and hence it reflects the number of surface hydroxyl groups. The BET c constant also reflects the adsorption affinity of water for stainless steel surfaces. Lower V_m and BET c values were obtained for all the surface-treated particles

TABLE 1. Atomic composition of the outermost surfaces of and BET analysis of water adsorption data onto the surface-treated stainless steel particles.

Stainless steel	Component (atom %)				H ₂ O adsorption	
	Cr	Fe	O	C	V_m (ml/m ²)	c (-)
Non-surface-treated ^a	3.6	4.2	54.7	23.5	0.32	26.3
30% HNO_3 -treated	8.9	1.9	54.2	21.9	0.21	17.2
30% HNO_3 +0.2% ozone-treated	7.4	1.7	52.1	29.1	0.20	13.9
0.2% ozone-treated ^a	3.3	3.5	51.9	22.7	0.28	24.8
Heat-treated (300°C)	2.5	6.2	53.1	28.4	0.23	21.0

For XPS analysis, argon ion etching was not conducted. Another major main spectrum was observed at 101.7 eV which was assigned to Si 2p. A linearized form of the BET equation was fitted to the water vapor adsorption data in the relative vapor pressure range of 0.05 to 0.30 based on a linear regression method.

^a Reported by Fukuzaki et al. (2001b).

FIG. 1. $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}}$ curves of non-surface-treated and surface-treated stainless steel particles as a function of pH. Titration curves were measured in the presence of 10^{-3} M KNO_3 . Symbols: $\circ$, Non-surface-treated; $\bullet$, 0.2 % ozone-treated; $\square$, heat-treated at 300°C ; $\triangle$, 30% HNO_3 -treated; $\blacktriangle$, 30% HNO_3 + 0.2% ozone-treated particles.

compared with those for non-surface-treated particles. These results indicated that passive films having smaller numbers of surface hydroxyl groups were formed on stainless steel particles, with varying degrees of dehydroxylation, by treatments with HNO_3 , gaseous ozone, and heating.

Figure 1 compares the curves of the relative adsorption density of H^+ and OH^- on non-surface-treated and surface-treated stainless steel particles ($[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}}$) as a function of pH. Stainless steel particles having different surface charge properties could be successfully prepared by the surface treatments examined. Non-surface-treated and all the surface-treated particles were positively charged ($[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}} > 0$) over the pH range of 2.5 to 5.0 although the absolute values of $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}}$ were very different. The surface charge of stainless steel is believed to be established by protonation ($\text{M}-\text{OH}_2^+$) or deprotonation ($\text{M}-\text{O}^-$) of surface hydroxyl groups, and therefore a variation in the $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}}$ curves appears to be correlated with the degree of surface dehydroxylation (see Table 1). Since protonated hydroxyl groups ($\text{M}-\text{OH}_2^+$) on stainless steel surfaces are considered to act as adsorption sites for negatively charged pectin, the surfaces having a high $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}}$ value would exhibit a higher affinity for pectin adsorption.

Charge property and molecular size of pectin

Figure 2 shows the curve of the relative adsorption density of H^+ and OH^- on pectin ($[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{pec}}$) and $[\eta]$ of pectin as a function of pH. The absolute value of $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{pec}}$ curve increased with incre-

FIG. 2. $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{pec}}$ curve and $[\eta]$ of pectin as a function of pH. Titration curve and specific viscosity (40°C) of pectin were measured in the presence of 10^{-3} M KNO_3 .

asing pH owing to the ionization of free carboxyl groups on the molecule (Fig. 2A). $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{pec}}$ was almost zero below pH 2.7, indicating that pectin was in unionized form. The $[\eta]$ value, a measure of the hydrodynamic volume occupied by the molecule, became the lowest at pH 2.5 to 2.7 and increased with increasing pH (Fig. 2B). Apparently, the $[\eta]$ value was correlated with $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{pec}}$. Fishman et al. (1984) reported that the size of pectins in aqueous solution depended on the degree of esterification of carboxyl groups and ionic strength, and hence on the strength of the coulombic repulsion. These results indicate that unionized pectin was a more compact random coil, whereas the distribution of negative charges along the pectin molecule induced an extended form through coulombic repulsion.

