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Changes in Functional Properties of Potato Starch by Oleylation Through Lipase-catalyzed Solid-phase Synthesis

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Abstract: Oleyl potato starch (OA-PS) was prepared by lipase-catalyzed solid-phase synthesis. PS retaining AY-Amano 30G as a lipase (PS/Lip) was prepared by immersing in the lipase solution, and by dehydrating with ethanol before air-drying. PS/Lip and oleic acid (OA) were incubated in *n*-hexane or without a solvent, and then unreacted OA was thoroughly eliminated by washing with *n*-hexane before air-drying to respectively obtain OA-PS or OA-PS (solvent-free). The OA-PS samples respectively showed the structural compositions of PS (as glucose residue) of OA = 30:1 and 286:1 (molar ratio). Their crystallinity evaluated by polarizing microscopic observation and X-ray diffractometry and their digestibility with α - and β -amylase were similar to those of PS. The OA-PS samples acquired significantly higher thermal structural stability, as evaluated by DSC, than that of the PS and control samples, and exhibited improved pasting properties in terms of a markedly lower swelling index and peak viscosity by RVA, as well as easier vaporization of water from the gelatinized suspension and higher heat-resistance for maintaining the swollen starch granules at 110°C for 30 min than those of the PS and control starches. In particular, OA-PS was superior to OA-PS (solvent-free) in these structural and pasting characteristics. OA-PS also exhibited a decreased surface tension with increasing concentration. Oleylation with lipase should thus be valuable for improving the multiple functional properties of starch.

Key words: potato starch, lipase-catalyzed acylation, multiple improvement, low swelling and viscosity, easy vaporization, endowment of surface activity

INTRODUCTION

Starch is widely used to build or modify the physical properties of many processed foods, and the effect of applying starch can only be revealed by being gelatinized. However, gelatinization by heating, particularly heating at a high temperature such as that involved in a retort treatment, produces extensive swelling and successive collapse of the swollen starch granules under physical stress accompanied by a drastic viscosity loss. Avoiding this problem by using augmented starch often results in a sticky and pasty texture and a decrease in the quality of starchy foods. Moreover, extensive swelling also produces a moist coating on fried foods, and results in rapid lost of crispness of the coatings due to water transportation from the internal food material after frying. It is thus very important to control the gelatinization behavior to inhibit swelling and maintain the resulting swollen starch granules.

A number of studies have been carried out to investigate the effects of many coexisting substances and control the gelatinization behavior and swelling by the chemical and physical modification of starch. We have studied such control by adding amino acids, particularly, charged amino

acids and charged amino acid-rich peptides,^{1–8)} and by conjugating or compounding non-starchy substances.^{9–12)} We have clarified that such charged amino acids as lysine (Lys) and glutamic acid (Glu) strongly elevated the gelatinization temperature, and reduced the viscosity and swelling of potato starch (PS),^{1,2)} depending on the binding strength of the amino acid to the starch chains.³⁾ It has also been revealed that adding charged amino acids could improve the degraded viscosity of retorted starch paste during storage.¹³⁾ However, such control by adding amino acids and peptides is insufficient, because changes in such conditions as pH, salt concentration, temperature, and additives occurring during practical food processing would have varied effects and result in unstable control of starch. It has recently been reported that compounding with Glu,^{9–11)} and conjugating with fatty acylated saccharide through Lys¹²⁾ by simultaneous modification with a heat-moisture treatment and the Maillard reaction increased gelatinization temperature, decreased solubility and swelling, endowed thermal resistance, and easy vaporization, reduced the digestibility with α -amylase, and reduced the viscosity of a wheat flour paste. However, since these modifications are performed under such severe condition as 120°C for 60–90 min, there is the possibility of producing unfavourable side reactions. It is thus desirable for the swelling of starch to be controlled for food use by such a mild treatment as enzymic modification. One of the

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effects of the Lys- and fatty acylated saccharide-PS conjugate just mentioned is believed to be due to hydrophobization of the starch chains with the fatty acid moiety of the conjugate. This suggests that binding fatty acid by acylation with an enzyme like lipase to the starch chains could result in effective and desirable control. It has previously been demonstrated that branched oligosaccharide phosphate oleyleted by lipase could markedly reduce the viscosity of PS with increasing concentration, and endow a surface active property;^{14,15} sericin, a silk protein, has exhibited good emulsifying ability by oleylating with lipase-catalyzed solid-phase synthesis.

