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Effects of lipids on enzymatic hydrolysis and physical properties of starch

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ABSTRACT

This study aimed to understand effects of lipids, including corn oil (CO), soy lecithin (SL), palmitic acid (PA), stearic acid (SA), oleic acid (OA), and linoleic acid (LA), on the enzymatic hydrolysis and physical properties of normal corn (NCS), tapioca (TPS), waxy corn (WCS), and high-amylose corn (HA7) starch, and to elucidate mechanisms of interactions between the starches and lipids. After cooking with the lipids (10%, w/w, dsb), NCS, TPS, and HA7 showed significant decreases in enzymatic hydrolysis, and their DSC thermograms displayed amylose–lipid-complex dissociation peaks except with the CO. ^{13}C NMR spectra of amylodextrin with CO showed downfield changes in the chemical shifts of carbons 1 and 4 of the anhydroglucose unit, indicating helical complex formation. Generally, free fatty acids (FFAs) reduced, but SL increased the peak viscosities of starches. FFAs and SL decreased, but CO increased the gel strength of NCS. These lipids displayed little impacts on the enzymatic hydrolysis and physical properties of WCS because it lacked amylose.

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1. Introduction

Starch and lipids are the major components of foods, and they play important roles in the caloric density, texture, and flavor of foods. Starch consists of two major components: amylose and amylopectin. Amylose is an essentially linear molecule with α-1,4 linked D-glucose units and a few branches of α-1,6 linkages, whereas amylopectin is a highly branched molecule with about 5% α-1,6 linkages (Hizukuri, Takeda, & Yasuda, 1981). Lipids are broadly defined as a group of compounds that are soluble in organic solvents. They can be further divided into three groups: simple lipids, such as monoglycerides, diglycerides, and triglycerides; compound lipids, such as phospholipids; and derived lipids, such as free fatty acids (FFAs) and long-chain alcohols (Duncan, 2000; McClements & Decker, 2008).

It is well known that amylose forms single-helical complexes with lipids and other guest compounds, such as iodine to give a blue color (Putseys, Lamberts, & Delcour, 2010). Structures and physical properties of the amylose-helical complexes vary with the structures of the guest compounds. Studies have shown that compounds with linear hydrocarbon chains (*e.g.*, *n*-butyl alcohol and FFAs) form amylose-helical complexes with 6 glucose-units per turn (Godet, Buleon, Tran, & Colonna, 1993; Jane & Robyt, 1984; Rappenecker & Zugenmaier, 1981), compounds with branched chains (*e.g.*, isopropyl alcohol and dimethyl sulfoxide) form amylose-helical complexes with 7 glucose-units per turn (Jane & Robyt, 1984; Nishiyama et al., 2010; Simpson, Dintzis, & Taylor, 1972), and compounds with even bulkier cross-sections (*e.g.*, 1-naphthol) form amylose-helical complexes with 8 glucose-units per turn (Yamashita & Monobe, 1971). Lipids that have been reported to form helical complexes with amylose include FFAs (Fanta, Shogren, & Salch, 1999; Raphaelides & Karkalas, 1988; Tufvesson, Wahlgren, & Eliasson, 2003b), monoglycerides (Krog, 1971; Tufvesson & Eliasson, 2000; Tufvesson, Wahlgren, & Eliasson, 2003a), and alcohols (Jane & Robyt, 1984; Kowblansky, 1985). Dissociation temperatures of amylose–lipid complexes (ALC), in general, increase with increasing length of hydrocarbon chains of the lipids, and decrease with increasing number of double bonds in the hydrocarbon chains (Eliasson & Krog, 1985; Karkalas, Ma, Morrison, & Pethrick, 1995; Kowblansky, 1985; Raphaelides & Karkalas, 1988; Tufvesson et al., 2003a, 2003b). After heating at a temperature above the dissociation temperature, amorphous ALC (form I) can further rearrange into lamellar crystallites (form II) (Biliaderis & Galloway, 1989; Kowblansky, 1985). The complex formation between amylose and lipids has been used to prepare starch products with improved properties for food application (Morgan, 2003; Yuan, 2000, 2001). In addition, after it is complexed with lipids and other compounds, amylose shows resistance to amylase hydrolysis (Jane & Robyt, 1984; Seneviratne & Biliaderis, 1991).

