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Improvement of the Functional Properties of Sucrose Stearate by Phosphorylation

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Phosphorylated sucrose stearate (SE-P) was prepared by dry-heating sucrose stearate (SE) with metaphosphoric acid. The main product was deduced to be a monophosphosucrose monostearate by chemical analysis and mass spectrometry. SE-P exhibited remarkably higher solubility and emulsifying properties than SE, especially in the acidic region and in the presence of NaCl, and SE-P bound Ca^{2+} at a 1:1 molar ratio (SE-P/Ca^{2+}). SE-P markedly reduced the viscosity of potato starch paste and inhibited retrogradation, whereas SE did not reduce it so much. It is thus expected that phosphorylation would be an appropriate method for improving the functional properties of SE and that SE-P could be used as a novel emulsifier and modifier with Ca^{2+}-binding ability for starchy foods.

KEYWORDS: Sucrose fatty acid ester; phosphorylation; solubility; emulsifying property; Ca-binding ability; starch

INTRODUCTION

The stabilization of lipids in foods is a large problem, because most foods contain oil and fat as important constituents. This problem has encouraged the development of various emulsifiers for food use. One of these is sucrose fatty acid ester (SE) (*1*, *2*), a nonionic emulsifier that has become widely used as an effective emulsifier for foods because of its safety. Various kinds of SE with a wide range of hydrophile−lypophile balance are commercially available to handle many requirements such as O/W or W/O type of emulsification, solubilization, foaming ability, modification of the physical properties of starch, and microbe regulation in the food, medical, and cosmetic industries.

However, SE has some major shortcomings such as its low solubility, which inhibits its emulsifying properties, particularly in the acidic region and in the presence of salt (*1*). To overcome these disadvantages, suitable modification of SE to increase its hydrophilicity is thought to be effective. For example, endowing acidic functional groups with low pK_a is considered to be suitable because it could prevent the decreased solubility under such conditions due to high hydrophilicity and ion-exchange ability. Because the phosphoryl group would be suitable for this purpose, phosphorylation of SE is likely to be effective for improving the solubility and emulsifying properties. Buchanan et al. (*3*) were first to report the phosphorylation of sucrose. However, their method was complicated with many reaction steps. Kim and Behrman (*4*) have reported the synthesis of sucrose 6′-phosphate in only two steps, but the phosphorylating reagent used selectively phosphorylated the primary hydroxyl. In the case of SE, the primary hydoxyl at the 6- or 6′-position of SE could probably not be phosphorylated, because it is lost by fatty acylation. Phosphorylation without position specificity is thus considered to be superior. In this respect, because dry-heating saccharides and proteins with phosphates as a phosphorylating reagent could phosphorylate the primary and secondary hydroxyls of these substances (*5*), the dry-heating method is thought to be advantageous as a mild procedure suitable for food use.

Considerable attention has recently been focused on the bioavailability of dietary calcium, because many processed foods contain a calcium-absorption inhibitor such as phosphate, phytate, or oxalate. Calcium needs to be soluble in the intestines for it to be absorbed by the body. These inhibitiors form an insoluble complex with calcium, resulting in reduced calcium absorption. The solubility of calcium can be controlled by various substances such as alginic acid (*6*), poly-L-glutamic acid (*7*), casein phosphopeptide (*8*), phosphorylated oligosaccharides (*9*, *10*), and citric acid (*11*) that form a soluble complex. However, SE has no ability to form a soluble complex with calcium ion, so it might be useful for SE to be endowed with acidic groups that would form such a soluble complex. Phosphorylating SE might therefore be effective for improving the Ca^{2+}-binding property.

It was well-known that emulsifiers having an alkyl chain such as SE, monoacylglycerol, and related lipids form a complex with starch and affect such thermal behavior as gelatinization

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and retrogradation (*12*−*15*). These emulsifiers are frequently used in making breads, cakes, and noodles as a modifier with an antistaling effect to extend the shelf life by delaying the retrogradation of starch (*1*, *16*, *17*). However, their effects on the gelatinization behavior appearing as a viscosity change varied according to the type of emulsifier and lipid, source of starch, and experimental conditions (*18*, *19*). These varying results are the cause of difficulties in applications to regulate gelatinization. It is thus desirable to develop a potent new modifier with a specific regulatory effect. Compared to nonionic emulsifiers such as monoacylglycerol and related compounds, ionic emulsifiers have an additional effect due to the nature of their charge (*20*). This indicates that if SE as a nonionic emulsifier could be converted into an ionic emulsifier, it would have powerful effect on the gelatinization behavior. Phosphorylated SE might be expected to enhance the regulatory effect on gelatinization such as viscosity of starch paste in comparison with native SE, because the phosphate could have an effect on the thermal behavior of starch, probably due to ionic interaction (*21*−*24*).

