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Effect of annealing on starch–palmitic acid interaction

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Abstract

The effects of annealing on the complexing of added palmitic acid (PA) and on the physicochemical properties of starch were studied. Palmitic acid was used because of its predominance in starch lipids. Starches naturally low in lipids, including potato, tapioca, and waxy corn, were subjected to the PA treatment. Common corn starch, which is high in lipids, was also included for comparison. More PA was complexed by annealed starches than by native starches and the amount of bound PA was mainly influenced by the amylose content. The introduction of PA decreased the gelatinization temperature and increased the gelatinization range of both native and annealed potato and tapioca starches. Leaching of amylose was reduced by the annealing treatment and further decreased by adding PA. Annealing did not change the swelling power of the PA-complexed starches. A small portion of the added PA formed complexes with amylopectin and still remained in the Naegeli dextrins after 10 days of acid hydrolysis. The reorganization of starch molecules with starch granules from annealing strongly affected the amount of complexed PA and some physicochemical properties of the introduced starches.

Keywords: Starch; Annealing; Naegeli dextrins; Fatty acid introduction; Physicochemical properties

1. Introduction

Starch annealing is often described as perfection of the amorphous and crystalline lamellae of starch granules. Annealing is defined as incubation of starch in an excess water at a temperature above the glass transition but below the gelatinization of the starch (Yost & Hoseney, 1986), which implies that amorphous glassy starch molecules become mobile and reorganize to form improved crystalline structure during annealing. Two possible mechanisms for this amorphous and crystalline perfection have been proposed: improved alignment of amylopectin double helices within the crystalline lamellae and enhanced glassy structure of the amorphous lamellae (Tester & Debon, 2000). However, there is still great uncertainty about the annealing process at the molecular level and the location of glass transition temperature.

Lipids are commonly present throughout starch granules and usually consist of a mixture of free fatty acids (FFAs) and lysophospholipids (LPL) (Morrison, Tester, Gidley, & Karkalas, 1993), which amount to 1.5% in cereal starches (Morrison, Tan, & Hargin, 1980). In Triticeae, such as wheat, barley, and ryes, lipids are mostly LPL with variable amounts of FFAs, whereas other cereal starches such as corn and rice contain a greater proportion of FFAs (Morrison, 1988). Lipids are associated with starch in several ways and can be classified into three groups: starch lipids, starch surface lipids, and non-starch lipids. Lipids inside native starch granules are called starch lipids; lipids on the surface of starch granules are called starch surface lipids; lipids other than the above two categories are called non-starch lipids, which normally come from aleurone and germ (Morrison).

FFAs in the starch lipids can be extracted with hot polar solvents such as aqueous alcohols but not with low polarity solvents such as ethanol and chloroform (Morrison & Coventry, 1985). Internal starch lipids have been shown to occur as inclusion complexes inside the helical segments of amylose (Morrison, 1981). A large amount of FFAs in starch is usually associated with a greater proportion of linoleic acid, whereas a smaller amount of FFAs is usually associated with a greater proportion of palmitic acid (PA) (Fujimoto, Nagahama & Kanie, 1972). A mixture of six types of FFAs (myristic acid, palmitic acid, stearic acid, oleic acid, linoleic, and linolenic acid) were artificially introduced into defatted potato, sweet potato, wheat, common corn, tapioca, and rice starches, and it was discovered that there was little difference

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among the starch samples in total quantity and composition of the fatty acids incorporated, but a great difference in the penetration rate (Fujimoto et al). A greater proportion of artificially introduced FFAs was found in the amorphous lamellae than in the crystalline lamellae in potato, teppo-yuri, and gajyutu starches (Kitahara, Suganuma, Fujimoto, & Nagahama, 1993). Hoover and Hadziyev (1981) also suggested that potassium salt of myristic acid was the best fatty acid to form complex with amylose; however, myristic acid is not a naturally occurring FFA in starch granules. More saturated fatty acids such as myristic acid (14:0) and PA (16:0) were introduced into starch granule than unsaturated fatty acids such as linoleic acid (18:2) and linolenic acid (18:3) (Fujimoto et al; Kaneda, Kitahara, Suganuma, & Nagahama, 1996). The steric hindrance from the double bonds in unsaturated fatty acids may decrease the amount of FFA introduced into starch granules (Hoover & Hadziyev).

The objective of this study was to investigate the effect of annealing on the amount and location of introduced PA. Starches naturally low in FFAs, including potato, tapioca, and waxy corn, were studied. Common corn starch was also included for comparison.

2. Materials and methods

2.1. Materials

Potato and tapioca starches were from Staley Manufacturing Company (Decatur, IL). Common corn and waxy corn starches were from Cerestar USA, Inc. (Hammond, IN). Palmitic acid was purchased from Sigma Chemical Co. (St Louis, MO).