As a major energy source for humans and animals, starch is hydrolyzed to glucose by amylolytic enzymes present in the gastrointestinal tract. Glucose is then absorbed in the small

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intestine and increases the blood glucose concentration. The starch digestive rate of starchy foods is expressed as glycemic index (Jenkins et al., 1981). Because of the growing population suffering with insulin resistance, diabetes, overweight, obesity, and other related metabolic syndromes, there are increasing demands for starchy foods that have reduced glycemic-index (Barclay et al., 2008; Livesey, Taylor, Hulshof, & Howlett, 2008). A novel Type 5 resistant starch (RS5) has been developed *via* processing high-amylose corn starch with FFAs. Compared with control bread made with wheat flour, ingestion of bread with palmitic-acid-complexed RS5 resulted in substantially less postprandial plasma-glucose and insulin responses in human subjects (Hasjim et al., 2010). Drum-drying has also been used to enhance the ALC formation in wheat flour to reduce its *in vivo* starch digestive rate (Björck et al., 1984).

How food lipids of different structures, such as triglycerides, lecithins, and FFAs, affect enzymatic hydrolysis and physical properties of starches with different structures are not well understood. Triglyceride is the major component of lipids present in foods. Lecithin, a mixture of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol present in soybeans and egg yolk, is commonly used as an emulsifier in foods. Palmitic acid (PA, C16:0), stearic acid (SA, C18:0), oleic acid (OA, C18:1), and linoleic acid (LA, C18:2) are the most common FFAs found in food lipids. The saturated FFAs (*e.g.*, C16:0 and C18:0) have straight-chain structures, whereas the unsaturated FFAs with double bonds (*e.g.*, C18:1 and C18:2) have bent-chain structures. Objectives of this study were to examine effects of different food lipids, including triglycerides (corn oil, CO), phospholipids (soy lecithin, SL), and FFAs of different chain-lengths and numbers of double bonds, on the enzymatic hydrolysis, pasting properties and gel formation of starches of different structures, *i.e.*, normal corn (NCS), tapioca (TPS), waxy corn (WCS), and high-amylose corn (HA7) starch. We also elucidated mechanisms of interactions between the lipids and starches.

2. Materials and methods

2.1. Materials

Normal corn starch (NCS, Cargill Gel™) and high-amylose corn starch (HA7, AmyloGel™) were purchased from Cargill Inc. (Minneapolis, MN). Tapioca starch (TPS) was a gift from Miwon Vietnam Co., Ltd. (Viet Tri City, Vietnam). Waxy corn starch (WCS) was a gift from Daesang Co. (Seoul, South Korea). Corn oil (CO) was purchased from a local grocery store. Soy lecithin (SL) was purchased from Thermo Fisher Scientific Inc. (Waltham, MA). Palmitic acid (PA), stearic acid (SA) and porcine pancreatic α-amylase (PPA, Type VI-B, 21.6 units/mg solid) were purchased from Sigma Chemical Co. (St. Louis, MO). Oleic acid (OA) and linoleic acid (LA) were purchased from Acros Organics (Geel, Belgium). Amyloglucosidase of *Aspergillus niger* (200 U/mL) and D-Glucose Assay Kit were purchased from Megazyme International Ireland Ltd. (Co. Wicklow, Ireland). Amylodextrin (average DP 25) was prepared following the method of Jane, Robyt, and Huang (1985).

2.2. Amylose content of starch

The amylose content of starch was determined by measuring the iodine affinity of defatted starch using a potentiometric autotitrator (702 SM Tirino, Metrohm, Herisau, Switzerland) (Song & Jane, 2000). The amylose content of the starch was calculated by dividing the iodine affinity by 0.2 (Takeda, Hizukuri, & Juliano, 1987). The analysis was done in duplicate.

2.3. Thermal properties of starch and effects of adding lipids

Native starch (~6 mg, dsb) with deionized water (3×, w/w, dsb) was scanned from 10 °C to 150 °C at a rate of 10 °C/min in a sealed stainless steel pan using a differential scanning calorimeter (DSC, Diamond, Perkin-Elmer, Norwalk, CT) (Li, Jiang, Campbell, Blanco, & Jane, 2008). Effects of adding lipids to starch on its thermal properties were analyzed by scanning the starch that was thoroughly mixed with each lipid (10%, w/w, dsb) using the same procedure. The analysis was done in duplicate.

2.4. Preparation of cooked starch with or without added lipids

Each starch (4.0 g, dsb) with or without 10% added lipids (0.4 g) was cooked with deionized water (3×, w/w, dsb) in a boiling water-bath (~95 °C) for 8 min under constant manual stirring to fully gelatinize starch.