In the present study, sucrose monostearate (a typical commercial SE) was phosphorylated with metaphosophric acid by dry-heating to improve its solubility, emulsifying properties, Ca^{2+}-binding property, and regulation on the gelatinization and retrogradation of starch.

MATERIALS AND METHODS

Materials. SE (S-1670) containing stearic acid as the main fatty acid was from Mitsubishi Chemical Co. (Tokyo, Japan). High-performance thin-layer chromatography (HPTLC) showed that the sucrose monostearate content of SE was ~70%. Metaphosphoric acid (MPA) was purchased from Wako Pure Chemical Industry (Osaka, Japan). Potato starch (PS; Hokuren Research Institute, Sapporo, Japan) was used after being repeatedly washed with distilled water at 4 °C and air-dried (17% moisture on a dry basis).

Preparation of Phosphorylated SE (SE-P). Phosphorylation of SE was carried out by dry-heating with MPA according to the method of Tarelli and Wheeler (*5*). SE (12.1 g) was dispersed in 200 mL of distilled water at 60 °C, and MPA solution (40.1 g/ 100 mL, adjusted to pH 5.5 with 5M NaOH) was added (SE/MPA = 1:10, molar ratio). After the pH had been readjusted to 5.5 with 0.1 M NaOH, the reaction mixture was lyophilized and then heated at 110 °C for 20 h to phosphorylate. The reaction product was washed with 200 mL of chilled water by centrifugation at 18000 rpm for 30 min at 4 °C to eliminate the unreacted MPA, and the resulting residue was lyophilized. The lyophilized sample was dissolved in 200 mL of chloroform/methanol (8:2, v/v) with stirring for 30 min to eliminate the residual MPA. After filtration, the filtrate was evaporated under a nitrogen gas stream to recover the crude SE-P. Silica gel chromatography was carried out with an Iatrobeads RS-8060 column (22 i.d. × 200 mm, Iatron Laboratories, Tokyo, Japan). Crude SE-P was applied to the column, which had been equilibrated with chloroform/methanol (8:2, v/v). After sucrose polystearates above the diester had been eliminated by elution with 400 mL of the same solvent, SE-P and the sucrose monoester were eluted with 80 mL of methanol, and the eluate was evaporated under reduced pressure before being dialyzed against distilled water and lyophilized. SE-P was isolated by anion-exchange chromatography with DEAE-Sepharose Fast Flow (Amersham Bioscience, Tokyo, Japan). The sample (2 g) was dissolved in 200 mL of distilled water (pH 7.0 with 0.1 M NaOH) at 60 °C while being stirred. DEAE-Sepharose Fast Flow (200 mL) regenerated with 0.1 M NaOH and thoroughly washed with distilled water (pH 7.0) was added to the sample solution and packed in a column (24 i.d. × 400 mm). The adsorbed fraction was eluted with 0.5 M KCl at a flow rate of 5 mL/min. The eluate was lyophilized and then washed with chilled water by centrifugation at 18000 rpm and 4 °C for 30 min four times. The resulting precipitate was lyophilized to obtain SE-P.

HPTLC. HPTLC was performed with silica gel 60 HPTLC sheets (Merck, Darmstadt, Germany). The sample (5 μL) dissolved in chloroform/methanol (8:2, v/v) or distilled water at 1% was applied and respectively developed with mobile phase A [chloroform/methanol/55% $CaCl_2$ (5:4:1, v/v/v)] or mobile phase B [chloroform/methanol/acetic acid/water (80:10:8:2, v/v/v/v)] to separate SE-P or SE. After development, phosphate was detected as a quenching spot with the aluminum−morin reagent (*25*) prior to detection of saccharides with the aniline−diphenylamine reagent (*26*).

Mass Spectra Measurement. The mass spectrum was recorded by an SX-102A instrument (JEOL, Tokyo, Japan), using FAB ionization. The sample was dissolved in distilled water at a concentration of 1%, and diethanolamine was used as a matrix.

Measurement of the Solubility. SE-P and SE (10 or 20 mg) were homogenized in 1 mL of distilled water with a Polytron PTA-7 homogenizer (Kinematica, Switzerland) at 24000 rpm for 1 min at 0 or 30 °C and then respectively dissolved at 0 or 30 °C for 1 h while stirring at 500 rpm. Each dispersion was centrifuged at 18000 rpm for 20 min to obtain the supernatant. After the supernatant had been filtered with a 0.45 μm membrane filter (Amersham Bioscience, Tokyo, Japan), the solubility was evaluated by determining the saccharide concentration of the filtrate according to the phenol−sulfuric acid method (*27*).

