Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion

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The autoxidation of soybean oil in a cyclodextrin emulsion system was studied in the presence of an emulsion stabilizer consisting of polysaccharides such as xanthan, tragacanth gum, and methylcellulose. Xanthan strongly inhibited the peroxidation of soybean oil containing tocopherols but showed no antioxidant activity on soybean oil without tocopherols in the emulsion. Xanthan did not have hydrogen-donating ability but expressed Fe^{2+}-binding activity. The Fe^{2+}-binding activity corresponded to the pyruvate content of xanthan. Depyruvated xanthan did not inhibit effectively the autoxidation of soybean oil. The Fe^{2+}-chelating structure of xanthan is discussed.

INTRODUCTION

The autoxidation of fats and oils in food causes deterioration of flavor and taste. Sugars such as pentose, hexose, and reducing disaccharide are strong prooxidants of methyl linoleate and linoleic acid in aqueous emulsion systems (Mabrouk and Dugan, 1961; Mabrouk, 1964; Yamaguchi and Yamada, 1981). Yamauchi et al. (1984) found that reducing sugars reduce transition-metal ions in an aqueous emulsion, and the resulting reduced metal ions accelerate lipid peroxidation. Sugar alcohols increase the stability of safflower oil (Sims et al., 1979; Yamauchi et al., 1982). The effects of mono- or disaccharides on lipid peroxidation in emulsions have been investigated extensively, but little is known of the effects of polysaccharides used widely as emulsion stabilizers in foods.

Recently, the emulsifying properties of cyclodextrins (CDs) have been used to improve food processing (emulsifier) in mayonnaise, dressing, whipping cream, and others (Okada, 1984). α- and β-CDs show excellent emulsifying properties in oil-in-water emulsion, and polysaccharides such as tragacanth gum and xanthan enhance the stability of CD–soybean oil emulsions (Shimada et al., 1991). Xanthan contributes to the stability of a soybean oil emulsion using egg yolk as the emulsifer (Hennock et al., 1984). To enhance stability, various polysaccharide stabilizers such as xanthan are used for products such as mayonnaise and salad dressings.

In this study, examination was made of the effects of polysaccharides (xanthan, tragacanth gum, methylcellulose) on lipid peroxidation in CD–soybean oil emulsion. Xanthan of the polysaccharides inhibits the peroxidation of soybean oil in a CD emulsion. Elucidation of the mechanism for this was attempted here.

MATERIALS AND METHODS

Materials. β-CD was provided by Nihon Shokuhin Kako Co. (Tokyo, Japan). Sucrose fatty acid ester (DK ester F-140; HLB = 13) was donated by Dai-ichi Kogyo Seiyaku Co. (Kyoto, Japan). Soybean oil was from Honen Corp. (Tokyo, Japan). Xanthan 1, methylcellulose 1 (350–550 cps; viscosity at 2% concentration in water, at 20 °C), and methylcellulose 2 (7000–10 000 cps; viscosity at 2% concentration in water, at 20 °C) were products from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Tragacanth gum was purchased from Nacalai Tesque (Kyoto, Japan). Xanthans 2–5, present on the market as emulsion stabilizers of foods, were obtained from Taiyo Kagaku Co. (2 and 3) (Mie, Japan) and Dainihon Seiyaku Co. (4 and 5) (Osaka, Japan). All other chemicals were of analytical grade.

Preparation of Soybean Oil without Tocopherols. Soybean oil without tocopherols was prepared by activated alumina column chromatography with an elution of a petroleum ether–acetone (95:5 v/v) mixture. Tocopherols were determined by high-performance liquid chromatography (HPLC), using 2,2,5,7,8-pentamethyl-6-hydroxychroman as the internal standard (Shimada et al., 1990).