2.5. Content of the amylose–lipid complex (ALC) of the cooked starch with or without added lipids

The content of ALC of the starch sample that was cooked with or without 10% (w/w, dsb) added lipids (described in Section 2.4) was analyzed using the DSC. The cooked starch samples with or without added lipids were dried at 45 °C in a convection oven, and then ground into powder using a Coffee Grinder (ID S77, Sunbeam Products Inc., Boca Raton, FL). The ground sample (~6 mg, dsb) was heated using the DSC, following the same procedure as described in Section 2.3 (first scan), cooled to 10 °C at 40 °C/min, and then heated again to 150 °C at 10 °C/min (rescan) to confirm the dissociation peak of ALC (Hasjim et al., 2010). The thermal transition was analyzed using a Pyris Software (Perkin-Elmer, Norwalk, CT), and the enthalpy change was calculated on the dry starch basis. The analysis was done in duplicate.

2.6. Enzymatic hydrolysis of the cooked starch with or without added lipids

The cooked starch sample (described in Section 2.4) containing 300 mg starch (db) was weighed and dispersed in a phosphate buffer solution (15.0 mL, 0.1 M, pH 6.9, containing 0.25 mM calcium chloride) using a homogenizer (T25 Digital Ultra-Turrax® Homogenizer, IKA® Works Inc., Wilmington, NC) at 10,000 rpm for 20 s. The dispersion was pre-incubated in a shaker water-bath (37 °C and 80 rpm) for 30 min. PPA (32 units) in the same phosphate buffer solution (5.0 mL) was then added to the starch dispersion to start the starch hydrolysis. An aliquot (0.4 mL) of the hydrolysate was withdrawn at time intervals of 10, 20, 30, 60, 90, and 120 min, and mixed with 0.6 mL 100% ethanol to stop the enzyme reaction. After centrifuging at 5200 × *g* for 5 min, the supernatant was collected, and soluble sugars in the supernatant were hydrolyzed to glucose and quantified using the GOPOD method (Setiawan, Widjaja, Rakphongphairoj, & Jane, 2010). The percentage of starch hydrolysis was calculated using the equation: % starch hydrolysis = 100 × total mass of glucose released/initial dry mass of starch × (162/180). The analysis was done in duplicate.

2.7. Pasting properties of starch with or without added lipids

Each starch (2.24 g, dsb), alone or thoroughly mixed with 10% of each lipid (0.224 g), was suspended in deionized water to make a total weight of 28.0 g (8% dry starch, w/w) and then analyzed using a Rapid Visco-Analyzer (RVA, Newport Scientific, Sydney, Australia) following the program reported by Ai, Medic, Jiang, Wang, and Jane (2011). The analysis was done in duplicate.

2.8. Gel strength of starch gel with or without added lipids

Each starch (9.60 g, dsb), alone or thoroughly mixed with 10% of each lipid (0.96 g), was suspended in deionized water to make a total weight of 120.0 g (8% dry starch, w/w) and then cooked under constant stirring (250 rpm) using a Micro Visco-Amylograph (C.W. Bradender Instruments, South Hackensack, NJ) from 30 °C to 95 °C at a rate of 5 °C/min and held at 95 °C for 5 min. The hot paste was poured into a petridish-shaped container (internal diameter = 45.0 mm, height = 25.0 mm). The height of the container was extended (5 mm) by taping a piece of aluminum foil around the outside wall (Takahashi & Seib, 1988). The sample was covered with a lid and stored at 4 °C for 72 h. A fresh surface of the starch gel was obtained right before the gel-strength analysis by removing the excess gel above the rim of the container using a wire cheese cutter. The gel strength of the starch gel was analyzed using a Texture Analyzer TA-XT2i (Texture Technologies, Scarsdale, NY) with Probe TA-11 (diameter = 25.4 mm) at a test speed 0.2 mm/s and a compression depth 4.0 mm. The peak force at 4.0 mm compression was defined as the gel strength. The analysis was done in five replicates.

2.9. Detection of ALC formation using ^{13}C nuclear magnetic resonance (NMR) spectroscopy

^{13}C NMR spectra of amylodextrin (average DP 25) in an aqueous solution (50 mg/mL) with or without the addition of CO, SL, or OA (25%, w/w, dry amylodextrin basis) were obtained after acquiring 2500 scans at 25 °C following the method of Jane et al. (1985). The changes in chemical shifts of carbons of the anhydroglucose unit were obtained using the equation: chemical shift change = chemical shift of amylodextrin with lipid − chemical shift of amylodextrin control.