In order to overcome a drastic viscosity loss of starch under physical stress during heating and low crispness of fried starchy coating due to low vaporization resulting from extensive swelling, and to endow an amphiphilic property as a new functionality, the objectives of this present study are to prepare oleylated PS with lipase, and to demonstrate the resulting improved functional properties in terms of increased structural thermal stability, reduced swelling and viscosity, enhanced thermal resistance, easy vaporization of water, and an endowed surface active property.

MATERIALS AND METHODS

Materials. Large granules of potato starch (PS; Hokuren Research Institute, Sapporo, Japan) were used after being repeatedly washed with distilled water at 4°C and air-dried (15.3% residual moisture in the recovered starch). Lipase (AY-Amano 30G) was supplied by Amano Enzyme Inc. (Nagoya, Japan). Oleic acid (OA; purity, greater than 60%), *n*-hexane, 3A 1/16 molecular sieves, α -amylase (20 U/mg) and the other reagents were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). β -amylase (sweet potato; 834 U/mg) was purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan).

Preparation of oleyl potato starch granules. PS was oleylated by lipase-catalyzed solid-phase synthesis by two methods: [A] an *n*-hexane system according to the method previously described,¹⁴⁻¹⁶ and [B] a solvent-free system.

[A] *n*-hexane system:

PS (10 g) was suspended in 10 mL of a lipase solution (200 mg/mL) at room temperature for 1 h, and then dehydrated by adding 20 mL of ethanol. After centrifuging at about $2,900 \times G$ for 5 min, the resulting precipitate was dispersed in 20 mL of ethanol and then re-centrifuged. This dehydration and centrifugation was conducted three times. The final precipitate was dispersed in 50 mL of dehydrated ethanol, and then filtered. The ethanol-dehydrated sample was dispersed in 50 mL of dehydrated *n*-hexane, and then centrifuged. This replacement process was conducted twice to obtain lipase-retaining PS (Lip/PS). A control sample (Cont PS) without lipase was also prepared. Lip/PS or Cont PS, 200 mL of dehydrated *n*-hexane, 5 mL of OA, and 10 g of molecular sieves were added into a 500-mL screw-capped Erlenmeyer flask, and then the atmosphere was replaced with nitrogen gas. The reaction mixture was incubated at 30°C for 45 h while shaking at 140 stokes/min. The reaction product was passed through a stainless steel mesh to remove the molecular sieves, and then filtered at reduced pressure to remove unreacted OA. This removal of unreacted OA by

dispersing in dehydrated *n*-hexane and the successive filtration was conducted three times. The final precipitate was air-dried to obtain oleyl PS (OA-PS) or Cont PS.

[B] solvent-free system:

Lip/PS and Cont PS prepared according to the procedure just described were air-dried over night, and further dried under reduced pressure at room temperature for 1.5 h. After adding 10 mL of OA to 10 g of the dried Lip/PS or Cont PS, the reaction mixture was thinly spread on a polypropylene tray (140 × 185 × 27 mm), and then incubated at 65°C for 16 h. The reaction product was dispersed in *n*-hexane to extract unreacted OA, and then centrifuged at about $2,900 \times G$ for 5 min. This removal of unreacted OA by dispersing in *n*-hexane and successively centrifuging was conducted three times. The final precipitate was air-dried to obtain OA-PS (solvent-free) or Cont PS (solvent-free).

Dehydrated ethanol and *n*-hexane were prepared by applying ethanol and *n*-hexane containing molecular sieves at room temperature more than three days. Molecular sieves were used after dehydrating by heating at 400°C for 4 h.

