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Studies of Collagen Binding with Immobilized *Salmonella enteritidis* and Inhibition with Synthetic and Naturally Occurring Food Additives by a Surface Plasmon Resonance Biosensor

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The binding interaction of *Salmonella enteritidis* with collagen was investigated using the surface plasmon (SPR) biosensor. Collagen I bound to *S. enteritidis* immobilized on the sensor surface. The mixture of collagen I and laminin generated a synergistic response in the binding interaction with *S. enteritidis*. *Escherichia coli* IFO 3301, pathogenic *E. coli* O157:H7, *S. aureus* IFO 3060 and 14 strains of *Salmonella* were immobilized on the gold surface of the sensor chip with different generated refractive index (RI) units. The treatment of *S. enteritidis* on the sensor surface with hexametaphosphate, citrate, mannose and alginate showed no inhibitory effect on the subsequent binding interaction of collagen I with *S. enteritidis*. On the other hand, the pretreatment of *S.enteritidis* with monoglycerol monocaprylate, monocaprate, monolaurate and monomyristate did not show significant effects on the binding interaction of collagen with *S.enteritidis*; however, the RI unit was largely decreased by the subsequent treatment with the same monoglycerol esters. After treatment of *S. enteritidis* immobilized on the sensor with natural colors such as San-red MR, San-red YM, San-yellow No.3L, Purple corn extract, Annatto AN and Curcumin "San-Ei," the collagen I binding with bacterial cells was strongly inhibited. When the mixture of additive and collagen was injected, the RI value was lower in the presence of hexametaphosphate, citrate, mannose, alginate, monoglycerol fatty acid esters, diglycerol monomyristate, Japanese horseradish extract, chili extract, peach extract, concentrated tomato juice, San-red MR, San-red YM, San-yellow No. 3L, and Annatto AN than that of the control.

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1. Introduction

Food-borne illness caused by *Salmonella* is of increasing concern in many countries and the disposal of contaminated products and medical treatment is extremely costly. *Salmonella* is a leading cause of food-borne bacterial illness involving a wide range of foods. An increasing number of outbreaks caused by *Salmonella* have been observed around the world. *Salmonella enteritidis* is the most frequently isolated as a causative species in salmonellosis. It was reported that 1928 outbreaks of food-borne diseases (25,862 cases) took place in Japan in 2001.[1] Of these outbreaks, 201 (4,789 cases) were caused by *Salmonella.* The factors that frequently contribute to outbreaks of salmonellosis are: improper cooling, contaminated raw ingredients, inadequate heat processing and cross contamination.[2] Salmonellosis is mainly linked to the consumption of undercooked contaminated meat, eggs, and dairy products.[3] Sometimes, the outbreaks of food-borne illness due to *Salmonella* are caused by the consumption of contaminated raw vegetables and fruits.[1] Since *Salmonella* are common in animal feces and wastes from slaughtering houses and poultry processing plants, the organisms sometimes reach fresh water. Outer surfaces of meats can be contaminated with *Salmonella* after slaughter. This surface is composed of a connective tissue which is present between the skin and skeletal muscle and can be the site for bacterial adhesion. It has been reported by Medina and Fratamico[4] that among extracellular matrix proteins, collagen I and laminin bound with the *Escherichia coli* O157:H7 surface but fibronectin exhibited very low binding while hyaluronic acid and chondroitin sulfate had no detectable interaction when studied with a surface plasmon resonance (SPR) biosensor. To reduce the level of bacterial contamination, it has been reported that rinse treatments with trisodium phosphate, one of the sanitizers, reduced the levels of *E. coli* O157:H7 attached to adipose tissue up to 2.7 log units.[5] There are a few reports on methods for reduction and inhibition of attachment of pathogen on meats.

The SPR biosensor allows direct real-time detection of the binding, association, and dissociation rates of the reactions without chemical labeling for signal generation.[6] The SPR biosensors have been applied to study bacterial cell adhesion onto the surface of animal cells. The attachment of collagen-binding adhesions of *Staphylococcus aureus* with cartilage and other host tissue was reported[7]. These reports suggest that bacterial attachment onto animal tissues can be elucidated using the extracellular matrix proteins.

In this study, we investigated the binding of *S. enteritidis* cells with collagen, the most abundant protein in connective tissues, using the SPR biosensor. We also determined the binding interactions of synthetic and natural food additives with collagen and the *S. enteritidis* immobilized on the sensor chip. The results obtained in this study will be used to develop the methods to control contamination of pathogen on meat surfaces.

