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Effect of Amorphization Methods on the Properties and Structures of Potato Starch-Monoglyceride Complex

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Recently, starch-based fat replacers (FRs) have emerged as unique ingredients, possessing few calories and high vascular scavenger function without adverse organoleptic changes. Here, a two-step modification method for the development of a starch-based FRs is reported. First, native potato starch is amorphized by grinding, alkali and ethanol treatment. Then, the amorphized starch is complexed with monoglyceride. The results show that alkaline amorphous potato starch (AAPS) has the best emulsifying activity; ethanol amorphous potato starch complex (EAPSC) has the highest content of resistant starch (RS) (21.49%), while grinding amorphous potato starch (GAPS) retains the granular structure of the original starch best. The amorphization reduces the amylose content of starch, leading to reduced swelling power and increased digestibility. Complexation, on the other hand, is more like attaching a layer of the hydrophobic membrane. Combined with DSC and XRD, amorphization reduces the value of enthalpy and crystallinity, while the complexation process does the opposite. Overall, EAPSC is the best candidate for novel FRs, due to its greater emulsion stability and enzyme resistance. The experimental results provide a theoretical basis for the application of a novel potato starch-monoglyceride complex in foods such as cakes and snack fillings.

1. Introduction

Hyperlipidemia is a major global public health problem affecting two billion people worldwide, causing a high morbidity of coronary heart disease.[1] A systematic review and meta-analysis of 35 observational studies revealed that fasting hypertriglyceridemia is significantly associated with cardiovascular death, cardiovascular events, and myocardial infarction.[2] To address these problems, several varieties of fast acting and effective lipid-lowering drugs have been introduced to regulate serum lipid profiles.[3] However, their use is limited by the differences between individual patients, poor tolerance, potential adverse effects, and drug dependence.[4] Therefore, reducing fat intake has been recognized as the primary dietary intervention.[5] Simple fat reduction causes quality reduction that impedes food acceptance. Developing foods with quality characteristics similar to their higher fat counterparts has been a significant challenge. A number of water-binding fibers and gums, termed fat replacers (FRs), have the capacity to replace fat and contribute a functional human-health component.[6]

FRs are compounds incorporated into food products to provide them with some qualities of fat (such as water-binding capacity [WBC], smooth texture, and structure stability), and can be carbohydrate-, protein-, or fat-based depending on their origin. Carbohydrate-based FRs can incorporate water into a gel-type structure, resulting in lubrication and flow properties similar to those of fat,[7] therefore, are also referred to as "texturizing agents," as they can mimic fat in both physical and organoleptic properties.

Common categories of carbohydrate-based FRs include starch-derived, cellulose-based, fiber-based, and gum-based ones, among others. To better simulate the behavior of lipids in food systems, native starches can be modified with sodium octenyl succinate (OSA), which introduces an emulsifying property and lipophilic flavor to the starch granules.[8] OSA starch is a good emulsifier and can be used in the digestive system without limitation. However, high viscosity reduces its use in solid-like food systems.[9] Additionally, OSA starch is prohibitively expensive when used as an ingredient rather than an additive.

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Besides OSA starch, maltodextrin and resistant starch (RS) are two other frequently used starch-based FRs. Maltodextrin is a non-sweet, nutritive saccharide polymer, with a dextrose equivalent less than 20, and is used as ingredient in dairy products, frozen desserts, and meat products due to its ability to form soft, spreadable, thermoreversible gels with melt-in-the mouth properties.[10] However, maltodextrin has a low WBC, so a large quantity is needed. RS is a novel FR which can supply high WBC and low digestibility, but its organoleptic properties are more like those of a bony fiber. A single FR with high WBC and emulsion capacity, and, most important, low cost is still needed.

In native starch granules only a minor part of the polymer is mobile; most chains are densely packed and thus isolated from bulk water.[11] In amorphous starches, chains are more accessible to water; their structures resemble hydrogels: hydrophilic 3D networks, held together by chemical or physical bonds.[12] Can amorphization-induced flexibility increase affect emulsion capacity of starch? Will the process of amorphization increase the starch's retrogradation behavior? Can starch-lipid FR shave the above mentioned properties? As reported, hydrophobic groups of lipids can be combined with the helical structure of amylose to form starch-lipid complexes, resulting in a decreased digestibility and retrogradation behavior.[13] To investigate these questions, a two-step modification process was designed, and the effect of amorphization and complexation on the water binding, emulsion capacity, and retrogradation behavior were measured. Additionally, the structure and properties of the two-step modified starch were evaluated with a combination of small-angle X-ray scattering (SAXS), DSC, XRD, and texture profile analysis (TPA). The aim of this study was to develop a highly digestion resistant, anti-retrogradation FR which also has a high emulsion capacity. The experimental results may provide a theoretical basis for the application of potato starch-monoglyceride complex in starch products such as cakes and fillings.

2. Experimental Section

2.1. Materials

Potato starch was purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai. The water was double distilled, and the other chemicals and solvents were of analytical grade. Two kits, amylose/amylopectin and resistant/non-resistant assay kit were purchased from an import agency Equo Co. Ltd., Shanghai.

2.2. Methods

2.2.1. Potato Starch-Monoglyceride Complex Preparation

A two-step method was used to prepare the complex. First, potato starch was amorphized with different solutions under distinct reaction conditions: for ethanol amorphous potato starch (EAPS), 50% ethanol at 80 °C for 1 h; for alkaline amorphous potato starch (AAPS), a mixture of 15% Na_2SO_4 and 3%NaOH at 40 °C for 20 min; for grinding amorphous potato starch (GAPS), no additional substrate with only manual grinding was applied for 100 min. In all three starch slurries, a concentration of 20% w/v was used. Three different complexes were then performed by continuous magnetic stirring with glyceryl monostearate (5%, w/w) for 1 h at 50 °C water bath, labelled as EAPSC (ethanol amorphous potato starch complex), AAPSC (alkaline amorphous potato starch complex), and GAPSC (grinding amorphous potato starch complex), respectively. Samples were then filtered and washed with distilled water twice and lyophilized.