2.2. Preparation of annealed starch

Starches were annealed by a multi-step process. One hundred grams of starch and 300 ml of distilled water were placed in a 250-ml beaker, covered with aluminum foil, and incubated at 40 °C for 24 h, 45 °C for 24 h, and finally at 50 °C for 24 h. After the annealing treatment, starch samples were filtered through a Whatman No. 4 filter paper and dried at room temperature.

2.3. Complexing of palmitic acid by starch

The complexing of palmitic acid (PA) to the starch granule followed the procedure of Kitahara et al. (1993). Unmodified or annealed starch (3 g, db) was suspended in 20 ml of 85% methanol containing 1% PA (v/v based on methanol). The suspension was stirred with a magnetic stirrer at 30 °C for 3 h and then at room temperature for an additional 24 h. After filtration with a Whatman No. 4 filter paper, the treated starch was washed with water-saturated 1-butanol (WSB, 33:67 water:1-butanol mixture) by shaking a suspension of starch (3 g) in WSB (50 ml) on a rotary shaker for 2 h to remove free lipids on the starch surface (Morrison, 1981), centrifuged at $1000 \times g$ for 10 min, washed with 10-fold volume of deionized water 3 times, centrifuged at $1000 \times g$ for 10 min, and dried at room temperature.

2.4. Determination of starch palmitic acid content

The amount of artificially introduced PA in the starch was determined using a NEFA-C test kit (Wako, Osaka). Starch (300 mg, db) was hydrolyzed with 2 ml of 0.1 M HCl at 100 °C for 2 h. After acid hydrolysis, the solution was cooled to room temperature and 2 ml isopropanol was added. Fifty microliters of hydrolyzed sample was transferred into a 10-ml screw cap test tube, and was added with 350 μl of deionized water and then 1 ml of color reagent A. Color reagent A consisted of 0.3 U ACS (acyl-coenzyme A synthetase), 3 U AOD (ascorbate oxidase), 0.7 mg CoA (coenzyme A), 3 mg ATP (adenosine triphosphate), and 0.3 mg 4-aminoantipryne in 1 ml phosphate buffer (pH 6.9). The sample was then incubated at 37 °C for 10 min, and 2 ml color reagent B was added. Color reagent B contained 6.6 U ACOD (acyl-coenzyme A oxidase), 7.5 U peroxidase, and 0.06 mM MEHA (3-methyl-*N*-ethyl-*N*-(β-hydroxyethyl)-aniline) in 10 ml phosphate buffer (pH 6.9). The solution was kept at room temperature for 20 min and then absorbance at 550 nm was measured. Solutions of PA (400 μl) of 5, 10, 20 mg/l were used to construct the standard curve. Hydrolyzed solutions of 200 μl with the addition of 200 μl deionized water were used for starch samples low in FFAs, including native and annealed potato, tapioca, and waxy corn, and PA-treated native and annealed waxy corn starches. Hydrolyzed solutions of 25 μl with the addition of 375 μl deionized water were used for starch samples high in FFAs, including native, annealed, and PA-treated common corn starches.

The principle of the NEFA-C test kit is that saturated and unsaturated FFAs can be quantified colorimetrically based on the acylation of CoA by fatty acids in the presence of added acyl-CoA synthetase. The acyl-CoA produced is then oxidized by acyl-CoA oxidase with generation of H_2O_2.In the presence of peroxidase hydrogen peroxide permits the oxidative condensation of MEHA with 4-aminoantipyrine to form a purple colored product that can be measured at 550 nm. The amount of fatty acids as measured by the NEFA-C test kit was reported as total FFAs. The amount of introduced PA was the difference in total FFAs before and after the PA treatment.

2.5. Starch gelatinization characteristics

The gelatinization characteristics of annealed and native starches after PA introduction were measured and recorded by a Perkin–Elmer Pyris-1 differential scanning calorimeter (Perkin–Elmer, Norwalk, CT). Starch (approximately 4.0 mg, db) was placed in an aluminum pan and deionized

water (8.0 μl) was added with a micro-syringe. The starch sample was sealed, equilibrated at room temperature for at least 1 h, and then scanned from 20 to 140 °C at a heating rate of 10 °C/min. The DSC software computed the onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy (ΔH) of the gelatinization endotherm.

2.6. Starch leaching and swelling characteristics

The amounts of leached amylose and swelling power of treated and untreated starches were determined. The sample preparation followed the method of Doublier (1981). Starch (0.5 g) was suspended in deionized water (18 ml) in a test tube placed in a water bath at 50, 60, 70, 80, 90 and 100 °C, and held for 30 min. After centrifugation at 1000 × g for 10 min, the amount of leached amylose was determined by a colorimetric method (Juliano, 1971). Swelling power was determined by dividing the sedimented paste weight by the dry starch weight.