Adsorption of pectin

Figure 3 shows the isotherms of pectin adsorption on non-surface-treated stainless steel particles at pH 2.5 and 3.5. The isotherms exhibited well-defined saturation values (Γ_s). The Γ_s values were almost constant when adsorption time was prolonged from 2 to 14 h (data not shown). A significantly higher Γ_s was observed at pH 2.5 compared with that at pH 3.5. To determine the effect of pH on Γ_s , the Γ_s values on all the stainless steel particles were determined over the pH range of 2.5 to 5.0. Figure 4 shows the Γ_s

FIG. 3. Adsorption isotherms of pectin on non-surface-treated stainless steel particles at pH 2.5 and 3.5. The adsorption experiment was conducted at 40°C in the presence of 10^{-3} M KNO_3 for 2 h with reciprocal shaking (140 oscillations per min). Symbols: ○, pH 2.5; ●, pH 3.5.

FIG. 4. Γ_s values on non-surface-treated and surface-treated stainless steel particles as a function of pH. For experimental conditions, see the legend for Fig. 3. Symbols: ○, Non-surface-treated; ●, 0.2 % ozone-treated; □, heat-treated at 300°C; △, 30% HNO_3 -treated; ▲, 30% HNO_3 + 0.2% ozone-treated particles.

values on non-surface-treated and surface-treated stainless steel particles as a function of pH. On all the stainless steel particles, Γ_s tended to increase with decreasing pH and showed the largest values at pH 2.5. On the other hand, Γ_s also depended on the kind of stainless steel particles. Higher Γ_s values were observed on the stainless steel particles having larger numbers of surface hydroxyl groups and higher $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}}$ values (see Table 1 and Fig. 3). It is more likely that Γ_s depends on the surface charge (or the degree of hydroxylation) of stainless steel particles rather than the surface chemical composition. The effect of the surface charge of stainless steel particles on Γ_s disappeared at pH above 4.0. The dependence

of Γ_s on solution pH is evidently attributed to the substantial differences in the degree of ionization and molecular size of pectin depending on pH (see Fig. 2). The phenomena that the net charge and size of polyelectrolytes affect their Γ_s on stainless steel and oxide surfaces is commonly found for protein adsorption (Fukuzaki et al., 1995 and 1996; Koutsoukos et al., 1983; Norde et al., 1986). The unionized pectin forms a more compact structure and shows little lateral electrostatic repulsion. These can lead to intermolecular hydrogen bonds between hydroxyl groups and/or hydrophobic interaction between the ester methyl groups (Fishman et al., 1984; Oakenfull and Scott, 1984), and hence to a closely packed arrangement on stainless steel surfaces. In contrast, an extended form of highly ionized pectin shows intermolecular electrostatic repulsion and requires a larger contact surface area per molecule for adsorption, resulting in smaller Γ_s values. Judging from both the well-defined saturation values of isotherms and the effect of the surface charge of stainless steel on Γ_s , it is thought that unionized and ionized pectins are probably adsorbed in a monolayer on stainless steel surfaces, where electrostatic interactions and hydrogen bonds contribute to the attractive force.