2. Materials and Methods

2.1 *Strains and culture conditions*

Escherichia coli IFO 3301, *Salmonella enteritidis* IFO 3313, *S. typhimurium* IFO 12529, *S. gallinarum* IFO 3163 and *Staphylococcus aureus* IFO 3060 were purchased from the Institute for Fermentation, Osaka, Japan. *Escherichia coli* (O157, VTEC), *S. aarhus*, *S,*

agona, *S. anatum*, *S. braenderup*, *S. heidelberg*, *S. infantis*, *S. london*, *S. sofia*, *S. thompson* were generously provided by Fukuoka City Institute of Hygiene and Environment, Fukuoka, Japan. *Salmonella enteritidis* FHC and *S. typhimurium* FHC were isolated from foods in our laboratory. Unless otherwise noted, these bacteria were cultured overnight in Tryptic Soy Broth (Difco Laboratories, Detroit, Mich., USA) at 37°C. *S. aureus* was cultured in Tryptic Soy Broth supplemented with 3% NaCl.

2.2 *Preparation of bacterial cells*

Bacterial cells were harvested by centrifugation at 10,000 × *g* for 3 min at room temperature from 1 ml of the overnight culture (approx. 5×10^9 cfu/ml). The cells were resuspended in 1 ml of phosphate buffered saline (PBS) and then harvested by centrifugation at 10,000 × *g* for 3 min at room temperature. The harvested cells were resuspended in 1 ml of PBS and the cell suspension was used for immobilization.

2.3 *SPR sensor*

Spreeta™ Evaluation Module System operated with Spreeta™ software (Texas Instruments, Inc. Dallas, Texas, USA) was used as a SPR biosensor in this study. The minimum resolvable refractive index change of the Spreeta™ sensor was 3×10^{-6} refractive index (RI) units.

2.4 *Determination of interaction of bacterial cells and proteins*

2.4.1 *Immobilization of bacterial cells*

The bacterial cells were immobilized on the gold surface of the sensor chip in the flow cell of the Spreeta™ sensor. To the flow cell equilibrated with PBS, 0.5 ml of the cell suspension was injected at a flow rate of 0.1 ml/min. After the injection, the flow was stopped for 20 min to immobilize bacterial cells onto the surface of the sensor chip. The excess amounts of the bacterial cells were removed by washing the flow cell with PBS (the running buffer) at a flow rate of 0.1 ml/min. Since the bacterial cell suspension contains various bacterial surface proteins leaked from bacterial cells, blocking of the gold surface was not performed after the immobilization. The flow cell was equilibrated with PBS. In all the steps of injection of solutions, washing and equilibration of the flow cell, the flow rate was set at 0.1 ml/min.

2.4.2 *Binding of proteins*

Collagen I (Nacalai tesque, Kyoto, Japan) was dissolved in 0.1 M acetate buffer (pH 4.0), and laminin (Harbor Bio-products, Norwood, MA, USA) and bovine serum albumin (BSA, Nacalai tesque, Kyoto, Japan) were dissolved in PBS. These proteins were analyzed at 0.1 mg/ml. To the flow cell, these solutions were injected in a volume of approximately 0.5 ml and the flow cell was washed and equilibrated with PBS.

2.4.3 *Regeneration*

The sensor surface was regenerated by washing with 0.1 N NaOH containing 1% Triton × –100 for 5 min followed by distilled water for 5 min and PBS for 5 min.

2.5 *Inhibition with food additives*

2.5.1 *Sequential injection*

Various food additives were tested. Various extracts, cranberry juice, tomato juice, and natural colors were kindly provided by San-Ei-Gen F.F.I., Inc. Osaka, Japan. Protamine, pectin hydrolysate, and Japanese horseradish extract were generously provided by Asama Chemicals, Co. Ltd., Tokyo, Japan. Lyso-lecitin was a gift from Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan. Anthocyanin extracted from purple sweet potato was generously provided by Institute of Food Development, JA Miyazaki, Miyazaki, Japan. All the other chemicals used were of analytical grade. After immobilization of *S. enteritidis* cells onto the sensor surface, the flow cell was equilibrated with PBS. The food additives dissolved or diluted with PBS were injected in a volume of about 0.5 ml, and the sensor surface was washed and equilibrated with PBS. The collagen solution (0.1 mg/ml) was then injected in a volume of about 0.5 ml. After washing and equilibrating with PBS, the same food additive was again injected in a volume of about 0.5 ml, washed and equilibrated with PBS. The RI was monitored throughout the experiment.