2.7. Susceptibility to acid hydrolysis

The susceptibilisty to acid hydrolysis of the native and annealed starches after the introduction of PA was determined. Starch was suspended in 15.3% H_2SO_4 (5 g dry starch/100 ml) in a 250-ml beaker. The beaker was covered with parafilm and placed in a water bath at 38 °C for 2, 4, 6, 8, 10, 20, and 30 days with gentle shaking by hand daily. The supernatant was analyzed for total carbohydrate by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The FFAs content of the Naegeli amylodextrins after 10 days of acid hydrolysis was analyzed using the NEFA-C test kit.

3. Results and discussion

3.1. Complexing of palmitic acid by starch

The amounts of FFAs and the PA introduced in different starches are presented in Table 1. The endogenous FFA content was high in common corn starch, low in tapioca starch, and very low in potato and waxy corn starches. The annealed starch contained a similar amount of FFAs inside the starch granule as its native counterpart, suggesting annealing did not change the FFA content in starch. The PA treatment increased the amount of FFAs for both native and annealed starches. The amount of PA introduced differed among different starches (common corn > potato > tapioca > waxy corn for both native and annealed starches) and more PA was bound by annealed starches than by native starches, except waxy corn starch. The apparent amylose contents of the native and annealed starches were determined and reported in our previous study (Nakazawa & Wang, 2003). The apparent amylose content of native common corn, potato, tapioca, and waxy corn starches were 28.0, 22.5, 17.2, and 0.6%, respectively, and the apparent amylose contents of annealed potato, tapioca, common corn, and waxy corn starches were 24.6, 18.1, 13.0, and 0.4%, respectively. There was a strong positive correlation between the apparent amylose content and the amount of PA complexed for different starches with a coefficient of determination (R^2) of 0.78 for the native starches and 0.91 for the annealed starches. Hence, the capacity of accommodating introduced FFAs in starches was primarily a property of amylose. Nevertheless, amylopectin was also involved in interacting with FFAs as evidenced by the presence of added PA in native (23.4 mg/100 g starch) and annealed (24.4 mg/100 g starch) waxy corn starches, indicating the occurrence of amylopectin–lipids complexes. It was previously demonstrated that a small amount of the introduced PA in 14 low-lipid starches was present in their respective Naegeli amylodextrins (Kaneda et al., 1996). Because Naegeli amylodextrins mainly consist of the acid-resistant crystalline lamellae, the presence of FFAs in Naegeli amylodextrins indicated the interaction between amylopectin branch chains with FFAs.

Annealing is described as the perfection of the crystalline and amorphous lamellae such as optimization of crystalline order (Yost & Hoseney, 1986). It was suggested that the capacity of the whole starch granules to complex FFAs was dependent on the capacity of the amorphous lamellae (Kitahara et al., 1993). Our previous work suggested that annealing could result in more porous and accessible structure in both the amorphous and the crystalline lamellae because annealing increased the acid susceptibility of native

Table 1
Free fatty acid contents (mg/100 g starch) of native, annealed, and palmitic acid (PA)-treated starches

Starch	Free fatty acid content (mg/100 g starch)					
	Native starch	Native starch after PA addition	PA addition in native starch	Annealed starch	Annealed starch after PA addition	PA addition in annealed starch
Potato	8.3 (0.6)	79.4 (0.8)	71.1	8.1 (0.6)	108.6 (1.2)	100.5
Tapioca	13.9 (0.4)	72.6 (0.6)	58.6	13.1 (0.6)	95.2 (1.1)	82.1
Common corn	434.4 (0.4)	574.6 (0.9)	140.1	433.4 (1.0)	628.2 (0.6)	194.8
Waxy corn	5.4 (0.5)	28.8 (0.4)	23.4	4.7 (0.2)	29.1 (0.2)	24.4

FFAs content of starches (means of three replicates and standard deviations in parentheses).