Determination of the Pyruvate Group and Preparation of Depyruvated Xanthan. Pyruvate group content in xanthan was measured using the 2,4-dinitrophenyl derivative (Duggan, 1969). Depyruvation of xanthan 1 was conducted by oxalic acid treatment (Holzwarth and Ogletree, 1979). Pyruvate groups were removed by heating a solution of xanthan (2 g/L in 1 mM oxalic acid, 0.1 M NaCl, pH 3.5) for 2 h at 95 °C, resulting in elimination by about 80%.

Autoxidation of Soybean Oil in Emulsion. A mixture of 0.8 mL of soybean oil and 3.2 mL of distilled water containing 1.5% emulsifier (β-CD or sucrose fatty acid ester) and 0.5% emulsion stabilizer (xanthan, tragacanth gum, or methylcellulose), unless otherwise noted, was homogenized at 22 000 rpm for 2 min at 20 °C with a laboratory disperser (Ystral-Mitamura Riken Kogyo). The effects of pH were examined in 50 mM sodium phosphate buffer (pH 7.0) or 50 mM sodium acetate buffer (pH 4.0) instead of distilled water. The emulsion was autoxidized at 37 °C for 50 days. Incubation of the emulsion containing soybean oil without tocopherols was carried out at 37 °C for 47 h. At intervals, peroxide value (POV) was determined directly for each 100 μL of emulsion according to the iodometric method (Yamauchi et al., 1984). Autoxidized soybean oil POV determined by the AOCS (1964) method was used to prepare a standard curve. POV was expressed in milliequivalents per kilogram of oil. Thiobarbituric acid (TBA) value was measured according to the method of Asakawa et al. (1975), adding 0.1 mL of 10 mM butylated hydroxytoluene to the reaction mixture. TBA value was calculated from the standard curve obtained using 1,1,3,3-tetraethoxypropane and expressed as millimoles of malondialdehyde per kilogram of oil.

Measurement of Hydrogen-Donating Activity and Fe^{2+}-Binding Activity. Hydrogen-donating activity was estimated according to the method of Kirigaya et al. (1971). After 5 mL of sample solution was added to 5 mL of 0.008% 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 50% ethanol, decolorization of DPPH donated H^+ was followed by measuring absorbance at 528 nm.

Fe^{2+}-binding activity was measured according to the method of Asakura et al. (1990) with minor modification. Polysaccharide was dissolved in 10 mM hexamine buffer containing 10 mM

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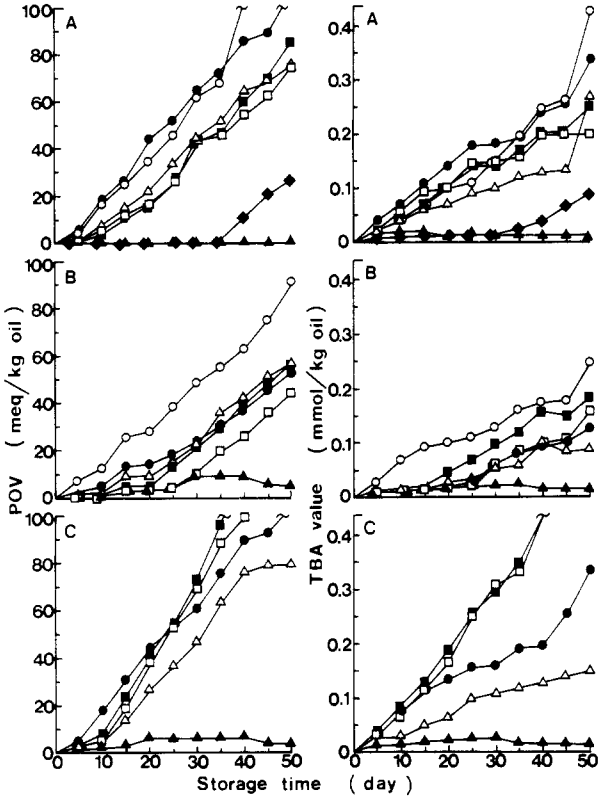

Figure 1. Effects of emulsifiers and emulsion stabilizers on the autoxidation of soybean oil in the aqueous emulsion system. (A) Distilled water; (B) 50 mM sodium phosphate buffer at pH 7.0; (C) 50 mM sodium acetate buffer at pH 4.0. (●) CD; (○) sucrose fatty acid ester; (▲) CD + xanthan 1; (△) CD + tragacanth gum; (■) CD + methylcellulose 1; (□) CD + methylcellulose 2; (♦) CD + EDTA-Na_2.