Measurement of the Emulsifying Property. SE-P and SE were dissolved in a 0.02 M citrate−0.01 M phosphate buffer (pH 4, 5, 6, 7, or 8) or in the same buffer containing NaCl (0.5 or 1.0%) to give a concentration of 0.4 mg/mL. The sample solution (2 mL) and 0.5 mL of corn oil in a test tube (18 i.d. × 85 mm) were homogenized by a Polytron PTA-7 homogenizer (Kinematica) at 24000 rpm for 1 min. The emulsion was diluted 50-fold with a 0.1% SDS solution 0, 10, 30, 60, and 120 min after emulsification, and the absorbance at 500 nm was measured according to the method previously described (*28*). The emulsifying activity index (EAI) was calculated by using the equations (*29*)

$$\mathrm{EAI} = 2T/\phi C \quad \text{and} \quad T = 2.3A/L$$

where A is the absorbance at 500 nm, L (light path) is 10^{-2} m, C is the concentration of a sample (4×10^2 g/m^3), and ϕ (oil phase volume) is 0.2.

Conductometric Titration. A conductometric titration was carried out to measure the calcium ions bound with SE-P. $CaCl_2$ (12.5 mM) was added to 16 mL of an SE-P or SE solution (0.5 mg/ mL) with small increments (50 μL at a time), and the conductivity was measured with an ES-12 conductometer (Horiba, Kyoto, Japan) at 30 °C. The end point for conductometric titration was determined as the intersection of the linear regression curves obtained from the initial titration and from the ultimate titration.

DSC. The SE-P or SE solution was added to 5 or 10 mg of potato starch (PS) in an anodized aluminum or silver capsule to give 5 w/w% of SE-P or SE on the basis of the dry PS weight and 15 or 30 mg of total weight, respectively. DSC was performed with a Seiko SSC-5020 DSC 6100 instrument (Seiko, Chiba, Japan) at a heating rate of 2 K/min under a helium gas stream (40 mL/min) as previously described (*30*). Distilled water was used as a reference. After DSC, the sample was preserved at 4 °C for 1 week to retrograde, and the DSC procedure was repeated.

Measurement of the Pasting Behavior. The viscosity of potato starch containing SE-P and SE was measured by a rapid RVA Super3 Viscoanalyzer (Newport Scietific Pty., NSW, Australia). The SE-P or SE solution was added to 2 g of PS in an aluminum sample container to give 0.2 or 0.5 w/w% of SE-P or SE on the basis of the dry PS weight. The suspension was held at 50 °C for 1 min and then heated to 95 °C within 8 min. After a 2 min hold at 95 °C, the suspension was cooled to 50 °C within 8 min. The peak viscosity, breakdown, final viscosity, and peak temperature were each recorded. Because three measurements for PS showed a high degree of reproducibility, the coefficient of variance of the peak viscosity being evaluated as only 0.19% (159.2 ± 0.3, mean ± SD), each measurement was taken only once. After the RVA measurement, the paste was preserved at 4 °C for 1 week before being dehydrated with ethanol to obtain a sample for analyzing the X-ray diffraction and degree of gelatinization.

X-ray Diffractometry. X-ray diffractometry of the retrograded starch samples after RVA was carried out by a Rint 2100 Ultima$^+$/PC X-ray diffractometer (Rigaku Co., Tokyo, Japan) with a copper target at 50 kV and 40 mA producing Cu Kα of 1.54 Å according to the method previously described (*31*).

Measurement of the Degree of Gelatinization. The degree of gelatinization of the retrograded starch after RVA was measured by using the BAP method (*32*). Starch sample (80 mg) was dispersed in 8 mL of distilled water with a Polytron PTA-7 homogenizer (Kinematica) at 24000 rpm for 1 min, and 2 mL of the dispersion was diluted to 25 mL with an 0.8 M acetate buffer (pH 6.0) for use an examination solution. Another 2 mL of the dispersion was completely gelatinized with 0.2 mL of 10 M NaOH, heated at 50 °C for 3 min, and then neutralized with 1 mL of 2 M acetic acid, prior to dilution to 25 mL with the same buffer. The enzyme solution (1 mL) was added to 4 mL of the examination or gelatinized solution and reacted at 40 °C for 30 min. After heating in boiling water for 5 min, the reaction product was diluted 5-fold, and the reducing sugar and total sugar were respectively determined by the Somogyi (*33*)−Nelson (*34*) method and the phenol−sulfuric acid method (*27*). A blank was prepared by adding 1 mL of the enzyme solution that had previously been heated in boiling water for 10 min. Pullulanase (170 mg, 2 units/mg; Hayashibara Biochemicals Laboratories Inc., Tokyo, Japan) and 9.59 mg of β-amylase (8.34 units/mg; Nagase Biochemicals, Tokyo, Japan) were dissolved in the 0.8 M acetate buffer (pH 6.0), diluted to 100 mL, and filtered to use the enzyme solution. The degree of gelatinization was calculated by using the equation