2.5.2 *Injection of mixture*

Food additive was mixed with collagen I at a final concentration of 0.1% and 0.1 mg/ml, respectively. After immobilization of *S. enteritidis* cells onto the sensor surface, the flow cell was equilibrated with PBS at a flow rate of 0.1 ml/min. The mixture was then injected in a volume of about 0.5 ml, and the sensor surface was washed and equilibrated with PBS at a flow rate of 0.1 ml/min. The RI was recorded throughout the experiment.

2.6 *Microscopy*

The gold surface of the sensor chip was investigated microscopically with a stereoscopic microscope Nikon SMZ-2T (Nikon, Tokyo, Japan) before and after immobilization of *S. enteritidis* cells.

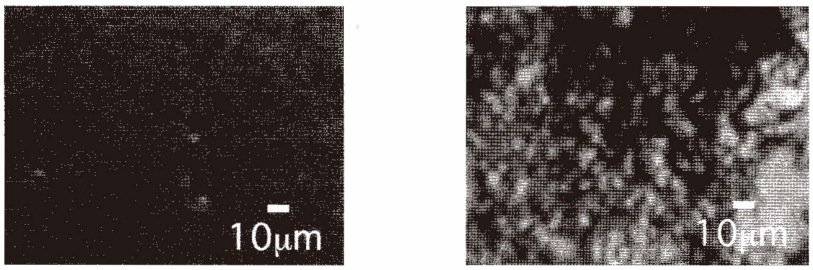
3. Results and Discussion

3.1 *Immobilization of bacterial cells on the sensor chip*

In this study, bacterial cells were directly immobilized on the surface of the sensor chip. Figure 1 shows the surface of the sensor chip before and after immobilization of *S. enteritidis* cells. As shown in Figs. 1(a) and 1(b), particles less than 3 μm in diameter were observed on the gold surface of the chip after immobilization of *S. enteritidis* cells, indicating that the immobilization of *S. enteritidis* on the gold surface was successful.

3.2 *Binding of collagen I and BSA with the sensor chip immobilized with* S. enteritidis

Typical sensorgrams of *S. enteritidis* immobilization and the binding interactions of collagen I and BSA with the sensor surface immobilized with *S. enteritidis* are shown in Fig. 2. In the separate experiments, immobilization of *S. enteritidis* cells generated 394 and 314×10^{-6} RI units. BSA and collagen I bound with the *S. enteritidis*-immobilized sensor surface with 353 and 513×10^{-6} RI units, respectively.



(a) Before immobilization (b) After immobilization

Fig. 1. Surface of the sensor chip (a) before and (b) after injection of *S. enteritidis* cells and washing with PBS and water. Into the flow cell equilibrated with PBS, 0.5 ml of the cell suspension was injected at a flow rate of 0.1 ml/min. After the injection, the flow was stopped for 20 min to immobilize bacterial cells on the surface of the sensor chip. The excess bacterial cells were removed by washing the flow cell with PBS at a flow rate of 0.1 ml/min.

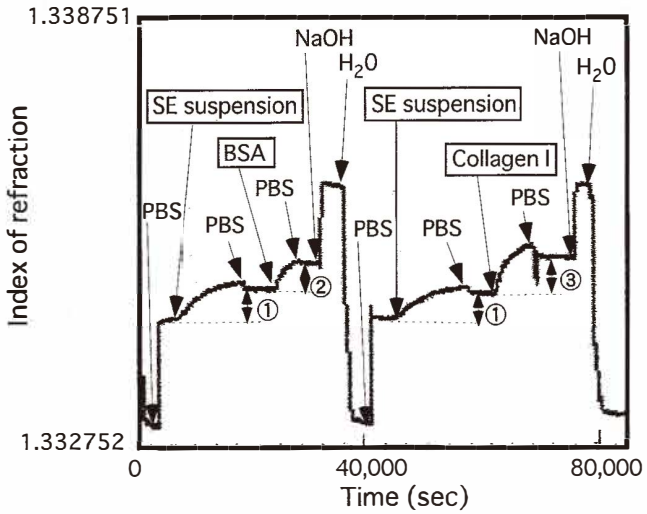


Fig. 2. Typical sensorgrams of *S. enteritidis* immobilization and the binding interactions of collagen I and BSA with the sensor surface immobilized with *S. enteritidis*. Solutions of collagen I and bovine serum albumin (BSA) were injected into the *S. enteritidis*-immobilized sensor surface in a volume of about 0.5 ml and the flow cell was washed and equilibrated with PBS. The RI was monitored.