KCl (pH 5.0). Two milliliters of polysaccharide solution was added to 2 mL of 10 mM hexamine buffer containing 10 mM KCl and 3 mM $FeSO_4$, and then 0.2 mL of 1 mM tetramethyl murexide (TMM) was added. Absorbance at 485 and 530 nm was measured. TMM was a chelating reagent, showing an absorption maximum at 530 nm, and formed a complex with free Fe^{2+} except Fe^{2+} bound by polysaccharide. The TMM-Fe^{2+} complex showed an absorption maximum at 485 nm. When 0.2 mL of 1 mM TMM was added to 4 mL of 0.3–2 mM $FeSO_4$ solution for the standard curve, the absorbance ratio (A_{485}/A_{350}) increased linearly with Fe^{2+} (TMM-Fe^{2+} complex) concentration. Fe^{2+}-binding activity was determined from the decrease in absorbance ratio in the presence of polysaccharide and expressed as millimolal of bound Fe^{2+}. The measurement was carried out at 20 °C to prevent Fe^{2+} oxidation.

RESULTS AND DISCUSSION

Effects of Emulsifiers and Emulsion Stabilizers on the Autoxidation of Soybean Oil in the Aqueous Emulsion System. Without an emulsion stabilizer, the effects of emulsifiers on the autoxidation of soybean oil were investigated by measuring POV and TBA value. The autoxidation of soybean oil in β-CD emulsion was found to be inhibited slightly more than that in a sucrose fatty acid ester emulsion after 40 days of incubation, using distilled water as the water phase (Figure 1A) and throughout 50 days in phosphate buffer at pH 7.0 (Figure 1B). The sucrose fatty acid ester could not be used as an emulsifier in acetate buffer at pH 4.0 since it could not be dissolved in acidic solution. The emulsions prepared by β-CD and sucrose fatty acid ester in distilled water were pH 5.0 and 7.7, respectively. Lipid peroxidation is accelerated at acidic pH by metal ions such as Fe^{2+} (O'Brien, 1969; Yamauchi et al., 1988). The autoxidation

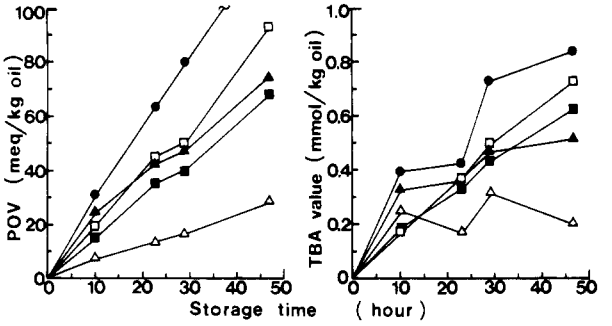

Figure 2. Effects of emulsion stabilizers on the autoxidation of soybean oil without tocopherols in the aqueous emulsion system (50 mM sodium phosphate buffer at pH 7.0 as water phase). The symbols are the same as in Figure 1.

of soybean oil was inhibited by 1 mM disodium ethylenediaminetetraacetate (EDTA-Na_2) in β-CD emulsion without polysaccharide (Figure 1A). Trace metal ions contaminating the emulsion system are thus shown to be involved in the oil peroxidation. However, the metal ions in this emulsion system were not measured. An emulsion containing β-CD in distilled water may not show antioxidative behavior at the initial stage of incubation because of more acidic pH than that of an emulsion containing the sucrose fatty acid ester. The reason for the slower autoxidation rate of β-CD emulsion is unclear.