X-ray diffractometry. X-ray diffractometry of the starch sample was carried out by an Ultima IV X-ray diffractometer (Rigaku Co., Tokyo, Japan) with a copper target at 40 kV and 30 mA producing CuK α radiation of 0.154 nm according to the method previously described.¹⁷

Digestion with amylases. The digestibility of each starch sample with α -amylase and β -amylase was evaluated as previously described.¹⁸ A sample suspension (2 mg/mL) heated at 100°C for 10 min, and α -amylase (0.02 U/mL) or β -amylase (1 U/mL) were incubated at 30°C for 120 min while being stirred at 100 rpm. The digestibility was evaluated by determining the saccharide concentration of each filtrate passed through a membrane filter with a 0.45- μ m pore size (cellulose nitrate; Advantec, Tokyo, Japan) by the phenol-sulfuric acid method.¹⁹

Differential scanning calorimetry (DSC). After adding 10 μ L of distilled water to 5 mg of the starch sample in an airtight anodized aluminum capsule, DSC was conducted to determine the gelatinization temperature and enthalpy by using an SSC-5020 DSC-6100 instrument (Hitachi High-Tech Science Co., Tokyo, Japan) as previously described.²⁰ Distilled water (15 μ L) was used as a reference. Each sample was heated from 5 to 100°C at 2°C/min. Triplicate measurements were taken.

Viscosity measurement. The pasting properties of the starch sample suspension (2.0 g/25 mL) were measured by an Rapid Super3 ViscoanalyzerTM (Newport Scientific, NSW, Australia) as previously described.¹⁷ Triplicate RVA measurements for potato starch had shown a high degree of reproducibility, the coefficient of variation of the peak viscosity being evaluated as only 0.19%,¹⁷ so each measurement was taken only once in this study.

Measurement of swelling index. The swelling index of the starch sample was measured as previously described.¹ The starch sample suspension (0.1 g/5 mL) was heated at 70°C for 30 min or at 93°C for 30 min while stirring at 500 rpm. After centrifuged at $31,000 \times G$ for 30 min at 20°C, the resulting supernatant was completely removed, and the remaining precipitate was weighed (W_w) and dried at 110°C for 16 h to obtain a constant weight (W_d). The swelling index was calculated by the ratio W_w to W_d .

Triplicate measurements were carried out.

Measurement of the surface tension. The starch sample suspension (0.2 g/100 mL) was put into several screw-capped test tubes (15 i.d. $\times$ 80 mm) and heated at 120°C for 30 min in an aluminum block bath (Scinics, Tokyo, Japan) while stirring at 500 rpm. After cooling to room temperature, the autoclaved sample was centrifuged at $31,000 \times G$ for 30 min at 20°C, and the resulting supernatant was lyophilized. The lyophilized sample suspension was dissolved by heating at 120°C for 30 min in a similar manner to that just described to give a 5% solution. The mother liquor was diluted with distilled water to 1 and 2.5% solutions, the specific gravity being determined by using a pycnometer. After heating at 80°C for 20 min and then quickly cooling to room temperature with running water, the surface tension was measured using the Young-Laplace method²¹⁾ with a CA-X surface tensiometer (Kyowa Interface Science Co., Saitama, Japan).

Microscopic observation. The starch sample (5 mg) in a screw-capped test tube (15 i.d. $\times$ 80 mm) was heated in 5 mL of distilled water at 110°C for 30 min or at 120°C for 20 min in an aluminum block bath (Scinics Corporation, Tokyo, Japan) while stirring at 500 rpm. A PM-10AD polarizing microscope (Olympus Corporation, Tokyo, Japan) was used to evaluate the swollen starch granules at a magnification of 100 times after staining with iodine as previously described.²²⁾

Measurement of vaporized water. Water vaporization from the starch sample was measured by a previously described method.⁹⁾ The starch sample suspension (0.14 g/mL) was heated at 100°C for 60 min while being stirred at 250 rpm. The water that had been vaporized from the resulting paste was quantified at 15-min intervals from the reduced weight of each sample. Duplicate measurements were taken.

Analytical methods. The total sugar content was determined as glucose by the phenol-sulfuric acid method.¹⁹⁾ The oleic acid content of the OA-PS samples was determined by gas-liquid chromatography with GC 4CM apparatus (Shimadzu Corporation, Kyoto, Japan) and a DEGS Chromosorb WAW column (GL Science Inc., Tokyo, Japan) after fatty acid methyl esters had been prepared from the OA-PS samples by methanolysis according to the method previously described.²³⁾

Statistical analysis. Multiple comparison by the Bonferroni/Dunn method was used to compare mean values of the data for DSC, swelling index, solubility, and surface tension at the 5% significance level.