2.2.2. Amylose/Amylopectin Analysis

Amylose content was measured using the Megazyme amylose/amylopectin assay kit on 0.5 g of starch. Briefly, starch samples were completely dispersed by heating in dimethyl sulfoxide. Lipids were removed by precipitating the starch in ethanol and recovering the precipitated starch. After dissolution of the precipitated sample in an acetate/salt solution, amylopectin was specifically precipitated by the addition of concanavalin A (Con A) and removed by centrifugation. The amylose, in an aliquot of the supernatant, was enzymically hydrolyzed to D-glucose, which was analyzed using glucose oxidase/peroxidase (GOPOD) reagent. The concentration of amylose in the starch sample was estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample to that of total starch samples.

2.2.3. Resistant Starch Analysis

Resistant starch fraction was assayed using 0.1 g starch in the Megazyme resistant starch/non-resistant starch assay kit. Briefly, 100 mg of milled sample were incubated with thermostable pancreatic α-amylase and amyloglucosidase (AMG) at 37 °C for 16 h. During this incubation, non-resistant starch (non-RS) was solubilized and hydrolyzed to glucose by the two enzymes. The reaction was terminated by the addition of equal volume of aqueous ethanol and the RS was recovered as pellet on centrifugation. The supernatants of this centrifugation and those of two consecutive washings were removed by decantation and then adjusted the volume to 100 mL. Digested starch was quantitatively determined at 510 nm using the GOPOD method. RS pellets were dissolved in 2 M KOH and stirred for 20 min in an ice/water bath over a magnetic stirrer. Sodium acetate buffer (1.2 M, pH 3.8) was added, and then RS samples were incubated with AMG for at 50 °C for 30 min. The reaction was terminated by the addition of equal volume of aqueous ethanol, and RS was similarly quantitatively determined at 510 nm using GOPOD method. Total starch was calculated as the sum of RS and non-RS.

2.2.4. Scanning Electron Microscopy

Starch surface morphology was examined using a FEI NOVA Nano SEM operating at an accelerating voltage of 5 kV. Prior to scanning, dried starch samples were deposited on copper stubs with adhesive and coated with 150 Å gold/palladium particles.

2.2.5. Swelling Power and Water Solubility Index

Swelling power (SP) and water solubility index (WSI) were determined following the modified method of Schoch.[14] In brief, 30 mL of 2% w/v starch (W_1) suspension were heated in a water bath at 80 °C for 30 min. The slurry was then cooled and centrifuged at 3000 × *g* for 30 min. The supernatant was evaporated in an air oven at 105 °C for 3 h. Then the wet sediment (W_2) and dried supernatant (W_3) were weighed. SP and WSI were calculated as follows.

$$\text{SP (g g}^{-1}) = \frac{W_2}{W_1} \quad (1)$$

$$\text{WSI (g 100 g}^{-1}) = \frac{W_3}{W_1} \times 100 \quad (2)$$

2.2.6. Water-Binding Capacity

0.5 g starch (W_1) at a concentration of 5% w/v starch suspension was prepared with continuous agitation at room temperature for 2 h. Then the starch slurry was centrifuged at 6000 × *g* for 10 min. The supernatant was carefully decanted, and the wetted starch was weighed as W_2. The WBC were calculated as follows.

$$\text{WBC (g g}^{-1}) = \frac{W_2 - W_1}{W_1} \quad (3)$$

2.2.7. Emulsifying Activity Index and Emulsifying Stability Index

The emulsifying activity index (EAI) and emulsifying stability index (ESI) values of emulsifiers were determined according to the literature with slight modification.[15] First, 1% starch slurry was completely gelatinized at 90 °C for 30 min. After the starch solution was cooled to room temperature, 4 mL soybean oil was added to 16 mL of starch solution, and the mixture was homogenized at 1000 rpm for 5 min. A portion of this emulsion (100 µL) was diluted 100 times with sodium dodecylsulfate solution (0.1%, w/w), and measured by a UV–vis spectrophotometer at 500 nm. The EAI and ESI were obtained using the following equations.

$$\text{EAI}_{\max} \text{ (m}^2 \text{ g}^{-1}) = \frac{2 \times 2.303 \times A_0 \times \text{DF}}{c \times l \times \phi \times 10\,000} \quad (4)$$

$$\text{ESI} = \frac{A_0}{(A_0 - A_1)} \quad (5)$$

where A_0 and A_1 are observed absorbance of emulsion at 0 min and 1 day; DF is dilution factor, c is emulsifier concentration (g mL^{-1}), l is optical path (0.01 m), and Φ is the volume fraction of the oil phase.

2.2.8. Texture Profile Analysis of Starch Gel

Starch slurries of 4% w/v concentration were heated at 90 °C for 30 min, and then cooled down to room temperature. Dry starch was added to the above solution to a final concentration of 20%. The suspension was stirred carefully and then sonicated at 50 °C for 40 min to make a homogeneous solution without obvious particles and foams. The degassed suspension was transferred to a mold and kept at 120 °C for 30 min in a sterilizer. After the starches were completely gelatinized and cooled, the starch gels were taken out of the mold. TPA of the fresh starch gels and stored gels were performed using a TA-XT PLUS texture analyzer, which measures texture attributes like hardness, cohesiveness, elasticity, resilience as described in ref. [16]. A P/50 cylindrical probe was used to compress the gel at a pre-test speed of 2 mm s^{-1} until 5 *g* force was achieved, then in the test stage the sample was compressed at 1 mm s^{-1} until 30% compression was reached. The waiting time between the first and second compression cycle was 2 s.