① Changes of refractive index (RI) units by immobilization of *S. enteritidis* (SE)
② Changes of RI units by binding of BSA to SE
③ Changes of RI units by binding of collagen I to SE

The affinity of BSA, collagen I and laminin with *S. enteritidis* and the combined effects of collagen I and laminin on binding were examined. Table 1 shows the affinity of binding of these proteins with *S.enteritidis* cells. Among 3 proteins tested, collagen I most strongly bound with *S.enteritidis*. The mixture of collagen I and laminin generated a synergistic response in the binding interaction with *S. enteritidis*. Even after binding of BSA with *S. enteritidis* cells, RI units increased by the injection of the mixture of collagen I and laminin. These results were consistent with that of Medina on *E. coli* O157:H7.[8] Kukkonen *et al.*[9] reported that the type-1-fimbriate strain SH401 of *S. enteritidis* showed good adherence to laminin, whereas the adherence to fibronectin, types I, III, IV, and V collagens was poor. The combined use of collagen I and laminin showed the higher binding interaction with *S.enteritidis* cells than that of the individual collagen I or laminin only. However, collagen I was used alone in the following study including the screening of the substance inhibiting the binding interaction of collagen I and *S. enteritidis,* since collagen I generated relatively high RI units in this study using *S. enteritidis.*

3.3 *Immobilization of various bacteria and binding of collagen I with them.*

Table 2 shows RI units generated after immobilization of *E. coli* IFO 3301 (type strain), pathogenic *E. coli* O157:H7 (*stx1 and stx2*), *S. aureus* IFO 3060 and 14 strains of *Salmonella* and binding responses of collagen I with the sensor surface immobilized with each of the bacteria. All the bacterial cells were immobilized onto the gold surface of the sensor chip with different generated RI units. Collagen I also bound with the surface of the bacteria-immobilized sensor chip with different RI units. Medina and Fratamico[4] also showed binding of collagen I with *E. coli* O157: H7 strain. It has been reported that collagen attracts bacterial cells of many species, such as *S. epidermidis* and *P. aeruginosa* with specific interactions.[10,11] The attachment of collagen-binding adhesions of *S. aureus* with cartilage and other tissues was reported by Patti and coworkers[12,13] Walls *et al.*[14] have described the binding of collagen molecules and *S. typhimurium.* Gonzalez *et al.*[15] reported that, among 13 strains of *Salmonella*, three rough variants showed higher binding

Table 1
Changes of RI units generated by injection of proteins into the *S. enteritidis*-immobilized sensor surface.

Protein (0.1 mg/ml)	Changes of RI units ($\times 10^{-6}$) after injection of proteins	
BSA*	371	
Collagen I	513	
Laminin	12	
Collagen + Laminin	1295	
BSA* → Collagen+Laminin	371	772

*BSA: Bovine serum albumin

Table 2
Changes of RI units generated after immobilization of *E. coli, S. aureus* and various *Salmonella* strains and injection of collagen I into the bacteria-immobilized sensor surface.

Bacteria (O serotype)	Changes of RI units after successive injection of ($\times 10^{-6}$)	
	Bacterial cells	Collagen I
Control	—	516
E. coli IFO 3301	689	349
E. coli O157:H7	196	851
S. aureus IFO 3060	2215	777
S. aarhus (O18)	583	453
S. agona (O4)	208	232
S. anatum (O3)	103	212
S. braenderup (O7)	212	264
S. enteritidis IFO 3313	465	817
S. enteritidis FHC	739	601
S. gallinarum IFO 3163	100	689
S. heidelberg (O4)	180	415
S. infantis (O7)	361	721
S. london (O3)	75	435
S. sofia (O4)	86	408
S. thompson(O7)	129	457
S. typhimurium FHC	727	306

with fibronectin and collagen than the corresponding smooth forms. There are no other reports on the binding interaction of collagen I and *Salmonella*. Our results indicated that collagen I alone had binding interactions with various *Salmonella* strains including non-motile *S. gallinarum*.