RESULTS AND DISCUSSION

Features of OA-PS.

The lipase (AY-Amano 30G)-retained PS (Lip/PS) prepared by immersing PS in a lipase solution, and then dehydrating with ethanol before air-drying was applied to produce oleylated PS by two methods [the *n*-hexane system (A) and solvent-free system (B): A, incubation of Lip/PS and oleic acid (OA) in *n*-hexane at 30°C for 45 h; B, incubation of Lip/PS and OA without the solvent at 60°C for 16 h], according to the previous study on oleylation of sericin with lipase-catalyzed solid-phase synthesis. In this study, acylation has been confirmed by appearing peaks in the ¹H-NMR spectrum assigned to the alkyl, acyl, and vinylene groups of the oleic

acid moiety.¹⁶⁾ The resulting oleylated PS samples by the method A and B are respectively referred to as OA-PS and OA-PS (solvent-free). The reaction conditions were selected from the results from preliminary experiments, being carried out by using three lipases [AY-Amano 30G, Lipzyme CALB L (*Candida antarctica*, Novozymes, Bagsvaerd, Denmark) and lipase from *Candida rugosa* (Amano Enzyme, Nagoya, Japan)] and varying the amount of the lipase (2 and 3 g/10 g of PS), the reaction temperature (30–60°C) and time (1 and 45 h) to give the lowest swelling index.

OA-PS and OA-PS (solvent-free) had the respective structural compositions of PS:OA = 100:5.8 and 100:0.6, indicating that the amount of acylated OA was limited. The respective weight ratios were converted to the molar ratios [PS (as the glucose residue):OA = 30:1 and 286:1], where the glucose residue content was estimated from the PS content. The molar ratio shows that the *n*-hexane system could be applied to more efficiently acylate than the solvent-free system did, probably due to easier approach of OA to the starch chains and lipase retained in the starch granule with permeation of *n*-hexane. Control samples by methods A and B without adding lipase were also prepared, and are referred to as Cont PS and Cont PS (solvent free).

Each OA-PS and Cont PS showed a clear polarized image similar to that of PS (Fig. 1), suggesting no substantial change in the crystallinity of PS by acylation with lipase, whether using *n*-hexane or not. X-ray diffractometry for OA-PS, Cont PS, and PS showed that the diffraction pattern and strength resembled each other (Fig. 2), as the respective peak strengths at $2\theta = 17^\circ$ of OA-PS, Cont PS and PS were 348, 432 and 381 cps with a 28–63 cps fluctuation range in the detection of X-ray photon during measurement. These results thus show no substantial difference in the crystallinity among the three starch samples.

The gelatinized OA-PS, Cont PS and PS samples were digested with α - and β -amylases. The digestibility showed no significant difference among the tested samples, except for the result of α -amylolysis for Cont PS (solvent-free) (Fig. 3), probably due to the low OA content attached with lipase. The independence of acylation in regard to β -amylolysis reaching about 41% digestion for OA-PS suggests that OA might have been attached in the amorphous region of the starch cluster in the starch granule.

The structural thermal stability of the OA-PS, Cont PS and PS samples was investigated by DSC. OA-PS showed a significantly higher gelatinization temperature (T_0) than that of PS or Cont PS (Fig. 4). OA-PS (solvent-free) also showed increased T_0 . The results of the peak temperature of gelatinization also showed a tendency similar to the results of T_0 . The gelatinization enthalpy (ΔH) of the OA-PS samples conversely decreased. The gelatinization entropy (ΔS) derived from T_0 and ΔH ($\Delta S = \Delta H/T$, where T_0 is the absolute temperature, and ΔH and ΔS are calculated on the basis of the glucose residue of starch) represents the rigidity of starch.^{15,24)} The ΔS values of the OA-PS samples were significantly lower than those of the PS and Cont PS samples, indicating that oleylation could improve the rigidity, *i.e.*, increase the structural thermal stability; in particular, oleylation through the *n*-hexane system proved useful to improve the structural thermal stability. Cont PS showed significantly higher rigidity than that of PS presumably due

Fig. 1. Polarized micrographs of the native and heated OA-PS samples as compared with those of the Cont PS and PS samples.