2.2.9. Differential Scanning Calorimetry Analysis

Starch was dispersed in distilled water (1:2; w/v) in an aluminum pan, which was then hermetically sealed and equilibrated overnight. The NETZSCH Phoenix DSC (NETZSCH 204 F1) was calibrated for temperature and for enthalpy measurements, and an empty pan was used as the reference. A DSC thermogram was recorded over the temperature range of 20–120 °C with a heating rate of 5 °C min^{-1}. The software provided by the manufacturer was used to calculate the enthalpy of the endothermic peak. The onset (T_o), peak (T_p), and completed (T_c) temperatures of the gelatinization peak were determined from the intersection of tangents fit to the leading and trailing flanks of the peak with the baseline.

2.2.10. X-Ray Diffraction Analysis

XRD experiments were performed with a Bruker-AXS D8 ADVANCE powder diffractometer using Cu-Kα radiation (wavelength $\lambda = 1.54$ nm) and operating at 40 kV and 20 mA. Data was collected by step scanning at 0.02° intervals over the 2θ range of 4–40°. The crystallinity degree was estimated using the nonlinear peak fitting method.[17]

2.2.11. Small-Angle X-Ray scattering analysis

Samples for SAXS measurements were prepared as aqueous starch pastes (≈45% w/v starch) centrifuged at 8000 × *g* for 10 min after an overnight equilibration. The SAXS experiments were conducted on beamline BL16B1 of the Shanghai Synchrotron Radiation Facility. An incident wavelength of 1.24 nm was used, and the sample-to-detector distance was set to 2150 mm. Scattering was detected in the q ranges of 0.06–1.88 nm^{-1} (where $q = (4\pi \sin\theta)/\lambda$, the scattering vector). The isotropic scattering patterns were radially averaged, empty scattering was subtracted, and the resulting SAXS intensity was analyzed as a function of the scattering vector q.

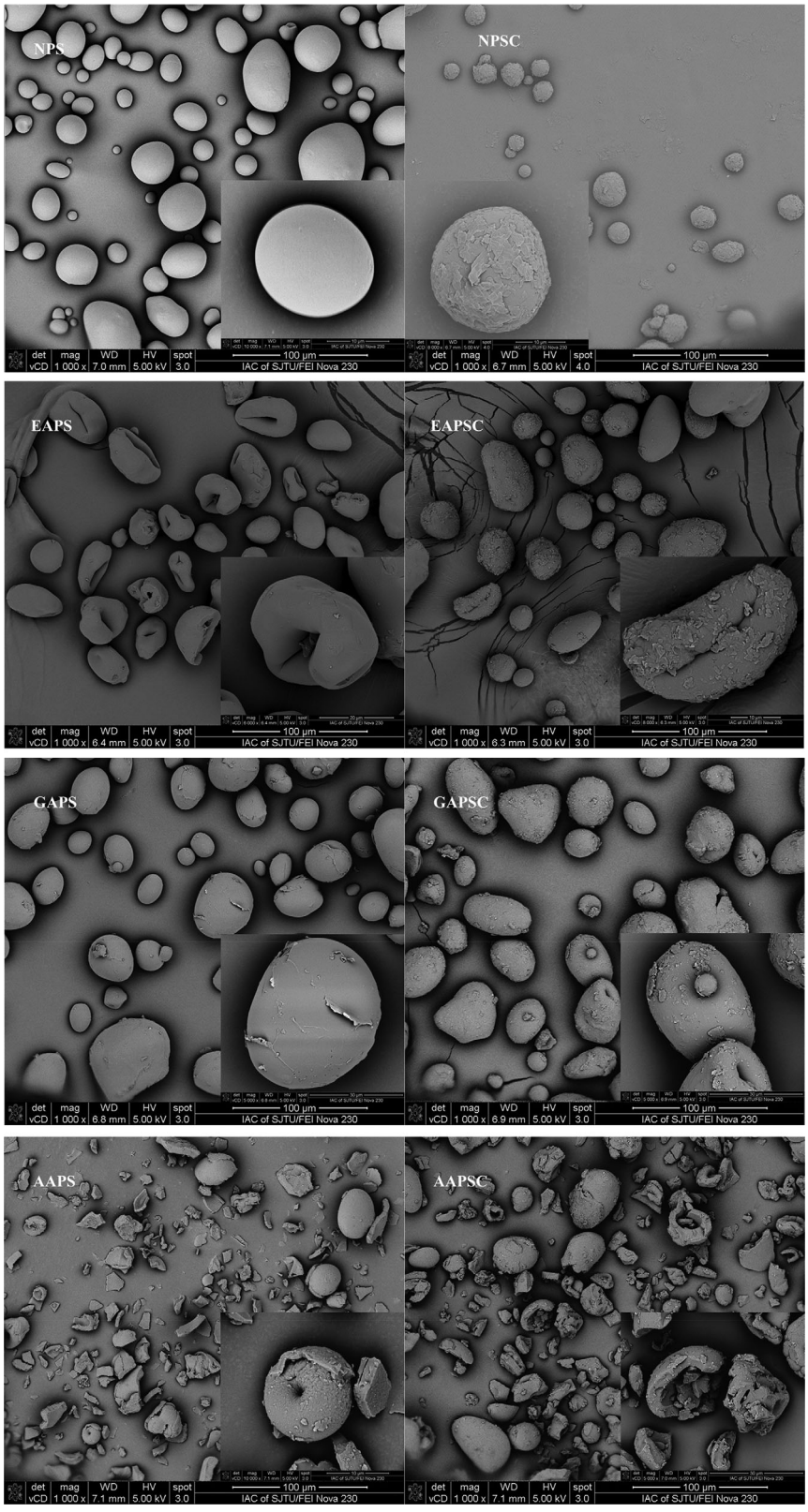

Figure 1. Surface morphology of native potato starch and its derivatives.