3.4 *Effects of various food additives on the binding of collagen I with* S. enteritidis

3.4.1 *Effects of synthetic food additives*

To determine the synthetic food additives and substances that inhibit the binding of *S. eneritidis* with meats, synthetic food additives with low toxicity were tested for the inhibition of collagen binding with *S. enteritidis*. Table 3 shows the changes of RI units generated by the sequential injection of a food additive, collagen I and the same food additive into the *S. enteritidis*-immobilized sensor surface. The treatment of *S.enteritidis* on the sensor surface with hexametaphosphate, citrate, mannose and alginate had no inhibitory effect on the subsequent binding interaction of collagen I with *S.enteritidis*. Furthermore, the bound collagen I was rarely removed by the treatment with the same additives. On the other hand, although the treatment of *S.enteritidis* with some monoglycerol fatty acid esters did not show significant effects on the binding interaction of collagen with

Table 3
Changes of RI units generated by sequential injection of synthetic food additive, collagen I and the same food additive into the *S. enteritidis*-immobilized sensor surface.

Food additives	Changes of RI units ($\times 10^{-6}$) after sequential injection of		
	Additive	Collagen I	Additive
Control	—	817	—
0.1% Na hexametaphosphate	–14	633	–29
1.0% Na citrate	–71	907	–66
1.0% Mannose	–1	705	16
0.1% Na alginate	–15	640	–11
0.1% Monoglycerol monocaprylate	256	413	–475
0.1% Monoglycerol monocaprate	38	621	–229
0.1% Monoglycerol monolaurate	351	430	–152
0.1% Monoglycerol monomyristate	–103	826	–93
0.1% Diglycerol monocaprylate	–110	949	–25
0.1% Diglycerol monocaprate	–353	136	289
0.1% Diglycerol monolaurate	17	439	–158
0.1% Diglycerol monomyristate	64	311	–490
0.1% Polygrycerol monomyristate	–14	665	73
0.1% Sucrose stearate (100% monostearate)	55	967	77
0.1% Sucrose stearate (70% monostearate)	207	659	83
0.1% Sucrose stearate (60% monostearate)	174	715	199
0.1% Sucrose stearate (45% monostearate)	–206	919	77
0.1% Sorbitan monocaprylate	80	343	–70
0.1% Sorbitan monolaurate	375	462	1088
0.1% Lyso-lecitin	25	507	–25

—: not done

S.enteritidis, the RI unit was largely decreased by the subsequent treatment with the same monoglycerol monofatty acid esters. Figure 3 shows the typical sensorgram of *S. enteritidis* immobilization and the sequential injection of monoglycerol monocaprylate, collagen I and monoglycerol monocaprylate. The monoglycerol ester having monocaprylate as a fatty acid, the monoglycerol ester having the shortest fatty acid among the monoglycerol esters tested, most strongly removed the collagen I bound with *S.enteritidis* cells. In the case of diglycerol fatty acid esters, diglycerol monomyristate ester, the diglycerol ester having the longest fatty acid among the esters tested, removed the collagen I that bound with *S.enteritidis* cells pretreated with the same diglycerol ester. Sucrose fatty acid esters and lyso-lecitin had no effect on the binding interaction of collagen I with *S. enteritidis*.

3.4.2 *Effects of naturally occurring food additives, extracts, and juices*

Table 4 shows the changes of RI units generated by the sequential injections of an additive, collagen I and the same additive again. The RI largely increased by the injection