The heated samples were prepared by heating at 110°C for 30 min and 120°C for 20 min while stirring at 100 rpm, before being stained with iodine. Polarized micrographs were taken at a direct magnification of 100 times. The arrow shows the elongated swollen granule.

Fig. 2. X-ray diffraction patterns for OA-PS, Cont PS and PS.

to retaining a little amount of OA during incubation in *n*-hexane, whereas there was no influence of the retention of OA on the digestibility of Cont PS with α - and β -amylases (Fig. 3).

Improved gelatinization behavior.

The swelling index of the OA-PS and Cont PS samples was evaluated at 70°C, because the conclusion temperature of these samples by DSC was about 67–70°C. The swelling index value of the OA-PS samples was significantly lower than those for the PS and Cont PS samples (Fig. 5), probably due to the inhibition of hydration of the starch chains by oleylation. This lower swelling corresponds well to the

Fig. 3. Digestibility of the OA-PS, Cont PS and PS samples with α - and β -amylase.

The sample suspension (pre-heated at 100°C for 10 min while stirring at 100 rpm) was digested with α - and β -amylase at 30°C for 120 min. The digestibility was evaluated by determining the saccharide concentration of each filtrate with the phenol-sulfuric acid method.¹⁹⁾ ■, α -amylase; □, β -amylase. Different letters (α -amylase, without apostrophe; β -amylase, with apostrophe) show significant difference ($p < 0.05$). Different letters (prime letter, the results with β -amylase; non-prime letter, the results with α -amylase) show significant difference ($p < 0.05$). Any omitted error bars were too close to show.

results of the improved rigidity with oleylation evaluated by DSC.

The pasting behavior of the OA-PS samples was investigated by RVA. The OA-PS samples exhibited a markedly

Fig. 4. Thermal characteristics (T_0 , ΔH and ΔS) of the OA-PS, Cont PS and PS samples evaluated by DSC.

T_0 , onset gelatinization temperature; ΔH , gelatinization enthalpy; ΔS , gelatinization entropy, being derived from T_0 and ΔH as previously described.^{15, 24} Different letters show significant difference ($p < 0.05$). Any omitted error bars were too close to show.

low peak viscosity (PV) and high pasting temperature (PT) as compared with those of PS and the control samples, while breakdown could not be observed (Fig. 6). The swelling indices for PS, OA-PS, and Cont PS were then evaluated at 93°C, close to the temperature at PV. The swelling index of OA-PS was significantly lower than those of PS and Cont PS

Fig. 5. Swelling indices of the OA-PS, Cont PS and PS samples.

The starch samples were heated at 70°C for 30 min (□) or at 93°C for 30 min (▨) while stirring at 500 rpm, and the resulting suspensions were then centrifuged at $31,000 \times G$ and 20°C for 30 min. The swelling index was estimated from the dry weight of the precipitate. Different letters (prime letter, the results at 93°C; non-prime letter, the results at 70°C) show significant difference ($p < 0.05$). Any omitted error bars were too close to show.

Fig. 6. Pasting behavior of the OA-PSs, Cont PS and PS samples evaluated by RVA.

Bar graphs by solid line, peak viscosity (PV); bar graphs by broken line, breakdown; $\triangle$, pasting temperature.

(Fig. 5). The higher PT and lower PV of values for OA-PS were thus due to the inhibited swelling resulting from oleylation. Cont PS also showed notably low PV value presumably due to retaining a little amount of OA during incubation in *n*-hexane, whereas there was no significant difference in the swelling index at 93°C from that of PS. However, the result of the PV value of Cont PS relatively well corresponds to those of the lowered swelling index at 70°C and ΔS value. Such different behaviors just described should be further investigated in a separate study. OA-PS by the *n*-hexane system showed a lower PV value than that of OA-PS by the solvent-free system, probably due to the higher OA content.