The effects of polysaccharides added as stabilizer of β-CD emulsion on the oil autoxidation are shown in Figure 1. When xanthan 1 was added to the water phase (distilled water or buffers at pH 7.0 and 4.0) in the β-CD emulsion, oil autoxidation was strongly suppressed. The autoxidation of soybean oil was not inhibited by the addition of other polysaccharides, such as tragacanth gum, methylcellulose 1, and methylcellulose 2. The viscosity of the water phase in emulsion was independent of oxidation rate as indicated by the results from methylcellulose 1 and 2. Antioxidative activity of xanthan 1 could not be determined for an emulsion using the sucrose fatty acid ester because of unstable emulsion formation.

Effects of Emulsion Stabilizers on the Autoxidation of Soybean Oil without Tocopherols in the Aqueous Emulsion System. α-, β-, γ-, and δ-tocopherols in soybean oil were present at 18.6 ± 0.4, 2.5 ± 1.2, 80.1 ± 0.3, and 17.6 ± 0.6 (mean ± SD, n = 3) mg/100 g of oil, respectively, as measured by HPLC. Soybean oil without tocopherols was prepared by alumina column chromatography. The effects of emulsion stabilizers on the autoxidation of soybean oil without tocopherols were investigated for aqueous emulsion (Figure 2). The autoxidation of soybean oil without tocopherols was inhibited by emulsion stabilizers, particularly tragacanth gum. The antioxidative activity of xanthan 1 was not high in the absence of tocopherols, indicating xanthan may possibly function as a synergist in the presence of tocopherols under the experimental conditions in Figure 1. The following two mechanisms may be proposed for the synergism: hydrogen-donating ability for regenerating the active form of tocopherol and metal chelation. Synergism is apparent when a phenolic antioxidant such as tocopherol is used together with a metal inactivator.

Measurement of Hydrogen-Donating Activity. Figure 3 shows the hydrogen-donating activity of ascorbic acid and polysaccharides. A reductone such as ascorbic acid mixed with DPPH decolorized DPPH due to its hydrogen-donating ability at a very rapid rate. Xanthan 1, methylcellulose 1, and methylcellulose 2 showed hardly any hydrogen-donating activity. Tragacanth gum had low

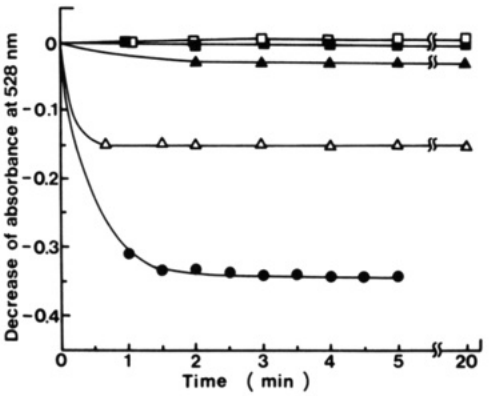

Figure 3. Hydrogen-donating activities of ascorbic acid (●), xanthan 1 (▲), tragacanth gum (△), methylcellulose 1 (■), and methylcellulose 2 (□). Final concentrations were 20 mM for ascorbic acid and 0.1% for polysaccharides.

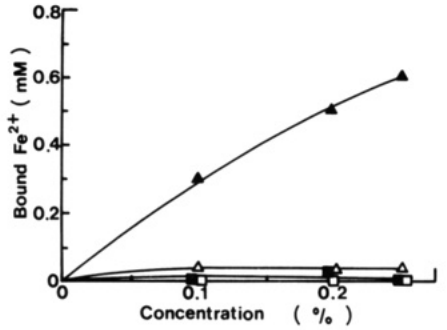

Figure 4. Fe^{2+}-binding activities of xanthan 1 (▲), tragacanth gum (△), methylcellulose 1 (■), and methylcellulose 2 (□).

hydrogen-donating activity. This may possibly be the cause for suppression of autoxidation in oil without tocopherols.