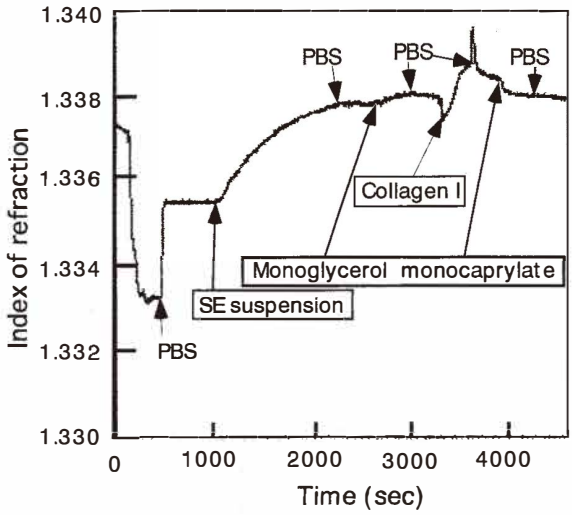


Fig. 3. Typical sensorgram of *S. enteritidis* immobilization and the sequential injection of monoglycerol monocaprylate, collagen I and monoglycerol monocaprylate. After immobilization of *S. enteritidis* cells on the sensor surface, the flow cell was equilibrated with PBS. The 0.1% solution of monoglycerol monocaprylate dissolved in PBS was injected in a volume of about 0.5 ml, and the sensor surface was washed and equilibrated with PBS. The collagen solution (0.1 mg/ml) was then injected in a volume of about 0.5 ml. After washing and equilibration with PBS, the same food additive was again injected in a volume of about 0.5 ml. The RI was monitored.

of protamine and decreased by the injection of collagen I, suggesting that protamine bound with the *S.enteritidis* immobilized on the sensor surface and a part of the bound protamine was removed by the injection of collagen I. Protamine remaining on the sensor surface might interfere with the binding of collagen I with *S.enteritidis*. Japanese horseradish extract showed the same effect. The binding interaction of collagen with *S.enteritidis* cells was reduced to some extent by the treatment of the cells with pectin hydrolysate, malt and mango extracts and concentrated tomato juice. In contrast, the RI value decreased by the reinjection of anthocyanin and nutmeg, grapefruit, peach, mango and lime extracts, though the RI value increased after the injection of collagen I. The results suggest that some compounds involved in the extracts are effective in removing collagen I which has bound with the surface of the bacterial cells.

3.4.3 *Effects of natural colors*

As shown in Table 5, after the treatment of *S.enteritidis*-immobilized sensor with San-red MR, San-red YM, San-yellow No.3L, Purple corn extract, Annatto AN and Curcumin "San-Ei," the collagen I binding with bacterial cells was strongly inhibited.

Table 4
Changes of RI units generated by sequential injection of a natural food additive, collagen I and the same food additive into the *S. enteritidis*-immobilized sensor surface.

Food additives	Changes of RI units ($\times 10^{-6}$) after sequential injection of		
	Additive	Collagen I	Additive
Control	—	817	—
0.1% Protamine	1510	–738	0
0.1% Anthocyanin	149	374	–113
0.1% Pectin hydrolysate	547	127	16
0.1% Japanese horseradish extract	158	–150	24
1.0% Malt extract	466	238	–13
1.0% Green tea extract	561	501	2300
1.0% Oolong tea extract	774	1160	1530
1.0% Tomato extract	–153	634	–36
1.0% Soybean germ extract	–32	365	–13
1.0% Nutmeg extract	–36	1174	–102
1.0% Parsley extract	17	521	–6
1.0% Chili extract	–27	427	–3
1.0% Ginger extract	116	465	—
1.0% Celery extract	–50	757	–8
1.0% Onion extract	–222	836	–8
1.0% Basil extract	–37	419	—
1.0% Blueberry extract	789	1001	1242
1.0% Blackberry extract	–4	991	–4
1.0% Kiwifruit extract	–31	714	–41
1.0% Grapefruit	–109	949	–165
1.0% Strawberry extract	1	775	–11
1.0% Peach extract	–185	489	–189
1.0% Mango extract	–3	206	–135
1.0% Lime extract	4	925	–107
1.0% Coffee extract	–43	492	—
1.0% Concentrated cranberry juice	1806	1515	1534
1.0% Concentrated tomato juice	239	228	45

—: not done

3.4.4 *Binding with S. enteritidis of collagen I in the presence of food additives*

Each of the food additives that had inhibitory effects on collagen binding with *S. enteritidis* in the previous experiments was mixed with collagen and the mixture was injected into the *S.enteritidis*-immobilized sensor. The results are listed in Table 6. Compared with the control experiment (collagen I without additives), the RI value decreased in the presence of hexametaphosphate, citrate, mannose, alginate, monoglycerol fatty acid esters, diglycerol monomyristate, Japanese horseradish extract, chili extract,

Table 5
Changes of RI units generated by sequential injection of a natural color, collagen I and the same color into the *S. enteritidis*-immobilized sensor surface.