$$\text{degree of gelatinization (\%)} = \{(A - a)/B\}/\{(A' - a)/B'\}$$

where A, B, and a are the respective reducing sugar contents of the examination sample, the gelatinized sample, and the blank and A' and B' are the total sugar contents of the examination sample and gelatinized sample.

Chemical Analysis. The fatty acid content was determined by gas−liquid chromatography (GLC) with a GC 4CM apparatus (Shimadzu, Kyoto, Japan) and a DEGS Chromosorb WAW column (GL Science, Tokyo, Japan) after fatty acid methyl esters had been prepared from SE-P by methanolysis according to the method previously described (*35*). The phosphoric acid and saccharide contents of SE-P were respectively determined by wet-ashing with sulfuric acid and perchloric acid and coloring with a commercial Phospher-C test kit (Wako Pure Chemical Ind., Osaka, Japan) and by the phenol−sulfuric acid method (*27*) as sucrose. The total sugar and reducing sugar were respectively determined by using the phenol−sulfuric acid method (*27*) and the Somogyi (*33*)−Nelson (*34*) method.

RESULTS AND DISCUSSION

Chemical Features of SE-P. SE-P was prepared from SE by dry-heating with MPA, through removal of unreacted MPA and SE with chilled water extraction, chloroform/methanol (8:2, v/v) dissolution, silica gel column chromatography, and final DEAE-Sepharose column chromatography. HPTLC of the SE-P preparation using mobile phases A and B for respectively separating SE-P and SE showed coincidental zonal spots of saccharide and phosphate moieties at R_f 0.24−0.7, whereas no spots for polystearate above the triester were apparent (data not shown). The yield of SE-P was ∼2%. The chemical composition of SE-P determined by GLC, the phenol−sulfuric acid method, and the molybdenum blue colorimetry showed that the molar ratio of sucrose, stearic acid, and phosphoric acid was 1:1.2:0.8, suggesting that SE-P should be a monophosphosucrose monostearic acid (**Table 1**). The main fatty acid of SE-P was stearic acid (69 wt %), whereas a small amount of palmitic acid derived from the SE preparation was also observed.

FAB-MS showed the mass spectrum of SE-P with signals at *m*/*z* 525, 659, and 687 (**Figure 1**), these respectively being the same as molecular weights of monophosphoglucose monostearate or monophosphofructose monostearate, monophosphosucrose monopalmitate, and monophsophosucrose monostearate. The signal at *m*/*z* 687 was strongest, indicating the main component of SE-P. Because sucrose is easily hydrolyzed under acidic conditions, monophosphoglucose monostearate or monophosphofructose monostearate is believed to have resulted from the phosphorylation of SE at pH 5.5. It is concluded from these results that SE-P could be prepared as monophosphosucrose monostearate by dry-heating SE with MPA.

Table 1. Chemical Composition of SE-P

moiety	chemical composition (%)
sucrose (S)[a]	34.3
fatty acid (F)[b]	35.5
palmitic acid	10.9
stearic acid	24.6
phosphoric acid (P)[c]	7.4
total	77.2
S:F:P	
weight ratio	1:1.1:0.2
molar ratio	1:1.2:0.8

[a] Determined by the phenol–sulfuric acid method. [b] Determined by gas–liquid chromatography. [c] Determined by molybdenum blue colorimetry.

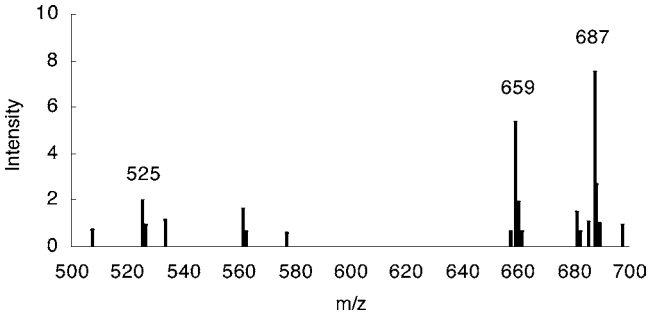

Figure 1. Mass spectrum of SE-P measured with a JEOL SX-102A instrument using FAB ionization.