Relationship between Fe^{2+}-Binding Activity and Pyruvate Content. Fe^{2+} ion is the most powerful prooxidant among various species of metal ions (O'Brien, 1969; Halliwell and Gutteridge, 1984; Yamauchi et al., 1988). Thus, Fe^{2+}-binding activity of polysaccharide was determined. Measurement of Fe^{2+}-binding activity was done for polysaccharide solutions ranging in final concentration from 0.1 to 0.25% (Figure 4). Fe^{2+}-binding ability was observed for xanthan 1 and increased with the concentration of xanthan 1. Other polysaccharides such as tragacanth gum, methylcellulose 1, and methylcellulose 2 showed hardly any Fe^{2+}-binding activity. Tragacanth gum, an acidic polysaccharide, does not have Fe^{2+}-binding ability, and thus the high activity of xanthan 1 may be correlated to the unique structure of xanthan in addition to its negative charge.

The primary structure of xanthan produced by *Xanthomonas campestris* consists of a cellulose-like β-1,4 glucan with (4,6-acetal-linked pyruvate)-β-D-Man*p*(1–4)-β-D-GlcA*p*(1–2)-α-D-Man*p*-6-OAc side chain at the O-3 position of alternate glucosyl residues (Jansson et al., 1975). Internal mannose of the side chain is substituted at O-6 with an acetyl group. The terminal mannose of the side chain is substituted with a pyruvate ketal, but the degree of substitution is not stoichiometric. About half or one-third of the side chains possess pyruvate substituents, depending on the culture conditions (Cadmus et al., 1978).

To investigate the relationship between Fe^{2+}-binding ability and the structure of xanthan, pyruvate content and Fe^{2+}-binding activity were measured for xanthan 1, xanthans 2–5 that are present on the market as food additives, and depyruvated xanthan 1 (Table I). An

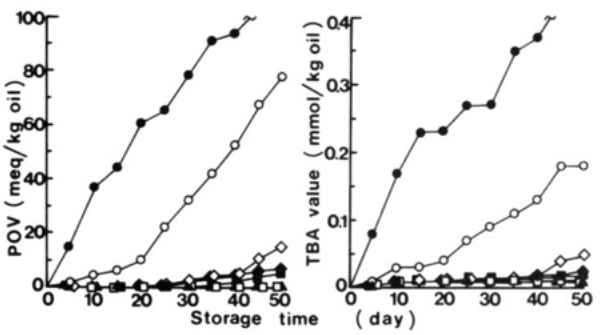

Figure 5. Effects of xanthans differing in pyruvate content on the autoxidation of soybean oil in the aqueous emulsion system (distilled water as the water phase). (●) CD; (○) CD + depyruvated xanthan 1; (▲) CD + xanthan 1; (■) CD + xanthan 2; (□) CD + xanthan 3; (♦) CD + xanthan 4; (◇) CD + xanthan 5.