3. Results and Discussion

3.1. Surface Morphology

In this study, scanning electron microscopy (SEM) was employed to examine the granular morphology changes of granular starches. Micrographs are shown in **Figure 1**. Native potato starch (NPS) were spherical or oval with smooth surfaces and significant variations in size. Smaller granules were more spherical, with sizes between 10 and 20 μm; while larger granules were oval and were sized between 20 and 55 μm. The process of amorphization made the starch granular surface more coarse, adding depressions and debris. Among the three amorphization methods, grinding is the mildest, leaving some slices on the smooth surface. This indicates that GAPS has a similar result to someone peeling the starch granules at a micrometer level. Compared to the native granules, there was a big and deep hole in EAPS leaving the starch granules like a hollow potato, but this hole does not start from a papillary which was found in maize.[18] In ethanol amorphization, ethanol has been shown to restrict the swelling of starch granules by decreasing the effective water concentration and can also serve as a complexing agent to stabilize the dissociated starch chains; in contrast, thermal treatment is able to destroy crystalline structure of the granules.[18,19] The EAPS sample is thus a balance between those two results. For AAPS, alkaline treatment was so severe that the starch granules swelled too much to recognize their shapes and outlines, leaving only debris of various sizes. Compared to amorphization methods, complexation had a similar result in GAPS, EAPS, and AAPS, the changes are more like a wrap of layers of monoglyceride.

3.2. Amylose Determination

The relative content of amylose and amylopectin were analyzed using megazyme kit. As shown in **Table 1**, both grinding and alkaline amorphization reduced amylose content, while no significant changes was observed in EAPS. This step thus might attribute to the amylose leaching. Similarly, the second step—complexation reduced amylose content due to the formation of amylose-glyceride complex. Compared to NPS, a reduction of 31.84% amylose was achieved in AAPSC, 20.34% in GAPSC, and 14.48% in EAPSC. It is easy to understand why AAPSC showed the lowest amylose content, as it was not in an intact granular form. Amylose chains are more flexible without structural limitation, and thus will leach out of the starch matrix easily during the first amorphization process. Among the three complexes, samples of GAPSC experienced the lightest treatment. However, the amylose content of GAPSC was not the highest; instead, fully swollen EAPSC had the highest amylose content. This result was very interesting, demonstrating that amylose content does not depend solely on the extent of amorphization. Compared to EAPSC, samples of GAPSC had a lower content of amylose but preserved the granular contour. Overall consideration of the SEM surface morphology was used to investigate the cause of this phenomenon. GAPS had a coarse surface with peeling starch, while EAPS was smoother with a middle hollow. Prior work reported that amylose was more concentrated at the periphery in potato starch. Mechanical grinding may then severely have destroyed the surface of potato starch, leading to amylose leaching.

Table 1. The amylose/amylopectin and resistant/non-resistant starch of potato starch and its derivatives.

Starch	Amylose [%]	Amylopectin [%]	Resistant starch [%]	Non-resistant starch [%]
NPS	18.09 ± 0.57	81.91 ± 0.57	14.25 ± 0.31	81.11 ± 1.77
NPSC	16.45 ± 0.49	83.55 ± 0.49	20.05 ± 0.93	70.21 ± 0.56
AAPS	16.01 ± 0.53	84.00 ± 0.53	1.44 ± 0.12	82.34 ± 1.10
AAPSC	12.33 ± 0.15	87.67 ± 0.15	12.81 ± 0.11	79.89 ± 0.56
GAPS	14.23 ± 1.16	85.72 ± 1.21	14.29 ± 0.00	74.04 ± 0.67
GAPSC	14.43 ± 0.01	85.58 ± 0.01	20.41 ± 0.28	70.96 ± 0.16
EAPS	17.84 ± 0.27	82.17 ± 0.27	11.81 ± 0.08	77.90 ± 0.16
EAPSC	15.47 ± 0.83	84.53 ± 0.83	21.49 ± 0.24	69.47 ± 0.51

3.3. Digestibility Analysis

Increased susceptibility toward digestion upon amorphization was noted in changes of RS, as shown in Table 1. Among the three amorphized potato starch, AAPS had the lowest RS due to disrupted starch crystallites. This can probably be ascribed to the increased molecular mobility by modification. Therefore, amorphization extent is not linearly correlated with amylose content but, but is linearly correlated with RS content. However, monoglyceride complexation significantly increased RS level. Compared to NPS (RS 14.25%), GAPSC and EAPSC had an RS of 20.41% and 21.49%, an increase of 43.23% and 50.81%, respectively. The added monoglyceride was complexed with amylose, thereby partly restricting accessibility of starch chains to the hydrolyzing enzymes. This low susceptibility to outer treatment makes RS an efficient nutritional ingredient. This characteristic is very similar to that of starch crystals. As such, the correlation between RS and crystallinity will be discussed carefully in the following section.

3.4. Physicochemical Properties

The physicochemical properties of the starches, such as SP, WSI, and WBC, are presented in **Table 2**. It is generally accepted that SP is closely related to the free space within and outside starch molecules, and amylose has been hypothesized to disturb the lamellar organization in starches, allowing increased water invasion.[20] However, in our system, this deduction is not always true. It follows that NPS has the highest SP, followed by AAPS and GAPS, then EAPS. The lowest SP was found in the three complexed starches (with nearly the same value). The low SP of EAPS compared with that of AAPS and GAPS, was ascribed to its big middle hollow, which do not have enough water retaining ability. The WSI of potato starch derivatives had the following order: EAPSC ≈ GAPSC ≈ AAPSC < EAPS ≈ GAPS ≈ NPS < AAPS. Hot water leaches amylose more easily from AAPS (11.01 g per 100 g) than NPS (8.75 g per 100 g), demonstrating

Table 2. The physicochemical properties of potato starch and its derivatives.