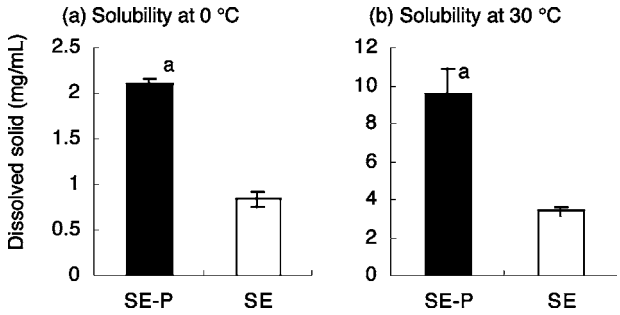

Figure 2. Comparative solubility of SE-P and SE at different temperatures. The solubility of SE-P in distilled water at (**a**) 0 °C or (**b**) 30 °C was determined as the saccharide concentration of the filtrate by using the phenol–sulfuric acid method. Each value is the mean ± SE (n = 3). "a" shows significant difference at $P < 0.05$ against SE.

Increased Solubility. The solubility of SE-P in distilled water was evaluated at 0 and 30 °C. SE-P exhibited significantly higher solubility than SE at 0 °C (**Figure 2**), whereas at 30 °C, the solubility of SE-P was 1.5 times as high as that of SE. It is thus concluded that phosphorylation was effective for improving the solubility of SE. Because the low solubility of SE inhibits ease of handling and adequate expression of the expected properties (*1*, *2*), it would be useful for SE-P to have good solubility, particularly at low temperature.

Improved Emulsifying Properties. The emulsifying properties of SE-P were evaluated by the absorbance at 500 nm of

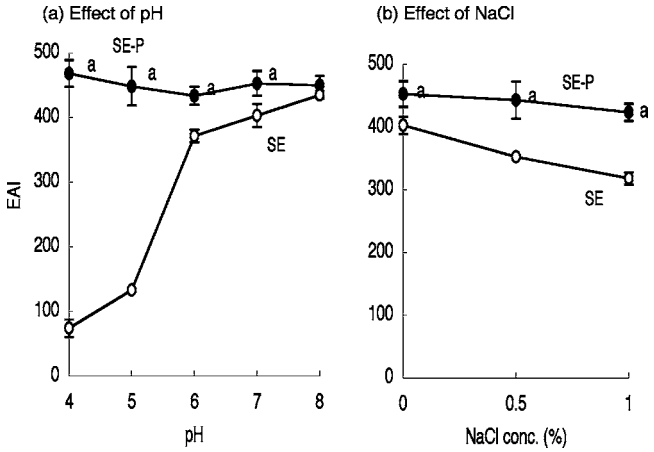

Figure 3. Comparative effects of pH and NaCl concentration on EAI of SE-P and SE. The emulsifying properties were evaluated as the emulsifying activity index (EAI) calculated from the absorbance at 500 nm of an O/W emulsion prepared (**a**) at pH 4.0–8.0 and (**b**) in the presence of NaCl (0.5 and 1.0%). Each value is the mean ± SE ($n = 3$). "a" shows significant difference at $P < 0.05$ against SE.

the O/W emulsions prepared in the range of pH 4.0−8.0. There was no significant difference between the EAI value of SE-P and that of SE in the neutral pH region (**Figure 3a**). However, in the acidic region, the EAI value of SE dropped markedly, indicating the disappearance of emulsifying properties. The emulsion stability evaluated by the absorbance at 500 nm of the emulsion 60 min after emulsification also markedly decreased. It is believed that a small amount of the alkaline salt of the fatty acid included in the SE preparation as a micellar stabilizer would result in the loss of the surface activity due to conversion to free fatty acid in the acidic pH range (*1*). In contrast, SE-P maintained a high EAI value and emulsion stability over the pH range from 4.0 to 8.0 due to its high solubility. The effect of NaCl on the emulsifying properties of SE-P was also examined. The EAI value and emulsion stability of SE decreased with increasing in concentration of NaCl. However, SE-P maintained an adequate EAI value and emulsion stability in the presence of NaCl (**Figure 3b**), probably due to the high hydrophilicity of the phosphoryl group, whereas there was no significant difference between the emulsion stability of SE-P and that of SE. It can thus be stated that phosphorylation is ideally suited to improving the emulsifying properties of SE in the acidic region and in the presence of NaCl. Because many foods have an acidic pH value, and contain some salt, the fact that SE-P exhibits good emulsifying properties under such disadvantageous conditions is valuable.