2.10. Statistical analysis

Statistical significance was analyzed using one-way ANOVA and multiple comparison test with Tukey's adjustment at p value <0.05. Correlations between the thermal properties of ALC and their effects on the enzymatic hydrolysis and physical properties of starches were analyzed using the Pearson correlation test. The statistical analyses were conducted in SAS (Version 9.2, SAS Institute, Inc., Cary, NC).

3. Results and discussion

Amylose contents and thermal properties of the NCS, TPS, WCS, and HA7 are shown in Table 1. The HA7 had the largest amylose content (68.4%), followed by the NCS (34.3%), TPS (29.0%) and WCS (1.9%). The HA7 displayed the highest conclusion gelatinization temperature (109.3 °C), which was above the temperature used for starch cooking (95 °C), whereas the others were similar, between 80.2 °C and 81.9 °C. The DSC results showed that gelatinization temperatures and enthalpy changes (ΔH) of all the starches were not substantially affected by physically mixing with most of the lipids.

The thermogram of the NCS displayed an ALC dissociation peak (T_p = 101.3 °C), which was separate from the starch gelatinization peak. The HA7, however, showed an ALC dissociation peak as the second peak (T_{p2} = 100.2 °C) (Jiang, Lio, Blanco, Campbell, & Jane, 2010), which partially overlapped with the starch gelatinization peak (T_{p1} = 75.7 °C) (Table 1). There was no ALC dissociation peak for the TPS and WCS because of lacking endogenous lipids and little amylose present in the WCS (Debet & Gidley, 2006; Kasemsuwan & Jane, 1996; Lim, Kasemsuwan, & Jane, 1994; Swinkels, 1985). With the addition of FFAs (PA, SA, OA, and LA) to the starch, ΔH of the ALC dissociation peak of the NCS increased, indicating increases in ALC. For the TPS, OA was the only added lipid to produce an ALC dissociation peak (ΔH = 0.6 J/g). This feature could be attributed to that OA was in a liquid form at room temperature (melting temperature = 13.3 °C) and it was an effective complexing agent for having only one double bond in the hydrocarbon chain. The liquid form of OA facilitated its penetration into starch granules after mixing with TPS, and, thus, OA could readily complex with amylose in the granule when the starch was gelatinized.

After the addition of SL, the peak temperature (T_p) and ΔH of the ALC dissociation peak of NCS decreased from 101.3 °C to 94.5 °C and from 0.9 J/g to 0.1 J/g, respectively, and the T_{p2} of HA7 decreased from 100.2 °C to 93.0 °C (Table 1) and the peak became smaller, indicating a decrease in ALC. These features could be attributed to that SL, having an amphiphilic characteristic, functioned as a detergent to remove endogenous lipids from the NCS and HA7 granules and, thus, resulted in reduced ΔH. The lower dissociation-temperatures corresponded to amylose–SL complex, which will be discussed later.

DSC thermograms of starch samples that were pre-cooked with lipids displayed no starch gelatinization peak, indicating complete gelatinization of starch (except HA7), but showed prominent ALC dissociation peaks, except for the WCS (Table 2). All the samples were rescanned to confirm the presence of ALC (Hasjim et al., 2010). Compared with the respective control, ΔH of the ALC dissociation peak increased in the NCS, TPS, and HA7 after cooking with SL and FFAs. For the FFAs, the amylose–SA (C18:0) complex displayed the highest dissociation-temperature, followed by PA (C16:0), OA (C18:1), and LA (C18:2) (Table 2). The different ALC dissociation temperatures were attributed to that SA (C18:0), with a straight and the longest hydrocarbon chain, had the strongest interaction with the hydrophobic cavity of the amylose helix, whereas PA with a straight but shorter hydrocarbon chain, OA with one double bond, and LA with two double bonds had weaker interactions with amylose.

Although the SL had an ability to remove endogenous lipids from the starch granules when physically mixed with starch (Table 1), it also formed complexes with amylose after cooking with the NCS, TPS, and HA7 as shown by the increases in ΔH of the ALC dissociation peaks (Table 2). The amylose–SL complex had dissociation temperatures substantially lower than the control but similar to the amylose–LA complex. The low dissociation-temperatures of amylose–SL complex could be attributed to steric hindrance caused by the two fatty acids on the SL molecule for the complex formation. Compared with that of physically mixed starch and lipids (Table 1), the substantially larger ALC contents of the starch samples previously cooked with lipids (Table 2) could result from better mixing and longer reaction time during cooking and subsequent drying. There was, however, no increase in ΔH of the ALC dissociation peak after starch was cooked with CO. Because of little amylose in the WCS, there was no ALC dissociation peak for the WCS with or without added lipids (Tables 1 and 2).