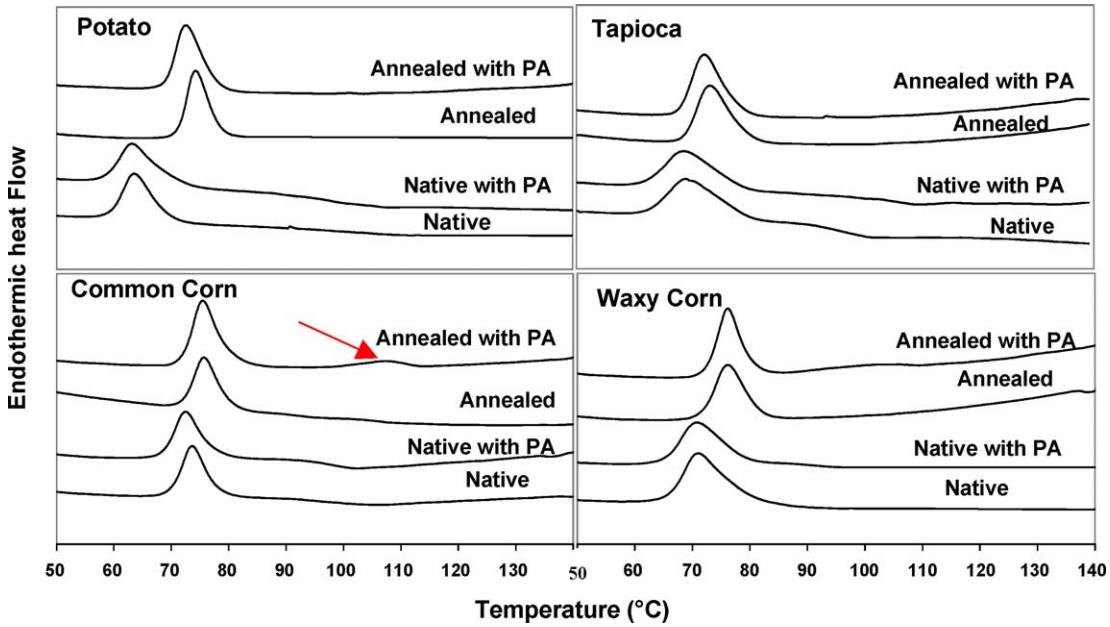

Fig. 1. Gelatinization characteristics of native, annealed, and palmitic acid (PA)-treated starches.

starches in the first (amorphous lamellae) and the second (crystalline lamellae) phases (Nakazawa & Wang, 2003). The present results of much increased complexed PA by annealed starches confirmed our previous finding because more PA was introduced into annealed starches to form complexes with amylose. During annealing amylose became mobile and the crystalline structure was improved. The perfection of the crystalline structure might produce void space, which permitted the penetration of more PA that could be introduced into the starches after annealing.

The amount of introduced PA was similar for both native and annealed waxy corn, implying PA interacted with amylopectin in a similar pattern in both native and annealed waxy corn starch. Although it was recently suggested that amylose might become more mobile and aggregate to form double helices during annealing (Jacobs, Eerlingen, Rouseu,

Table 2
Gelatinization characteristics of native, annealed, and palmitic acid (PA)-treated starches: onset (T_o), peak (T_p), and conclusion (T_c) temperatures, and enthalpy (ΔH)

Starch		Native starch	Native starch with PA	Annealed starch	Annealed starch with PA
Potato	T_o (°C)	60.4^{c}	59.5^{c}	71.4^{a}	69.1^{b}
	T_p (°C)	64.4^{c}	63.5^{d}	74.5^{a}	72.8^{b}
	$T_c - T_o$ (°C)	9.3^{b}	10.8^{a}	7.1^{d}	8.4^{c}
	ΔH (J/g)	16.9^{b}	16.7^{b}	18.5^{a}	18.7^{a}
Tapioca	T_o (°C)	63.7^{c}	62.8^{d}	69.5^{a}	68.4^{b}
	T_p (°C)	69.6^{c}	68.7^{d}	73.3^{a}	72.5^{b}
	$T_c - T_o$ (°C)	13.8^{b}	15.4^{a}	10.0^{c}	10.5^{c}
	ΔH (J/g)	10.7^{c}	10.7^{c}	13.5^{a}	13.4^{a}
Common corn	T_o (°C)	70.0^{b}	69.2^{c}	72.6^{a}	72.0^{a}
	T_p (°C)	74.4^{b}	73.4^{c}	76.2^{a}	76.2^{a}
	$T_c - T_o$ (°C)	8.4^{b}	8.3^{b}	8.0^{c}	9.3^{a}
	ΔH (J/g)	12.7^{b}	12.6^{b}	14.6^{a}	14.9^{a}
Waxy corn	T_o (°C)	66.5^{b}	66.5^{b}	72.3^{a}	72.6^{a}
	T_p (°C)	71.6^{b}	71.8^{b}	76.7^{a}	76.4^{a}
	T_cT_o (°C)	12.8^{a}	11.8^{b}	10.0^{c}	9.3^{c}
	ΔH (J/g)	15.5^{b}	15.3^{b}	18.1^{a}	17.9^{a}

Mean values of three replicates in the same row with different superscript letters are significantly different ($P < 0.05$).

Colonna, & Delcour, 1998), aggregated amylose might not possess a similar capacity as the free amylose in terms of complexing with FFAs.