Starch	WSI [g^{-1} 100 g^{-1}]	SP [g^{-1} g^{-1}]	WBC [g^{-1} g^{-1}]	EAI [m^2 g^{-1}]	ESI
NPS	8.75 ± 1.26 b	36.81 ± 0.42 a	5.29 ± 0.14 a	117.45 ± 5.18 c	0.02089 ± 0.0028 d
NPSC	2.39 ± 0.04 e	7.67 ± 0.36 d	2.82 ± 0.23 c	124.36 ± 6.91 c	0.02335 ± 0.0004 cd
AAPS	11.01 ± 1.02 a	16.23 ± 0.78 b	0.68 ± 0.05 e	346.60 ± 44.91 a	0.02595 ± 0.0012 cd
AAPSC	5.19 ± 0.21 d	7.49 ± 0.63 d	1.77 ± 0.02 d	159.48 ± 4.61 c	0.07323 ± 0.0058 a
GAPS	8.53 ± 0.74 bc	15.52 ± 1.03 b	3.72 ± 0.05 b	133.00 ± 2.31 c	0.02870 ± 0.0013 cd
GAPSC	4.32 ± 0.21 de	7.63 ± 0.19 d	3.01 ± 0.12 c	125.23 ± 8.92 c	0.04765 ± 0.0029 b
EAPS	6.43 ± 0.33 cd	12.33 ± 0.69 c	1.80 ± 0.03 d	216.48 ± 12.67 b	0.0112 ± 0.0005 e
EAPSC	2.79 ± 0.21 e	7.32 ± 0.73 d	2.84 ± 0.09 c	133.42 ± 4.03 c	0.03087 ± 0.0013 c

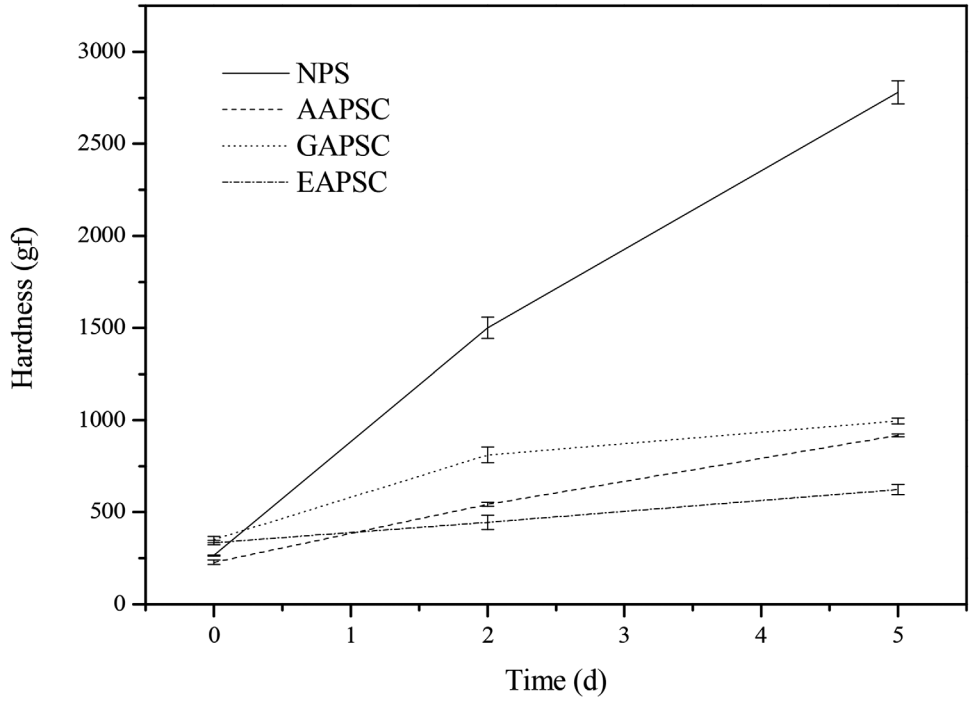

Figure 2. Hardness changes of native potato starch and its derivatives.

that the granular structure limits the dissolution of starch molecules. As stated in the first section, the monoglyceride complexation is similar to attaching a layer of hydrophobic membrane to that of starch granules, thus it reduced WSI. For EAPS and AAPS, monoglycerine complexation slightly increased the WBC, due to the hydrophobic membrane water holding behavior. For NPS and GAPS, the integrity of the starch granules restricted the WBC.

3.5. Emulsifying Activity and Emulsifying Stability

The EAI and ESI values of potato starch derivatives are also summarized in Table 2. The EAI value of the emulsion stabilized by AAPS is the highest, followed by EAPS, and there was no significant difference between the remaining starches. AAPSC had the highest ESI value, then GAPSC, with the ESI similar among the remaining starches. Amorphization increased EAI value, probably due to the increased starch chain flexibility. Complexation, on the other hand, reduced EAI value and increased ESI, in all samples aside from EAPS, due to the steric repulsion and hydrophobic force from long chain alkyl chains.[21]

3.6. The Texture Profile Analysis

The TPA test revealed no significant difference in elasticity and cohesiveness between different starches, and thus in this study, only hardness and resilience were used for texture analysis since gumminess and chewiness are the comprehensive behavior of hardness, elasticity, and cohesiveness. The texture properties of potato starch and its derivatives are shown in **Figure 2**. It is clear that modification significantly reduced the hardness of starch gels. A marked decrease in hardness was observed when the

Table 3. The gelatinization behavior and crystallinity of potato starch and its derivatives.