Enzymatic-hydrolysis rates of starches previously cooked with or without added lipids are shown in Fig. 1. After incubation with PPA for 120 min, the percentage hydrolysis of cooked TPS alone was 77.5%, followed by WCS (76.3%), NCS (72.0%), and HA7 (35.0%). The very low enzymatic-hydrolysis of cooked HA7 was a result of the starch not being fully gelatinized because of its very high gelatinization temperature (T_c = 109.3 °C, Table 1). After cooking with lipids, the TPS showed significant decreases (p < 0.05) in the percentage enzymatic-hydrolysis at different time intervals. The percentage hydrolysis of the cooked TPS at 120 min was reduced from 77.5% to 65.5% with the addition of SA, followed by PA (66.1%), CO (66.4%), OA (66.8%), SL (69.5%), and LA (72.0%) (Fig. 1). Enzymatic-hydrolysis rates of the NCS and HA7 decreased in similar orders with the addition of those lipids, but the ranges of reductions were smaller than that of the TPS.

Table 1
Amylose contents[a] and thermal properties of native starches and starches with addition of 10% (w/w, dsb) different lipids.[b]

Sample[c]		Amylose (%)	Starch gelatinization[d]					Dissociation of amylose–lipid complex[d]			
			T_o (°C)	T_{p1} (°C)	T_{p2} (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
NCS	Control	34.3 ± 0.4	69.1 ± 0.5	74.0 ± 0.4	–	80.2 ± 0.5	13.4 ± 0.1	90.1 ± 0.6	101.3 ± 0.7	108.2 ± 1.5	0.9 ± 0.0
	+CO	–	69.7 ± 0.2	75.0 ± 0.1	–	80.6 ± 0.2	14.0 ± 0.5	90.4 ± 0.1	101.3 ± 0.7	109.1 ± 0.2	0.9 ± 0.1
	+SL	–	68.5 ± 0.1	74.3 ± 0.1	–	81.2 ± 0.1	14.0 ± 0.2	91.9 ± 0.0	94.5 ± 1.7	101.8 ± 0.6	0.1 ± 0.0
	+PA	–	N.A.[e]	74.8 ± 0.6	–	80.8 ± 0.5	N.A.	89.8 ± 0.3	103.8 ± 0.0	109.6 ± 0.3	1.8 ± 0.1
	+SA	–	N.A.	N.A.	–	N.A.	N.A.	89.8 ± 0.0	101.5 ± 0.1	109.0 ± 0.4	1.2 ± 0.1
	+OA	–	68.6 ± 0.2	73.7 ± 0.1	–	80.5 ± 0.0	15.3 ± 0.7	90.9 ± 0.4	102.2 ± 0.0	110.2 ± 0.2	1.9 ± 0.1
	+LA		68.8 ± 0.6	73.5 ± 0.7	–	79.9 ± 0.6	14.2 ± 0.3	90.5 ± 0.9	96.7 ± 0.0	103.0 ± 0.2	1.7 ± 0.4
TPS	Control	29.0 ± 0.1	67.4 ± 0.5	73.9 ± 0.5	–	81.9 ± 0.6	14.7 ± 0.2	N.D.[f]	N.D.	N.D.	N.D.
	+CO	–	68.0 ± 0.6	73.9 ± 0.0	–	82.2 ± 0.0	14.2 ± 0.3	N.D.	N.D.	N.D.	N.D.
	+SL	–	67.6 ± 0.3	74.2 ± 0.4	–	86.0 ± 0.7	14.5 ± 0.1	N.D.	N.D.	N.D.	N.D.
	+PA	–	N.A.[e]	73.6 ± 0.2	–	81.4 ± 0.5	N.A.	N.D.	N.D.	N.D.	N.D.
	+SA	–	N.A.	N.A.	–	N.A.	N.A.	N.D.	N.D.	N.D.	N.D.
	+OA	–	68.0 ± 1.0	72.5 ± 1.6	–	81.3 ± 0.3	14.2 ± 0.7	90.4 ± 0.7	100.7 ± 0.1	108.4 ± 0.0	0.6 ± 0.1
	+LA	–	67.6 ± 0.3	73.5 ± 0.1	–	81.3 ± 0.2	14.8 ± 1.1	N.D.[f]	N.D.	N.D.	N.D.
WCS	Control	1.9 ± 0.0	66.3 ± 0.1	73.7 ± 0.4	–	81.5 ± 0.4	15.3 ± 0.1	N.D.[f]	N.D.	N.D.	N.D.
	+CO	–	66.6 ± 0.4	74.3 ± 0.4	–	83.0 ± 1.1	15.3 ± 0.2	N.D.	N.D.	N.D.	N.D.
	+SL	–	67.4 ± 0.1	75.1 ± 0.5	–	81.2 ± 0.0	15.1 ± 0.7	N.D.	N.D.	N.D.	N.D.
	+PA	–	N.A.[e]	73.6 ± 0.2	–	81.1 ± 0.3	N.A.	N.D.	N.D.	N.D.	N.D.
	+SA	–	N.A.	N.A.	–	N.A.	N.A.	N.D.	N.D.	N.D.	N.D.
	+OA	–	66.2 ± 0.1	73.9 ± 0.0	–	81.9 ± 0.1	15.9 ± 0.4	N.D.	N.D.	N.D.	N.D.
	+LA	–	66.3 ± 0.5	73.2 ± 0.6	–	81.3 ± 0.0	16.1 ± 1.2	N.D.	N.D.	N.D.	N.D.
HA7	Control	68.4 ± 1.4	70.6 ± 0.0	75.7 ± 0.1	100.2 ± 0.1	109.3 ± 0.6	12.1 ± 1.2	N.A.[g]	N.A.	N.A.	N.A.
	+CO	–	70.9 ± 0.1	76.7 ± 0.3	100.5 ± 0.4	109.7 ± 1.6	12.5 ± 0.4	N.A.	N.A.	N.A.	N.A.
	+SL	–	71.4 ± 0.2	76.5 ± 0.7	93.0 ± 1.7	105.3 ± 0.4	11.5 ± 1.9	N.A.	N.A.	N.A.	N.A.
	+PA	–	N.A.[e]	N.A.	101.8 ± 0.0	109.7 ± 0.1	N.A.	N.A.	N.A.	N.A.	N.A.
	+SA	–	N.A.	N.A.	99.6 ± 0.1	109.6 ± 0.3	N.A.	N.A.	N.A.	N.A.	N.A.
	+OA	–	70.6 ± 0.3	75.7 ± 0.2	100.6 ± 0.1	109.6 ± 0.0	12.6 ± 0.0	N.A.	N.A.	N.A.	N.A.
	+LA	–	69.8 ± 0.8	74.0 ± 0.0	98.4 ± 0.5	107.0 ± 0.0	14.4 ± 0.6	N.A.	N.A.	N.A.	N.A.