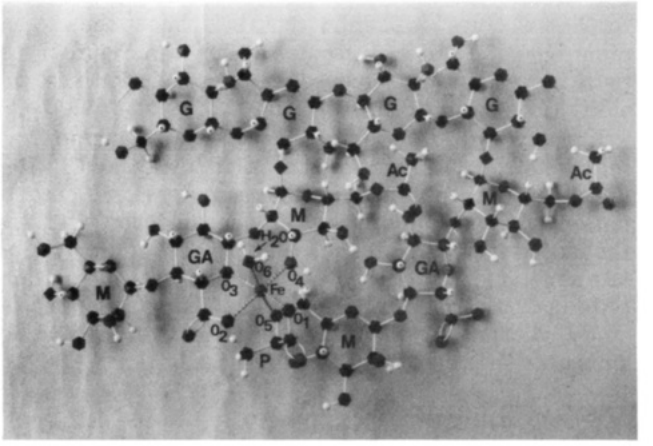

Figure 6. Proposed structure of the xanthan–Fe^{2+} complex. (G) D-Glucose; (M) D-mannose; (GA) D-glucuronic acid; (Ac) *O*-acetyl; (P) acetal-linked pyruvate; (Fe) ferrous ion; (H_2O) water molecule; (O_1) charged oxygen atom of pyruvate residue; (O_2) carboxyl oxygen of D-glucuronic acid; (O_3) hemiacetal oxygen of D-glucuronic acid; (O_4) oxygen atom of OH-3 of internal D-mannose; (O_5) oxygen of pyruvate; (O_6) oxygen of water molecule. Striped bond shows a coordinate bond.

increase in Fe^{2+}-binding activity corresponded to that of pyruvate content. The effects of these xanthans on the autoxidation of soybean oil in the emulsion system were investigated (Figure 5). Depyruvated xanthan 1 did not inhibit effectively autoxidation compared to xanthans containing high pyruvate group content. Pyruvate residues in xanthan are thus shown to possibly be related to antioxidation by metal ion chelation. Although the autoxidation of soybean oil in an emulsion was investigated after 20 μM $FeSO_4$ was added, the rate was not accelerated even without xanthan (data not shown here). The added Fe^{2+} ions may likely have been oxidized rapidly, so that resulting Fe^{3+} ions became insoluble and unable to contribute to oil peroxidation. However, the synergistic activity of xanthan at known Fe^{2+} concentrations in different experimental systems remains to be determined. Further, the investigation for the effects of xanthan concentration on autoxidation is in progress in our laboratory.

Figure 6 shows the structure of the xanthan–Fe^{2+} complex assembled with a molecular structure model. The coordination number of the Fe^{2+} ion is normally six, and its ionic radius is 0.75 (low spin) or 0.92 Å (high spin). The ionic and van der Waals radii of an oxygen atom are 1.4 and 1.5 Å, respectively (Huheey, 1978). When a constituent oxygen atom in xanthan is linked by a coordinate bond to an Fe^{2+} ion, the interatomic distance may approximate 2.15–2.42 Å from the sum of both atomic

Table I. Fe^{2+}-Binding Activity and Pyruvate Content of Xanthan

	xanthan					
	1	2	3	4	5	depyruvated
bound Fe^{2+},[a] mM	0.60[b]	0.68	0.68	0.62	0.58	0.32
pyruvate content, %	4.52	5.46	5.33	4.92	3.64	0.96

[a] Fe^{2+}-binding activity at 0.25% xanthan. [b] Data shown in Figure 4.

radii. The charged oxygen atom (O_1 in Figure 6) of pyruvate residue is located near the carboxyl oxygen (O_2) of D-glucuronic acid in an adjacent side chain. The two charged oxygen atoms may chelate an Fe^{2+} ion in combination with a hemiacetal oxygen atom (O_3) of the D-glucuronic acid residue, an oxygen atom (O_4) of HO-3 of internal D-mannose residue, and an acetal oxygen atom (O_5) of the pyruvate residue, and the sixth bond of Fe^{2+} for the six-coordinate complex may be linked to the oxygen atom (O_6) of a water molecule as shown in Figure 6. If pyruvate residues are removed, Fe^{2+} ion chelation between side chains may become difficult.

In conclusion, xanthan used as an emulsion stabilizer inhibits strongly the autoxidation of soybean oil in the β-CD emulsion system. Xanthan suppresses oil peroxidation synergistically in the presence of tocopherols by the inactivation of metal ions such as Fe^{2+} and may chelate a metal ion between two side chains with a pyruvate residue. Although β-CD was used as an emulsifier in this study, xanthan may likely have an antioxidative effect on emulsions prepared in other emulsifiers.

ACKNOWLEDGMENT

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Registry No. Fe, 7439-89-6; xanthan gum, 11138-66-2; tragacanth gum, 9000-65-1; methylcellulose, 9004-67-5; β-cyclodextrin, 7585-39-9; pyruvic acid, 127-17-3.