Starch	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J g^{-1}]	Crystallinity
NPS	59.46	62.9	68.2	9.308	24.43
NPSC	53.35	57.46	61.23	5.836	19.61
AAPS	/	/	/	/	0
AAPSC	53.39	55.55	59.02	0.6905	5.16
GAPS	52.44	55.80	59.67	5.519	12.42
GAPSC	54.18	58.58	60.88	3.129	17.16
EAPS	/	/	/	/	12.91
EAPSC	54.09	55.09	56.53	0.741	17.66

gels were stored at 4 °C for 2 days; the reduction was 70.36% for EAPSC, 63.83% for AAPSC, and 45.95% for GAPSC. The two-step modification process will impede retrogradation, which can be confirmed by reduced gel hardness compared to NPS. As reported,[22] long chain molecules (amylose and super long chain amylopectin) could provide favorable inter/intramolecular interactions of starch with itself, thus affecting the rigidity of the starchy food. As such, the hardness increased as amylose content increased.

3.7. Differential Scanning Calorimetry

It has been reported that gelatinization properties of starch is closely related to the melting properties of crystallites.[23] Therefore, these crystallites may have a significant influence on the physicochemical properties of modified starches. The parameters determined from the DSC thermograms (i.e., onset temperature [T_o], peak temperature [T_p], completion temperature [T_c], and enthalpy of gelatinization [ΔH]) are summarized in **Table 3**. Peak temperature T_p, which corresponds with gelatinization temperature, varied within the potato starch derivatives. Native starch has the highest value of peak gelatinization temperature, which suggests that more thermal energy is required to initiate gelatinization. However, no gelatinization peak was observed in the samples of AAPS and EAPS; these two treatments were so severe that they caused fragmentation of starch structure, disrupting short-range order and making gelatinization peaks disappear. However, the complex prepared from these two starches and monoglyceride did show two weak peaks (shown in **Figure 3**). The transition enthalpy, ΔH, which reflects the loss of double helical and crystalline order, was also compared. Both amorphization and the complexation process reduced the value of enthalpy. The decrease in ΔH in samples of potato starch derivatives relative to that of NPS suggests that double helices that are aligned

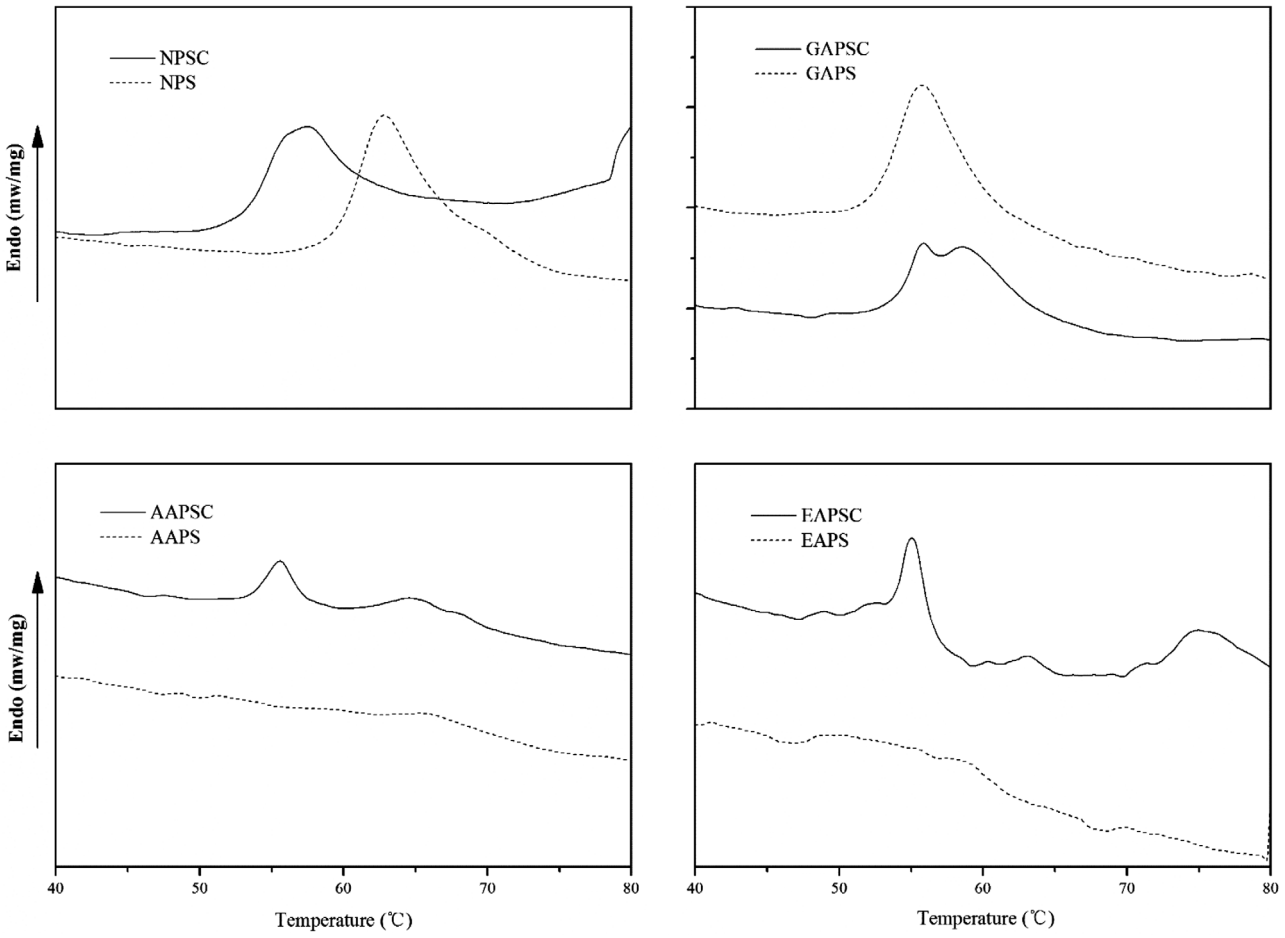

Figure 3. Thermograms of native potato starch and its derivatives.