[a] Measured using iodine potentiometric titration method.
[b] Measured using differential scanning calorimetry.
[c] NCS, normal corn starch; TPS, tapioca starch; WCS, waxy corn starch; HA7, high-amylose corn starch; CO, corn oil; SL, soy lecithin; PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid.
[d] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy change.
[e] N.A., not available because of the overlapping between the melting peak of free fatty acid and starch gelatinization peak.
[f] N.D., not detected.
[g] N.A., not available because of the overlapping between the starch gelatinization peak and amylose–lipid complex dissociation peak of HA7.

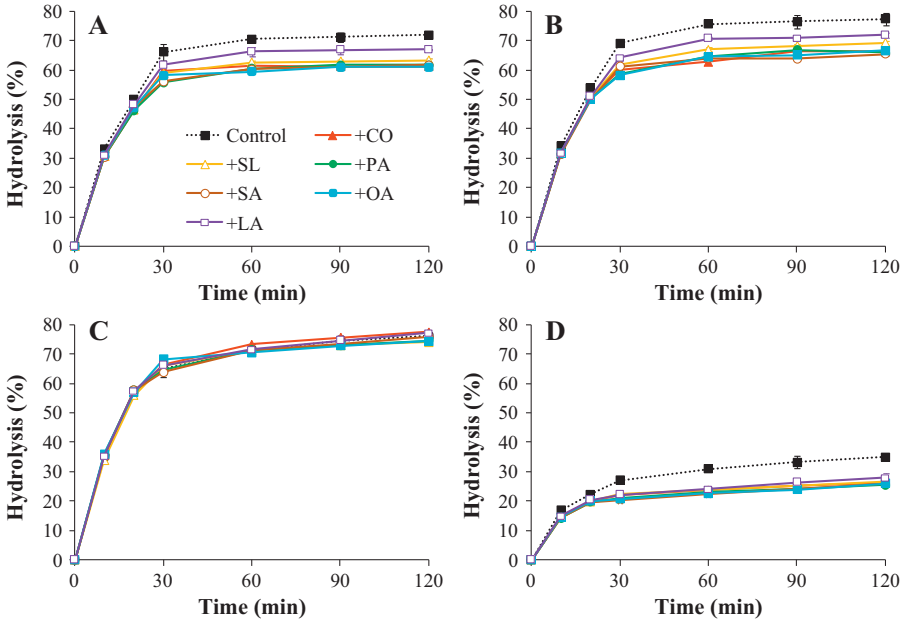

Fig. 1. Enzymatic-hydrolysis rates of cooked starches and starches cooked with 10% (w/w, dsb) different lipids. PPA was used for the hydrolysis at 37 °C, pH 6.9. % Starch hydrolysis = 100 × total mass of glucose released from soluble sugars/initial dry mass of starch × (162/180). (A) Normal corn starch, (B) tapioca starch, (C) waxy corn starch, (D) high-amylose corn starch. CO, corn oil; SL, soy lecithin; PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid.

Table 2
Dissociation of amylose–lipid complex of pre-cooked starch alone (control) and starch pre-cooked with 10% (w/w, dsb) different lipids.[a,b,c]

Sample[d]		First scan[e,f]				Rescan[e]			
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
NCS	Control	89.2 ± 0.2 a	99.6 ± 0.2	107.0 ± 0.1	2.1 ± 0.1	83.4 ± 0.2 d	92.3 ± 0.2	101.3 ± 1.8	0.5 ± 0.2
	+CO	88.0 ± 0.1 ab	100.1 ± 0.6	107.1 ± 0.1	2.1 ± 0.0	83.0 ± 0.0 d	92.3 ± 0.1	103.4 ± 0.6	0.9 ± 0.0
	+SL	75.1 ± 0.0 c	91.4 ± 0.1	99.5 ± 0.1	3.3 ± 0.2	76.2 ± 0.1 e	88.7 ± 0.4	96.5 ± 0.2	2.2 ± 0.0
	+PA	88.2 ± 0.0 ab	100.7 ± 0.2	109.0 ± 0.3	3.9 ± 0.0	88.4 ± 0.1 b	102.5 ± 0.1	109.3 ± 0.1	5.0 ± 0.0
	+SA	89.1 ± 0.8 a	101.2 ± 0.2	109.9 ± 0.2	4.3 ± 0.1	92.5 ± 1.0 a	100.0 ± 0.1	109.9 ± 0.0	3.7 ± 0.1