The OA-PS, Cont PS and PS suspensions were autoclaved at 110°C for 30 min while stirring at 500 rpm to observe the morphological change. The PS and Cont PS samples heated under such condition lost the swollen starch granules and completely dissolved, whereas the OA-PS samples was incompletely dissolved, with elongated swollen granules (Fig. 1), indicating higher heat-stability, probably due to the inhibited hydration of the starch chains resulting from the increased hydrophobicity by oleylation. It is thus concluded that oleylation with lipase could provide a conjugated starch with the improved pasting properties in terms of markedly low swelling index and viscosity, and high heat-stability. However, autoclaving at 120°C for 30 min resulted in

Table 1. Linear regression equations and determining coefficients (r^2) for water vaporization of the OA-PS and Cont-PS samples.

Sample	Regression equation	r^2	Ratio of the slope
Distilled water	$Y = 1.601X - 0.386$	0.9998	1.00
PS	$Y = 1.143X - 0.12$	0.9980	0.714
OA-PS	$Y = 1.304X - 0.298$	0.9965	0.814
Cont PS	$Y = 1.193X - 1.548$	0.9977	0.745
OA-PS (solvent-free)	$Y = 1.267X - 1.276$	0.9988	0.791
Cont PS (solvent-free)	$Y = 1.062X - 0.442$	0.9995	0.663

The sample suspension was heated at 100°C while stirring at 250 rpm. The relationship between vaporized water (%), Y and heating time (min, X) was analyzed.

complete dissolution (Fig. 1), probably enabling use for liquid foods without swollen starch granules and starch aggregates resulting from their collapse.

Improved water vaporization.

Water vaporization of the OA-PS and Cont PS suspensions was evaluated by determining the weight loss of each suspension during heating at 100°C while stirring. The external water of the swollen starch granules could be preferentially vaporized in this experimental system. Each starch suspension showed that the amount of vaporized water increased with increasing heating time. Consequently, the relationship between the vaporized water (%), Y and heating time (min, X) could be expressed by a linear regression equation with a high determination coefficient (Table 1). The slope of the regression equation represents the vaporization rate. The PS and control PS pastes showed about a 30% lower vaporization rate than did distilled water, due to large swelling and collapse of the swollen starch granules by stirring. In contrast, the pastes of the OA-PS samples exhibited a higher vaporization rate than did the PS and control PS pastes, being relatively close to that of water. The relationship between the vaporization rate (Y , Table 1) and ΔS value (X , Fig. 4) could be expressed by a linear regression equation with a relatively high determination coefficient [$Y = -0.168X + 1.09$ ($r^2 = 0.759$)]. This suggests that the vaporization rate depends on the rigidity of starch. The OA-PS samples having high rigidity could thus provide a paste with easy vaporization of water, due to the reduced swelling and lack of collapse of the swollen starch granules, corresponding well to the vaporization of the lysine and fatty acylated saccharide-conjugated PS,¹²⁾ and charged amino acid-compounded PS.⁹⁾ It is a specific characteristic that the OA-PS samples could be enzymatically prepared, substantially different from the conjugated or compounded PS sample prepared by autoclaving. The OA-PS samples should therefore be valuable as a food material giving a dry texture to fried coatings by the easy vaporization of water, when fried in hot oil, or recovering the crispness of moistened fried foods by re-heating in a microwave oven.

Endowed surface active property.

The OA-PS, Cont PS and PS samples were autoclaved at 120°C for 30 min to completely dissolve the starch and to evaluate the surface tension. PS and Cont PS showed a surface tension similar to that of distilled water, independent of their concentrations (Fig. 7), indicating no surface activity. However, OA-PS exhibited a decreased surface tension

Fig. 7. Surface tension of the OA-PS, Cont PS and PS samples.

The starch samples were heated at 120°C for 30 min while stirring at 500 rpm, and then centrifuged at 1,000 × G for 30 min at 20°C before being lyophilized. The lyophilized sample suspension was dissolved by heating at 120°C for 30 min, and then the surface tension was measured by a surface tensiometer. Any omitted error bars were too close to show.

with increasing concentration. It is thus concluded that oleylation with lipase-catalyzed solid-phase synthesis could endow a surface active characteristic to PS, suggesting the contribution to revealing its emulsifying ability.

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