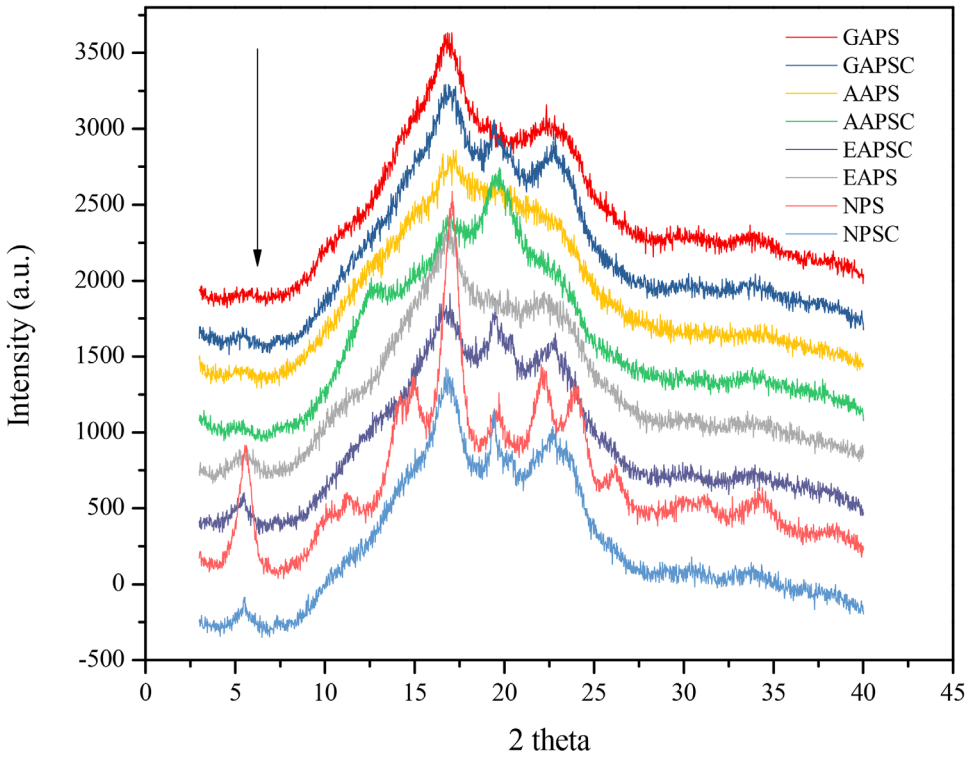

Figure 4. The X-ray diffraction diagram of potato starch and its derivatives.

within the crystalline lamella are more strongly associated in NPS.

3.8. XRD Analysis

The crystal types of the starches are divided into three types (A, B, and C) based on their XRD patterns. A strong doublet peak at $2\theta \approx 17$–$18°$ is characteristic of A-type allomorphs, whereas a pronounced peak at $2\theta \approx 5°$ and a broad peak at $2\theta \approx 23°$ is characteristic of B-type allomorphs.[24] Potato starch is typically a B-type starch, with strong reflections at 5° and 17° 2θ and a broad double peak with medium intensity at 23° 2θ, as shown in **Figure 4**. Samples of AAPS did not exert any crystalline pattern due to the complete disruption of starch crystallites. However, a crystallinity increment of 5% (exactly the amount of added monoglyceride) was observed after monoglyceride complexation. Therefore, we can infer that complexation did not have any synergistic effect on the crystallinity.

All complexed starch analyzed exerted a stronger diffraction peak at $2\theta \approx 20°$, which was ascribed to the presence of amylose-lipid complex (Vh type).[25] As shown in Table 3, the degree of crystallinity differed significantly between these starches. NPS has crystallinity of 24.43%, which is in agreement with the values determined using the two-phase method, but is considerably lower than the values determined using the crystal-defect method.[26] Samples of EAPS and GAPS have similar crystallinity with varying amylose content, demonstrating that amylose is not the only one factor that influence the crystallinity.

3.9. SAXS Analysis

SAXS can provide information about hydration-induced changes in amorphous starches. Therefore, in order to investigate the structural changes of potato starch during the two-step modification, SAXS analysis was employed. **Figure 5** shows the SAXS morphology of potato starch and its derivatives, the last of which is monoglyceride. From the figure we can see that a lamellar peak of approximately $q = 0.65$ nm^{-1} appeared in samples of NPS, NPSC, GAPS, and GAPSC; no clear peak was observed in samples of EAPS, EAPSC, AAPS, and AAPSC. Though EAPS has a similar crystallinity to GAPS, SAXS, and XRD are based on a different structural scale. XRD is a powerful tool for evaluating short-range ordering structures, while SAXS is useful for the characterization of long-range ordering structure. Besides AAPSC and GAPSC, the absorption peaks of monoglyceride appeared at $q \approx 1.30$ nm^{-1}. This might be due to the complexation mechanism; in the samples of EAPSC and NPSC, monoglycerides are loosely linked with starch granules, maintaining the long-range ordering of the two molecules. Due to the amorphization, it is inappropriate to use 1D correlation function to infer the detailed lamellar structure, so discussion here is limited to SAXS morphology differences.