	+OA	87.4 ± 0.0 b	96.4 ± 0.5	108.1 ± 0.5	4.1 ± 0.1	86.8 ± 0.0 c	95.6 ± 0.4	108.3 ± 0.4	6.7 ± 0.1
	+LA	72.8 ± 0.3 d	91.7 ± 0.2	103.6 ± 0.4	6.8 ± 0.0	75.8 ± 0.2 e	86.5 ± 0.2	102.8 ± 0.3	5.4 ± 0.3
TPS	Control	N.D.[g]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+CO	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+SL	76.1 ± 0.1 c	89.3 ± 0.5	98.6 ± 0.4	2.9 ± 0.2	76.9 ± 0.0 d	86.9 ± 0.0	94.9 ± 0.9	1.2 ± 0.1
	+PA	90.2 ± 0.4 a	102.5 ± 0.5	109.0 ± 0.0	3.8 ± 0.0	89.7 ± 0.2 b	103.1 ± 0.0	109.1 ± 0.1	3.4 ± 0.1
	+SA	91.7 ± 0.5 a	103.4 ± 0.5	109.9 ± 0.6	2.1 ± 0.3	94.3 ± 0.5 a	101.0 ± 0.1	110.1 ± 0.0	1.7 ± 0.0
	+OA	88.0 ± 0.8 b	100.2 ± 0.2	108.2 ± 0.2	3.9 ± 0.0	87.8 ± 0.1 c	99.9 ± 0.1	108.1 ± 0.1	5.0 ± 0.0
	+LA	76.7 ± 0.3 c	92.1 ± 0.1	100.6 ± 0.1	4.1 ± 0.1	77.7 ± 0.3 d	92.0 ± 0.0	100.1 ± 0.1	3.9 ± 0.1
WCS	Control	N.D.[g]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+CO	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+SL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+PA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+SA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+OA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	+LA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
HA7	Control	90.2 ± 0.2	94.7 ± 0.2	109.2 ± 0.1	6.5 ± 0.2	85.3 ± 0.5 bc	94.7 ± 0.2	106.4 ± 0.1	4.1 ± 0.1
	+CO	93.6 ± 0.2	92.1 ± 0.1	111.3 ± 0.4	5.3 ± 0.4	86.7 ± 0.4 b	92.1 ± 0.1	103.8 ± 0.1	2.2 ± 0.1
	+SL	N.A.[h]	N.A.	N.A.	N.A.	75.1 ± 0.5 d	86.9 ± 0.1	96.2 ± 0.4	3.0 ± 0.3
	+PA	92.9 ± 0.3	101.6 ± 0.4	109.2 ± 0.3	7.7 ± 0.1	85.4 ± 0.3 bc	95.8 ± 0.2	108.6 ± 0.1	9.4 ± 0.6
	+SA	94.4 ± 0.1	103.0 ± 0.0	109.9 ± 0.0	7.4 ± 0.1	88.6 ± 0.3 a	98.3 ± 0.2	109.9 ± 0.0	7.6 ± 0.5
	+OA	90.7 ± 0.0	100.3 ± 0.6	108.8 ± 1.1	7.6 ± 0.5	84.8 ± 0.1 c	94.6 ± 0.8	107.9 ± 0.7	8.7 ± 0.1
	+LA	N.A.[h]	N.A.	N.A.	N.A.	74.4 ± 0.1 d	86.7 ± 0.1	102.7 ± 0.0	6.4 ± 0.1