Calcium Ion-Binding Properties. The interaction with calcium in vitro is generally evaluated by measuring the soluble Ca concentration of the centrifuged supernatant of a mixed suspension containing a Ca-interacting substance, soluble calcium salt, and inorganic phosphate, by which the inhibitory effect of the Ca-interacting substance on the formation of an insoluble Ca complex can be estimated. However, because SE-P and SE can disperse insoluble substances, it was impossible to evaluate the calcium ion-binding ability of SE-P and SE by the foregoing method. In the present experiment, the interaction of SE-P with calcium was therefore examined by conductometric titration with a $CaCl_2$ solution. The SE solution showed a linear titration plot passing through the origin with a high correlation coefficient ($r = 0.9995$; **Figure 4**), indicating that SE could not bind to Ca^{2+}. However, SE-P exhibited a curved titration plot that could be resolved into two straight lines. The initial

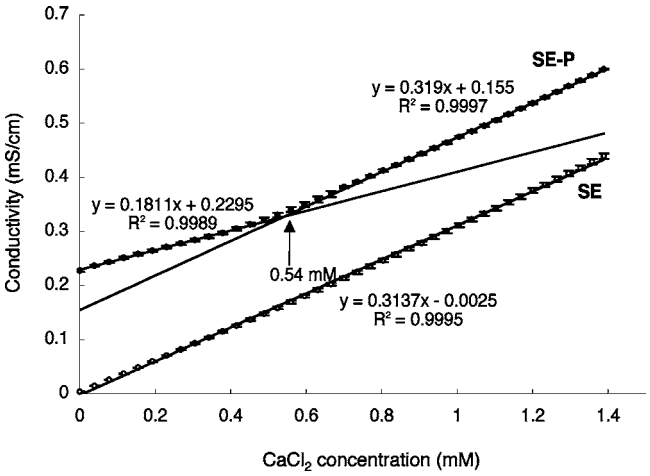

Figure 4. Comparative conductometric titration curves of SE-P and SE solutions in the presence of $CaCl_2$. Conductometric titration was carried out by adding small increments (50 μL at a time) of 12.5 mM $CaCl_2$ to 16 mL of an SE-P or SE solution (1.0 mg/mL) at 30 °C. The end point of titration of SE-P was determined as the intersection between two linear regression curves obtained from the values of 0–12 and 22–40 points. Each value is the mean ± SE ($n = 3$).

Table 2. Comparative Effects of SE-P and SE on the Thermal Characteristics of PS[a]

(a) Gelatinization Behavior

	gelatinization temperature (°C)			
sample	T_o	T_p	T_c	ΔH_1 (mJ/mg)
PS	57.4 ± 0.2	61.2 ± 0.1	71.5 ± 0.1	19.4 ± 0.4
PS/SE-P	57.6 ± 0.2 b	62.8 ± 0.5 a	70.6 ± 0.3 ab	15.0 ± 1. 3a
PS/SE	57.2 ± 0.1	62.2 ± 0.1	70.3 ± 0.3 a	17.0 ± 0.5 a

(b) Regelatinization Behavior

sample	T_p (°C)	ΔH_2 (mJ/mg)
PS	58.8 ± 0.9	8.8 ± 1.6
PS/ SE-P	56.4 ± 0.9 ab	6.0 ± 1.3 a
PS/SE	58.0 ± 0.3	6.4 ± 1.1 a

[a] SE-P or SE was added to give 5 w/w% on the basis of the dry PS weight. The concentration of PS was 25 w/w%. Each value is the mean ± SE ($n = 3$). Letters "a" and "b", respectively, following entires show significant difference at $P < 0.05$ against PS and SE. After the first run, the samples were retrograded by preserving at 4 °C for 1 week, and then the second run was carried out to evaluate the regelatinization behavior.

titration line had a gentle slope due to the binding of Ca^{2+} to SE-P. The second regression line had a steeper slope similar to that of SE above the saturated concentration of $CaCl_2$ after complete Ca-binding to SE-P. The end point of titration was thus determined as the intersection between the two regression lines. Consequently, the bound Ca^{2+} was calculated to be 8.8 μmol. Because the amount of phosphate residue of SE-P in the test solution was 7.8 μmol, the binding molar ratio of Ca^{2+} to SE-P was evaluated to be 1.1:1. It is thus concluded that Ca^{2+}-binding ability could be endowed by phosphorylating SE.