Food additives	Changes of RI units ($\times 10^{-6}$) after sequential injection of		
	Additive	Collagen I	Additive
Control	—	817	—
1.0% San-brown (powder)	1909	1076	2037
1.0% San-brown KA	910	812	1704
1.0% San-brown AC	318	476	1753
1.0% San-beet L	–117	1239	120
1.0% San-red G	1194	1016	1131
1.0% San-red ELA	473	626	374
1.0% San-red No.5	538	769	499
1.0% San-red MR	93	179	408
1.0% San-red YM	45	22	66
1.0% San-red RCFU	235	327	122
1.0% San-yellow No. 2AU	12	126	84
1.0% San-yellow No. 3L	–128	–297	–114
1.0% Purple corn extract	351	110	298
1.0% Annatto AN	–458	–231	61
1.0% Rycopene base No.33644	215	465	79
1.0% Curcumin "San-Ei"	2435	–65	1323

—: not done

peach extract, concentrated tomato juice, San-red MR, San-red YM, San-yellow No. 3L, and Annatto AN.

Hexametaphosphate and citrate had a strong inhibitory effect on the binding of collagen I with *S. senteritidis* when added as a mixture with collagen (Table 6). These compounds did not bind with *S. enteritidis* cells (Table 3). Hexametaphosphate has a chelating effect and can release some outermembrane proteins, lipopolysaccharides and Mg^{2+} from *E. coli* cells.[16] Polyphosphates such as hexametaphosphate show strong antibacterial activity against gram-positive bacteria.[17] Citrate also exhibits a chelating activity and shows antibacterial activity against some bacteria such as *Campylobacter jejuni*.[18,19] It seems that the damage on the cell envelope caused by these compounds rarely affected the binding affinity of *S. enteritidis* with collagen I. Only in the presence of these compounds does the binding of collagen with *S.enteritidis* seem to be inhibited. Mannose also slightly inhibited the collagen binding with *S. enteritidis*. Buck *et al.*[20] reported that the adhesion of *S. enteritidis* to isthmal secretions was blocked by the addition of mannose and demonstrated the existence of mannosylated glycoproteins in the isthmus glandular cells. They also revealed that the binding was mediated by type I fimbriae.[20] It seems that in the binding interaction of collagen I with *S. enteritidis*, type I fimbriae is partly involved in the interaction.

Table 6
Changes of RI units generated by the injection of mixture of a food additive and collagen I into the *S. enteritidis*-immobilized sensor surface.

Food additives	Changes of RI units ($\times 10^{-6}$)
Control	925
0.1% Na hexametaphosphate	–429
0.1% Na citrate	286
0.1% Mannose	238
0.1% Na alginate	–125
0.1% Monoglycerol monocaprylate	–7
0.1% Monoglycerol monocaprate	–682
0.1% Monoglycerol monolaurate	–1064
0.1% Diglycerol monolaurate	719
0.1% Diglycerol monomyristate	–258
0.1% Sorbitan monocaprylate	654
0.1% Protamine	1383
0.1% Anthocyanin	160
0.1% Japanese horseradish extract	–715
0.1% Chili extract	–767
0.1% Peach extact	100
0.1% Mango extract	717
0.1% Concentrated tomato juice	49
0.1% San-red MR	–1113
0.1% San-red YM	–234
0.1% San-yellow No.2AU	457
0.1% Purple corn extract	760
0.1% San-yellow No.3L	–320
0.1% Annatto AN	–1199

In the presence of alginate, the collagen binding with *S. enteritidis* was also inhibited. The results were consistent with those of Medina's study on the binding of collagen I with *E. coli* O157:H7.[21]

Glycerol fatty acid esters showed unique behavior in the inhibition of collagen binding with *S. enteritidis*. The reinjection of the monogrycerol esters removed the bound collagen I and the activity increased with a decrease in the length of fatty acid bound to glycerol (Table 3). In contrast, when injected as a mixture with collagen I, the RI values decreased with an increase in the length of fatty acid (Table 6). Although the reason for the unique behavior is not clear, amphipatic compounds may have an inhibitory effect on the binding interaction of collagen with *Salmonella* as well as monoglycerol fatty acid esters and diglycerol monomyristate. The surface of collagen I molecules is relatively hydrophobic.[22] It seems that the hydrophobic region in the glycerol esters bound with collagen and covered the hydrophobic surface of collagen I. As a result of the hydrophobic interaction

of collagen and glycerol ester, it seems that the hydrophilic region of the ester molecules was distributed at the outermost part of the collagen-glycerol ester complex and decreased the binding interaction of collagen with *S. enteritidis*.