3.10. Amorphization and Complexation Mechanism

By combining the physicochemical properties presented in Tables 1 and 2 and the debris stages in Figure 1, the breaking

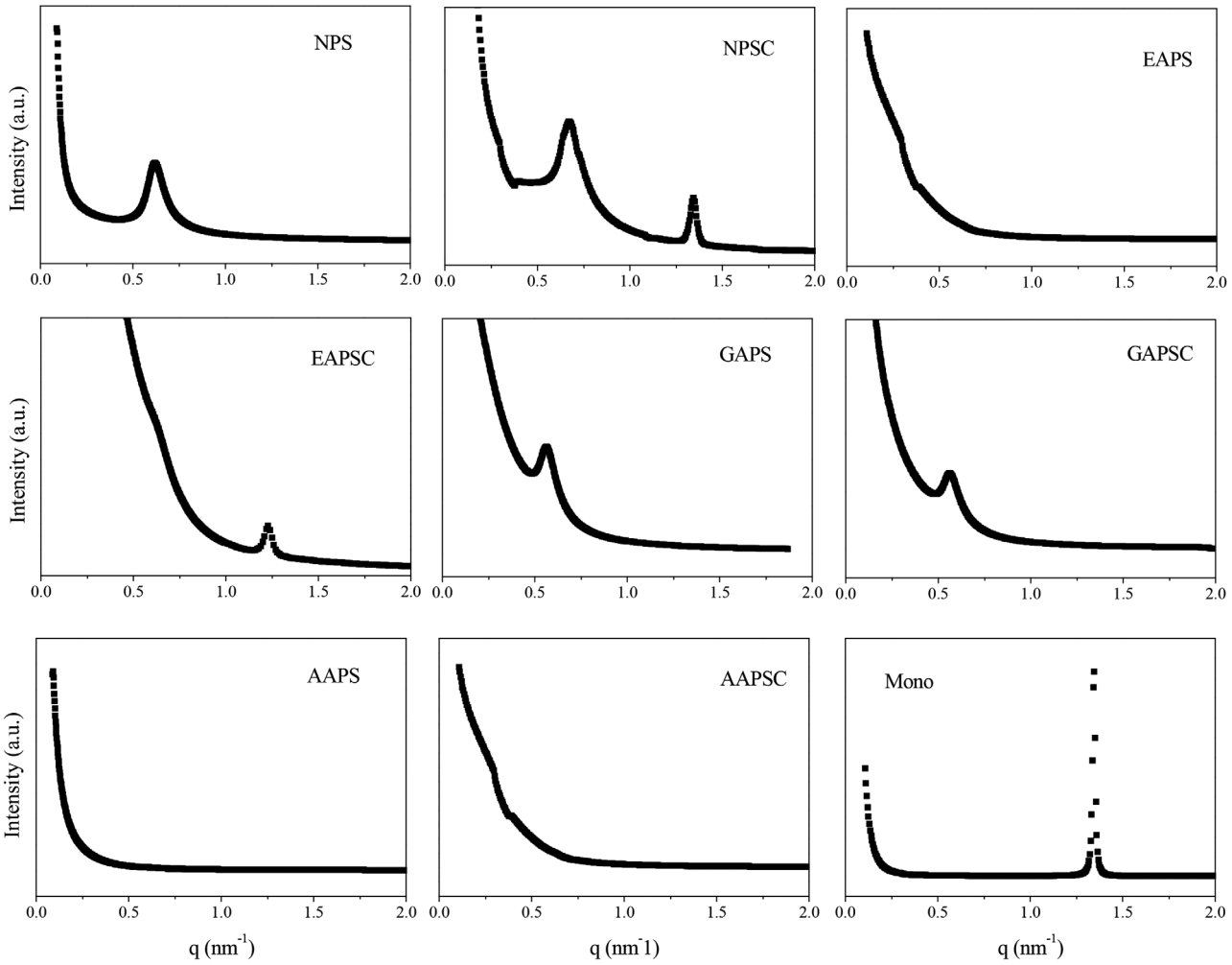

Figure 5. SAXS patterns of potato starch and its derivatives.

mechanism of starch granules can be predicted. It is clear that starch granules first become swollen, followed by the disappearance of Maltese cross-like birefringence, and then a hollow in the middle of granules was formed due to the energy absorbed from the heat treatment or external pressure. When the growth of the hollow extended to the other side of granular surface, it resulted in a disruption of granules. Whether the starch granules are completely disrupted depends on the amount of energy absorbed. Energy absorbed by granules not only swells the starch granules, but also facilitates "rearrangement," or formation of new bonds between molecule; under certain conditions, it will also melt crystallites. During the second modification step, monoglyceride was complexed to the surface of amorphous starch, leading to a coarse surface with fragment outside. In the samples of GAPSC, EAPSC, and AAPSC, the starches were surrounded by monoglyceride fragment. The result was very similar to the cassava starch-monoglyceride complex reported previously.[27] Therefore, it was inferred that simple complexation does not exert severe changes on the starches; it is more like attaching a layer of hydrophobic membrane. Compared to gelatinization, the process of amorphization shifts the peak temperature to a lower value, probably due to the breakdown of starch crystallites. The complexation process then helped maintain the starch crystallites. This conclusion could be confirmed by the crystallinity determined from XRD.

4. Conclusion

As a whole, we have introduced a two-step modification to develop a starch-based fat replacer with high emulsifying activity and anti-retrogradation behavior. On the one hand, the added monoglyceride formed a complex with amylose, thereby partly restricting accessibility of starch chains to the hydrolyzing enzymes. On the other hand, amylose-monoglyceride complex acts as an amphiphilic molecule, yielding a good balance between emulsifying activity and stability. Further work is underway to obtain the separation and wettability assessment of the amylose-monoglyceride complex, which might aid in the discovery of the formation mechanism and the development processes for designing novel fat replacer.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

amorphous, emulsify, fat replacers, monoglycerides, potato starch

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