Regulation of the Thermal Behavior of PS. Gelatinization of the micelle structure and the pasting property of PS containing SE-P were respectively investigated by DSC and RVA. DSC showed that the gelatinization temperature (T_p) of PS containing SE-P was significantly higher than that of PS (**Table 2**), whereas the conclusion temperature (T_c) was significantly lower, resulting in reduced enthalpy. In the case of SE being added, PS showed no significant difference. The authors have previously reported

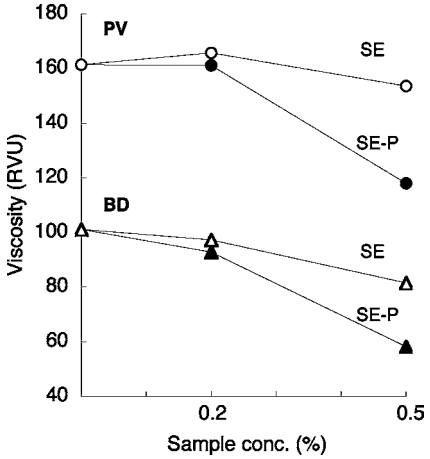

Figure 5. Comparative effects of SE-P and SE on the viscosity of a PS paste. The viscosity of PS paste samples containing 0, 0.2, or 0.5% SE-P based on the dry PS weight is represented by the peak viscosity (PV) and breakdown (BD) of the RVA viscogram.

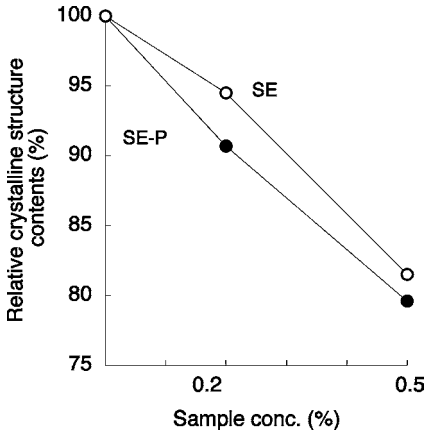

Figure 6. Comparative effects of SE-P and SE on the crystalline structural content of a sample retrograded after RVA. The crystalline structural content of each sample was estimated by the peak intensity at $2\theta = 17°$, and the relative crystalline structural content was calculated as the ratio of each intensity to that of PS.

that phosphates elevated the gelatinization temperature of potato starch (*21*). The peculiar regulation by SE-P of the gelatinization behavior is thus probably caused by the phosphate moiety in SE-P.

The addition of SE-P resulted in a marked decrease in the peak viscosity and breakdown (the decrease in viscosity after the peak viscosity) of PS without any changes in the pasting and peak temperatures depending on the level of addition (**Figure 5**), whereas the addition of SE did not result in these large changes. These results indicate that the addition of SE-P could effectively inhibit the swelling and successive collapse of the PS granules during gelatinization.

The effect of SE-P on the retrogradation of PS was also investigated by DSC, X-ray diffractometry, and enzymatic digestibility. X-ray diffractometry and enzymatic digestibility were carried out on samples that had been retrograded by preserving the paste after RVA at 4 °C for 1 week. Because the X-ray diffraction pattern showed only a small peak at $2\theta = 17°$ due to the crystalline structure that recovered during retrogradation, the relative crystalline structural content was defined in this experiment as the relative ratio of each diffraction intensity to that of PS (**Figure 6**). It was recognized that added SE-P or SE could inhibit the reconstruction of the crystalline

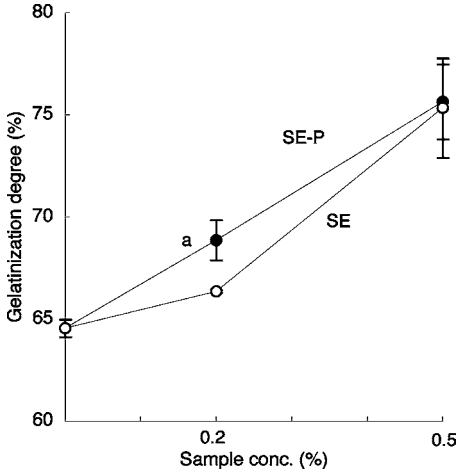

Figure 7. Comparative effects of SE-P and SE on the enzymatic digestibility of a sample retrograded after RVA. The degree of gelatinization was evaluated as the enzymatic digestibility by using the β-amylase–pullulanase method. Each value is the mean ± SE ($n = 3$). "a" shows significant difference at $P < 0.05$ against SE.