3.2. Starch gelatinization characteristics

The gelatinization properties of PA-complexed native and annealed starches as measured by DSC are shown in Fig. 1 and summarized in Table 2. Annealing treatment is known to yield starches with increased gelatinization temperatures and narrowed gelatinization ranges (Gough & Pybus 1971). It was noted that the introduction of PA decreased the onset and peak gelatinization temperatures and increased the gelatinization range ($T_c - T_o$) of both native and annealed potato and tapioca starches and for native common corn starch compared with the untreated counterparts. Nevertheless, this phenomenon was not observed for native and annealed waxy and annealed common corn starches. It was suspected that the small amount of introduced PA in waxy corn starch was not sufficient to induce any change in gelatinization characteristics, whereas the large amount of endogenous lipids in common corn starch already affected gelatinization changes even prior to the addition of PA. It was generally accepted that amylopectin determines the gelatinization properties of starch (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). The reduction in gelatinization temperature after the PA addition also suggested the possible occurrence of amylopectin–lipid complex, which destabilized the crystalline lamellae. This destabilization effect from fatty acid introduction was more pronounced for annealed starches with a greater reduction in onset and peak temperature. It was assumed that a more ordered structure in annealed starches would be more affected by the occurrence of amylopectin–lipid complex. Nevertheless, the majority of the introduced PA presumably complexed with amylose. It has been reported that amylose complexes with lipids during plant growth (Fujimoto et al., 1972). Recently, Tufvesson, Wahlgren, and Eliasson (2003) observed that most of the artificially added FFAs complexed with amylose during heating and became more ordered structure during cooling as measured by DSC. Therefore, the bound PA in this study possibly complexed weakly with amylose and upon heating PA became more tightly complexed with amylose. The amylose–lipid complex peak was observed at a temperature of around 110 °C (arrow) for common corn starch after the introduction of PA. This amylose–lipid peak was not observed for potato, tapioca and waxy corn starch, presumably because of their small amounts of FFAs. Two types of amylose–lipid complex arrangement exist, namely complex I with a melting temperature below 100 °C and complex II with a melting temperature above 100 °C (Biliaderis & Galloway, 1989). Their difference in melting temperature is attributed to the degree of organization in the solid state. Complex I crystallizes at a low temperature (50–60 °C) with a random distribution of helical segments. In contrast, complex II crystallizes at a higher temperature (>90 °C) with a more ordered crystallite. The presence of amylose–lipid complex II in PA-treated annealed common corn starch supported our hypothesis that annealed starches consisted of more voids that allowed for the formation of more aligned amylose–lipid complexes.

3.3. Starch leaching and swelling behavior

The leached apparent amylose contents of different starches at different temperatures are shown in Fig. 2. The annealed potato starch leached significantly less amylose ($P < 0.05$) than its native counterpart, which might be due to that approximately 4% amylose already leached out during the annealing process (Nakazawa & Wang, 2003) and/or amylose became partly involved in double helical structure with amylopectin and/or with amylose chains, which consequently became less soluble. Nevertheless, the interaction between amylose and amylose/amylopectin would not affect amylose and lipids interaction because probably only a fraction of amylose interacted with lipids. After the addition of PA, the amount of leached amylose further decreased for both native and annealed

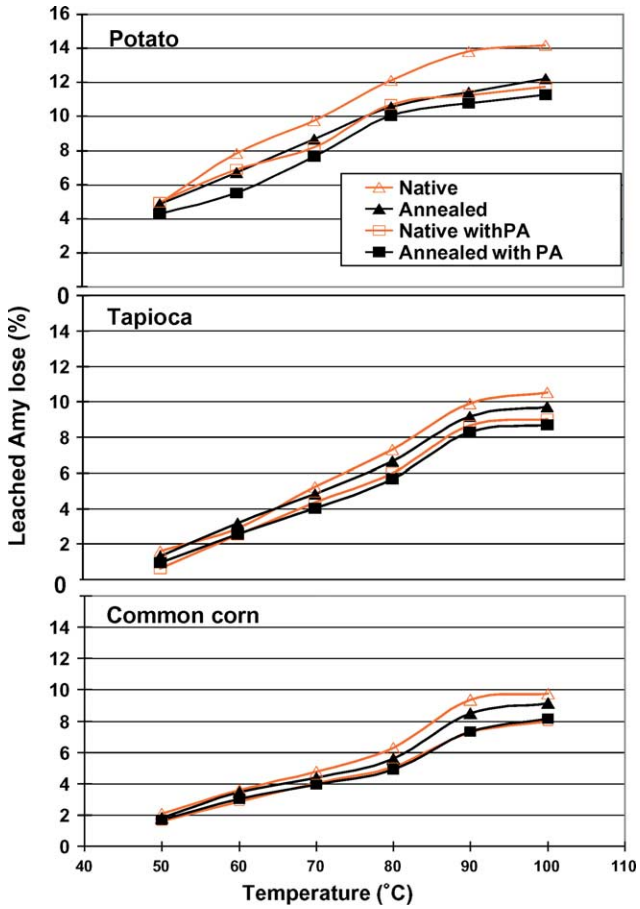

Fig. 2. Amylose leaching behavior of native, annealed, and palmitic acid (PA)-treated starches.

starches, although the difference was only statistically significant for common corn starch, again confirming the occurrence of amylose–lipid complexes. Lipids inside the starch granule could prevent amylose from leaching out and no lipids were detected in the leached carbohydrate (Tester & Morrison, 1990). Amylose–lipid complexes were insoluble in water and did not dissolve until temperature reached 94–98 °C.