In our study, protamine largely increased the RI value in both experiments (Table 4 and 6). The protamine bound with *S. enteritidis* was partially removed by the injection of collagen (Table 4). Protamine is one of the basic proteins. It shows antibacterial activity against gram-negative bacteria and binds with the bacterial cell surface through electrostatic interaction.[23] Our result was supported by the facts reported by Hansen and Gill.[23]

Japanese horseradish extract, chili extract, peach extract, concentrated tomato juice, San-red MR, San-red YM, San-yellow No. 3L, and Annatto AN decreased the RI value in both the experiments, that is sequential injection and injection of the mixture with collagen (Tables 4, 5 and 6). These extracts and colors contain various compounds, and in particular, each of the natural colors used in this study contains specific colored compounds such as carotenoides, anthocyanins, and flavonoids. The main bodies of the inhibitors included in the extracts and natural color products will be identified and reported in the near future. These food additives and extracts will be used in the studies on detachment and inhibition of reattachment of *Salmonella* on beef, pork and poultry.

This paper indicates that an SPR biosensor is highly effective for the screening of substances having interaction with microorganisms. The results obtained through this study will contribute to the development of the processing method to control the contamination of pathogenic bacteria on meats.

References

1 Inspection and safety division, Department of Food sanitation, Ministry of Health, Labor and Welfare: Food Sanitation Research **52** (2002) 118.
2 F. Bryan: J. Food Protection **43** (1980) 859.
3 F. Bryan: J. Food Protection **44** (1981) 394.
4 M. Medina and P. Fratamico: Biotechnology Techniques **12** (1998) 235.
5 P. Fratamico, F. Schultz, R. Benedict, R. Buchanan and P. Cooke: J. Food Protection **59** (1996) 453.
6 M. Medina: Food testing & analysis **3** (1997) 14.
7 J. Patti, K. House-Pompeo, J. Boles, N. Garza, S. Gurusiddapp and M. Hook: J. Biol. Chem. **270** (1995) 12005.
8 M. Medina: SPIE **3544** (1998) 97.
9 M. Kukkonen, T. Raunio, R. Virkola, K. Lahteenmaki, PH. Makela, P. Klemm, S. Clegg and T. Korhonen: Molecular Microbiol. **7** (1993) 229.
10 E. Trafny, M. Stepinska, M. Antos and J. Grzybowski: Antimicrobe agents Chemother. **39** (1995) 2645.
11 B. Westerlund and T. Korhonen: Mol. Microbiol. **9** (1993) 687.
12 J. Patti, J. Boles and M. Hook: Biochemistry **32** (1993) 11428.
13 J. Patti, K. Hopuse-Pompeo, J. Boles, N. Garza, S. Gurusiddappa and M. Hook: J. Biol. Chem. **270** (1995) 12005.
14 I. Walls, P. Cooke, R. Benedict and R. Buchanan: Food Microbiol. **10** (1993) 387.
15 E. Gonzalez, S. Baloda, J. Blanco and T. Wadstrom: Medical Microbiology, Infectious Diseases, Virology, Parasitology **269** (1988) 437.

16 T. Miyamoto, K. Ohyama, M. Yoshimoto and S. Hatano: Agric. Biol. Chem. **53** (1988) 655.
17 K. Isshiki, M. Tsutsumi, K. Nishimura and T. Watabnabe: J. Food Hygienics Society of Japan **18** (1977) 335.
18 M. Nose, I. Hirata, T. Arai, K. Ohta and S. Sakai: J. Food Hygienics Society of Japan **29** (1988) 38.
19 K, Imai, and T. Iijima: J. Appl. Microbiol. **17** (1971) 457.
20 J. Buck, F. Immerseel, G. Meulemans, F. Haesebrouck and R.Ducatelle: Vet. Microbiol. **93** (2003) 223.
21 M. Medina: Int. J. Food Micribiol. **69** (2001) 199.
22 P. Bornstein and W. Traub: The Proteins Vol. IV, ed. H. Neurath and R. Hill (Academic Press, New York, 1979) p. 411.
23 L. Hansen and T. Gill: J. Appl. Microbiol. **88** (2000) 1049.