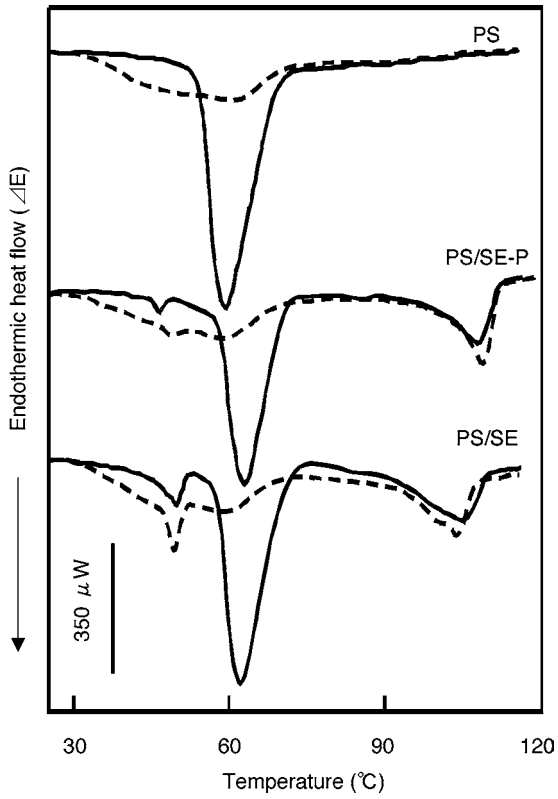

Figure 8. DSC curves for PS containing SE-P or SE. DSC of PS containing 0.5% SE-P or SE based on the dry PS weight was carried out from 5 to 120 °C at a heating rate of 2 K/min to evaluate the respective gelatinization and retrogradation behavior with the first and second runs (after preservation at 4 °C for 1 week). The first and second runs are, respectively, shown by the continuous and broken lines.

structure depending on the level of addition and that the inhibitory effect of SE-P was somewhat greater than that of SE. The degree of gelatinization evaluated by the enzymatic digestibility was significantly increased by SE-P or SE depending on the level of addition (**Figure 7**) and tended to increase a little more with SE-P than with SE. After DSC measurements for the first gelatinization, the samples were preserved at 4 °C for 1 week and then analyzed again to evaluate by DSC the

extent of the ordered structure reconstituted during retrogradation. The broad endothermic peak overlapped by a very small peak was observed in a lower temperature range than that of the first gelatinization. Because the small peak could be identified to be due to the remelting of SE-P and SE that had solidified during cooling, the retrogradation enthalpy (ΔH_2) was corrected by subtracting it. ΔH_2 values of SE-P- and SE-added PS were significantly lower than that of native PS, SE-P-added PS showing lower ΔH_2 than SE-added PS. These results indicate that SE-P and SE inhibited the reconstitution of the ordered structure including the crystalline structure due to retrogradation corresponding to changes in the degree of gelatinization and that the inhibitory effect of SE-P was larger than that of SE.

It is well-known that fatty acids form a complex with a starch chain, resulting in the inhibition of the retrogradation (*36*). DSC curves for the SE-P- and SE-added PS, which were obtained by using a silver capsule as a sample capsule, showed another endothermic peak at ~110 °C that was different from the first gelatinization peak, and endothermic transition was detected again after retrogradation, which was not the case in the DSC curve for PS (**Figure 8**). This endothermic peak seems to have been caused by thermal transition of the starch−SE-P or starch−SE complex via the fatty acid moiety, because it has been reported that fatty acid could form a complex with starch (*15*) and that thermal transition of the complex could be observed above 100 °C (*13*). It is therefore concluded that SE-P and SE could each inhibit retrogradation of starch by forming a complex with a starch chain via the fatty acid moiety and that the inhibitory effect of SE-P was greater than that of SE, probably due to inhibition of the rearrangement of starch chains by electrostatic repulsion between phosphate residues and/or steric hindrance to the phosphate groups. The implication of these results is that SE-P would be a more effective modifier of starch than SE.

Concluding Remarks. Monophosphosucrose monostearate (SE-P) could be prepared from sucrose monostearate (SE) by dry-heating with metaphosphoric acid. SE-P exhibited higher solubility than SE, outstanding emulsifying properties under such undesirable conditions as acidic pH and the presence of NaCl, and Ca^{2+}-binding ability. SE-P improved thermal behavior indicated by an increase in the gelartinization temperature, decrease in viscosity, and inhibition of retrogradation. These findings show that SE-P could be a useful material with novel emulsifying, Ca^{2+}-binding, and starchy food-modifying properties.

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