The results of swelling powers of native and treated starches are displayed in Fig. 3. Because starches treated with 85% methanol were reported to have one-half to one-fifth of their original swelling power (Kitahara et al., 1993), both native and annealed starches were measured for the swelling power before and after being treated with 85% methanol in this study. There was a significant difference ($P < 0.05$) in swelling power between potato, tapioca, and common corn starches with added PA and without, except for waxy corn starch. However, no differences were noted between native and annealed starches and between the two treated starches. Although annealed starches leached less amylose than did their native ones, both native and annealed starches showed a similar swelling power. These results confirmed that swelling power is primarily a property of amylopectin and amylose acts as a diluent (Tester & Morrison, 1990). It is well known that swelling power is closely related to leaching behavior of starch (Tester & Morrison). The starches in this study generally followed a similar trend of a greater amount of leached amylose accompanied by a larger swelling power. The added PA inhibited swelling through inclusion complexes with part of the amylose (Tester & Morrison) and possibly with amylopectin as well. Tufvesson et al. (2003) demonstrated that lipids formed complexes with amylose upon heating. Even though PA was introduced at room temperature in this study, it still complexed with amylose and the quantity of PA–amylose complexes were further enhanced during heating.

3.4. Susceptibility to acid hydrolysis

Annealed starches were hydrolyzed to a greater degree than were native starches and the PA treatment did not change the extent of acid hydrolysis (Fig. 4). During the first phase, acid rapidly hydrolyzed the amorphous lamellae and the second hydrolysis was slow because acid hydrolyzed the crystalline lamellae (Robin et al., 1975). The differences between native and annealed starches were assumed to be a result of the perfection of the crystalline lamellae, confirming our previous results (Nakazawa & Wang, 2003). Some researchers reported that amylose was largely hydrolyzed at the first 3–5 days of acid hydrolysis (Robin, Mercier, Charbonniere, & Guilbot, 1974; Robin

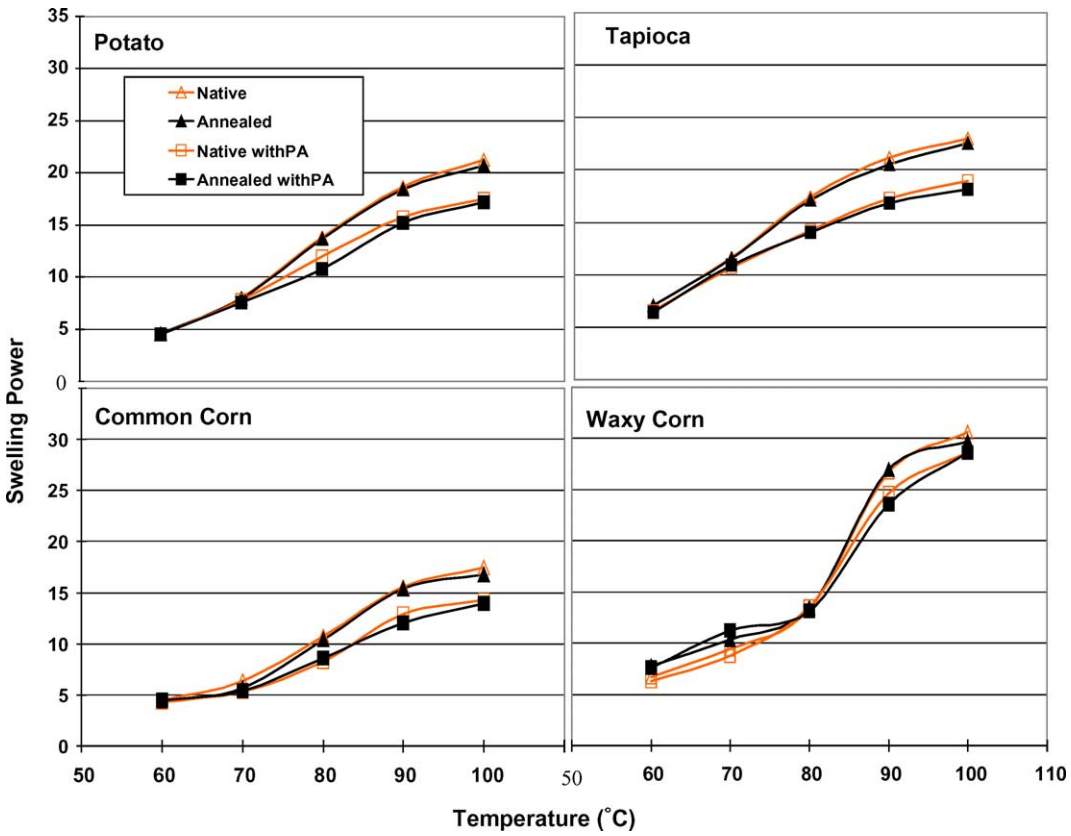

Fig. 3. Swelling power of native, annealed, and palmitic acid (PA)-treated starches.