Interactions between pectin and stainless steel surfaces

A cleaning experiment was conducted to assess the effect of adsorption pH on the relative binding strength of pectin. Figure 5 shows the desorption curves of pectins that had been adsorbed on non-

FIG. 5. Effect of adsorption pH on the desorption behavior of pectin from non-surface-treated stainless steel particles during cleaning with NaOH solution of pH 13.1. Each cleaning experiment was conducted in a stainless steel column at 40°C by continuously feeding the NaOH solution at τ of 1.6 min. Symbols (adsorption pH): ○, 2.50; ●, 2.75; △, 3.00; ▲, 3.25; □, 3.50.

surface-treated stainless steel particles at pH 2.5 to 3.5 ($\Gamma = \text{ca. } 0.7 \text{ mg/m}^2$) during cleaning with NaOH solution of pH 13.1. Before feeding the NaOH solution, approximately 20 to 23% of the fragments of the pectin molecules, corresponding to $\ln(S/S_0)$ at time zero, were eluted from the dried pectin-adsorbed particles with KNO_3 solution. The initial rate of pectin desorption increased slightly by decreasing the adsorption pH. In addition, the equilibrium amount of residual pectin (S_r) after 55 min of cleaning decreased from 0.16 to 0.10 mg/m^2 with decreases in the adsorption pH from 3.5 to 2.5. The difference in the desorption behavior depending on the adsorption pH was probably attributed to the degree of the ionization of pectin. The ionized pectin interacts with the stainless steel surface through the formation of ion pairs at the contact region. Certainly, an increase in the degree of the ionization of pectin can enhance the opportunity of these bonding events, resulting in the greater magnitude of the forces of electrostatic interaction. On the other hand, the attractive forces between unionized pectin and stainless steel surfaces would be predominantly hydrogen bonds between polar groups ($-\text{COOH}$, $-\text{OH}$) on the pectin molecule and surface hydroxyl groups on stainless steel particles. In this adsorption state, pectin is weakly adsorbed and it can be readily desorbed during alkali treatment.

Pectin was adsorbed on non-surface-treated and surface-treated stainless steel particles ($\Gamma = \text{ca. } 1.3 \text{ mg/m}^2$) at pH 2.5, where pectin was unionized, and

FIG. 6. Effect of surface treatments of stainless steel particles on the desorption behavior of pectin during cleaning with NaOH solution of pH 12.1. Pectin was adsorbed on the surface-treated stainless steel particles at pH 2.5. For cleaning conditions, see the legend for Fig. 5. Symbols: ○, Non-surface-treated; ●, 0.2 % ozone-treated (0.2%); △, 30% HNO_3 -treated; ▲, 30% HNO_3 + 0.2% ozone-treated particles.

then each of the pectin-adsorbed particles was cleaned with NaOH solution of pH 12.1 (Fig. 6). The desorption behavior of unionized pectin was also found to be affected by the surface charge of stainless steel particles. Significant differences were observed in the S_r value. The S_r values on non-surface-treated, heat-treated, HNO_3 -treated, and $\text{HNO}_3 + \text{O}_3$ -treated particles were 0.29, 0.24, 0.19, and 0.15 mg/m^2 , respectively. This result suggests that the relative binding strength of unionized pectin depends on positive $[\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-}]_{\text{st}}$ value, i.e., the number of basic hydroxyl groups on stainless steel surfaces for hydrogen bonding. It is conceivable that the magnitude of forces of hydrogen bonds between unionized pectin molecules and hydroxyl groups on stainless steel surfaces depends on the strength of the basicity of hydroxyl groups on stainless steel and that it might become greater on protonated hydroxyl groups ($\text{M}-\text{OH}_2^+$) on stainless steel because of the contribution of electrostatic forces.

The results showed that the electrostatic nature of stainless steel surfaces, as well as the degree of the ionization of pectin, was an important factor determining the adsorption behavior of pectin at stainless steel-water interfaces. The adsorption affinity of pectin for stainless steel could be reduced by modifying stainless steel surfaces to an electrostatically inert nature. Among the surface treatments studied, HNO_3 treatment was the most effective to reduce the number of hydroxyl groups and hence the surface charge density of stainless steel, rendering the surface less susceptible to pectin adsorption. These findings suggest that controlling the surface charge of stainless steel is of importance in improving resistance to the adsorption of other ionic compounds, including bacterial extracellular polymers.

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