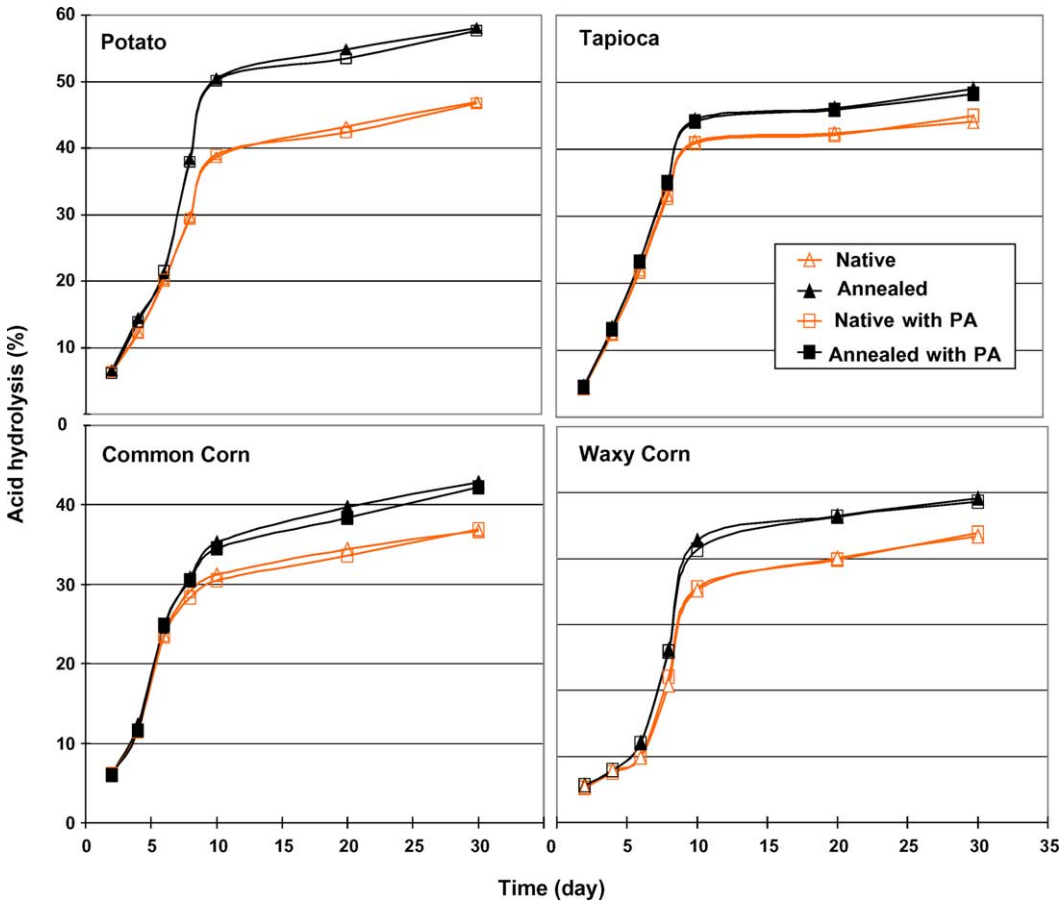

Fig. 4. Acid hydrolysis of native, annealed, and palmitic acid (PA)-treated starches.

et al., 1974). Kaneda et al. (1996) found that most introduced FFAs disappeared after acid hydrolysis. Morrison et al. (1980) proposed that those introduced FFAs inside starch granules did not complex with amylose and even if those artificially introduced FFAs complexed with amylose, they did not affect the degree of acid hydrolysis in the first phase. We proposed that the added PA did form complexes with amylose and amylopectin, however, these complexes apparently did not contribute to any improved perfection or disrupt the ordered arrangement in the amorphous and crystalline lamellae, thus resulting in no change in the degree of acid hydrolysis. Annealing resulted in more changes in the amorphous and crystalline lamellae than did the addition of PA.

The fatty acid contents in Naegeli amylodextrins and the ratios of fatty acid content in the acid-soluble fraction and in the Naegeli amylodextrins of native and treated starches are shown in Table 3 and Fig. 5, respectively. There was no difference in FFAs between Naegeli dextrins of native and annealed starches, hence the proportion of FFAs in the acid-soluble fraction was the same for native and annealed starches. After the addition of PA, the ratio of FFA in

Table 3
Fatty acid contents (mg/100 g starch) of Naegeli amylodextrins of native, annealed, and palmitic acid (PA)-treated starches after 10 days of acid hydrolysis (means of three replicates and standard deviations in parentheses)

Naegeli amylodextrin	Free fatty acid content (mg/100 g starch)					
	Native starch	Native starch after PA addition	PA addition in native starch	Annealed starch	Annealed starch after PA addition	PA addition in annealed starch
Potato	4.0 (0.3)	27.5 (0.2)	23.5	3.9 (0.4)	32.5 (0.8)	28.6
Tapioca	7.6 (0.4)	22.9 (0.8)	15.3	6.9 (0.2)	24.4 (0.5)	17.5
Common corn	207.6 (0.4)	211.1 (0.5)	3.5	200.2 (0.9)	221.0 (1.1)	20.8
Waxy corn	1.7 (0.3)	5.3 (0.3)	3.6	1.4 (0.3)	5.0 (0.4)	3.6

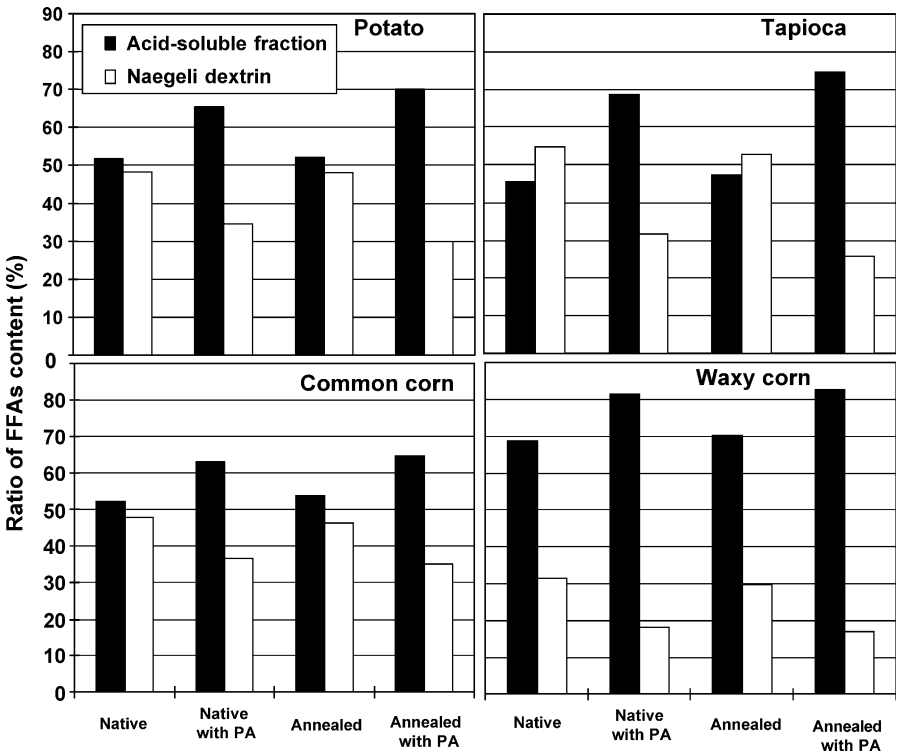

Fig. 5. Ratios of free fatty acid content in acid-soluble fraction and in Naegeli amylodextrin for native, annealed, and palmitic acid (PA)-treated starches.

the acid-soluble fraction greatly increased. In comparison with the fatty acid content before acid hydrolysis (Table 1), more than 60% of total fatty acids was present in the acid-soluble fraction for PA complexed starches. Approximately 70% of total fatty acids was in the acid-soluble fraction for both treated and untreated waxy corn starch. The residual fatty acid content in Naegeli amylodextrins was higher for the annealed starches than for the native starches after PA addition with the exception of waxy corn starch. These results supported our hypothesis that annealing enhanced the capacity of starch to complex more PA in both the amorphous and crystalline lamellae.

Tester and Debon (2000) explained the plasticization effect of water on annealing of starch based on the model proposed by Waigh et al. (1997). Nevertheless the changes in molecular arrangement of amylose and amylopectin during annealing were not discussed in detailed. According to results from our previous study (Nakazawa & Wang, 2003) and this study, we propose that the transition of the crystalline lamellae from a rigid glassy state to a mobile rubbery state from the presence of excess water facilitated the reorganization of amylose and amylopectin double helices in both the amorphous and the crystalline lamellae, and the perfection of imperfect helices in the crystalline lamellae. The perfection of the crystalline structure produced void space, which increased the susceptibility of annealed starches to acid hydrolysis and the amylose capacity to complex with PA.

4. Conclusions

Annealing increased the capacity of starch to complex more PA, which was proposed to result from more porous structure of annealed starches due to reorganization of the amorphous and the crystalline lamellae. Introduced PA generally decreased the gelatinization temperature, leached amylose content, and swelling power. A small portion of the introduced PA penetrated into the crystalline lamellae and formed complexes with amylopectin.

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