[a] The samples were pre-cooked in a boiling water-bath, dried at 45 °C, and ground before the scanning with water (3×, w/w, dsb) to 150 °C.
[b] Measured using differential scanning calorimetry.
[c] Values of the same starch with the same letter in a column are not significantly different at $p < 0.05$.
[d] NCS, normal corn starch; TPS, tapioca starch; WCS, waxy corn starch; HA7, high-amylose corn starch; CO, corn oil; SL, soy lecithin; PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid.
[e] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy change.
[f] For HA7, the peak in the first scan was a combination of starch gelatinization and amylose–lipid complex dissociation.
[g] N.D., not detected.
[h] N.A., not available because of the overlapping between the melting peak of retrograded starch and amylose–lipid complex dissociation peak.

The reductions in enzymatic-hydrolysis rates of the NCS, TPS, and HA7 after cooking with SA, PA, OA, SL, and LA could be attributed to complex formation between amylose and the lipids as shown in Table 2. The amylose-helical complex is known to be resistant to amylase hydrolysis (Jane & Robyt, 1984; Seneviratne & Biliaderis, 1991). The ALC also restricted the swelling of starch granules, which further reduced the starch hydrolysis (Cui & Oates, 1999; Hasjim et al., 2010; Tester & Morrison, 1990). The decreases in percentages enzymatic-hydrolysis (at 120 min) of the NCS, TPS, and HA7 after cooking with the SL and FFAs positively correlated with T_o of ALC as shown in Table 2 ($r = 0.88$, $p = 0.047$; $r = 0.94$, $p = 0.02$; $r = 0.85$, $p = 0.071$ for the NCS, TPS, and HA7, respectively). These results were consistent with previously reported data showing that ALC with higher dissociation-temperatures were more resistant to amylase hydrolysis (Eliasson & Krog, 1985). Although the DSC results showed no ALC formation after the starches were cooked with CO (Table 2), enzymatic hydrolyses of NCS, TPS, and HA7 were significantly reduced ($p < 0.05$) by CO (Fig. 1). The enzymatic-hydrolysis rate of WCS was not affected by the addition of those lipids (Fig. 1), which correlated well with the results of no ALC dissociation peak detected after cooking the WCS with any of the lipids (Table 2).

Pasting properties of starch heated with or without 10% (w/w, dsb) added lipids are shown in Fig. 2. The NCS displayed a higher pasting-temperature (80.1 °C) but lower peak- and breakdown-viscosities (185.7 RVU and 61.6 RVU, respectively) than the TPS (69.1 °C, 269.8 RVU and 172.7 RVU, respectively) and WCS (70.0 °C, 277.3 RVU and 177.6 RVU, respectively). The differences were attributed to the lack of endogenous lipids in the TPS and WCS (Debet & Gidley, 2006; Kasemsuwan & Jane, 1996; Lim et al., 1994; Swinkels, 1985) and little amylose in the WCS. The ALC present in the NCS granules restricted the swelling of starch granules (Debet & Gidley, 2006; Swinkels, 1985; Tester & Morrison, 1990) and resulted in its higher pasting-temperature and lower peak-viscosity. Without endogenous lipids and ALC, the TPS and WCS granules swelled freely during cooking and eventually dispersed. Thus, the TPS and WCS displayed large breakdown-viscosities but small setback-viscosities. The less swelling of the cooked NCS could result in its slower enzymatic-hydrolysis rate (72.0% at 120 min) than the cooked TPS (77.5%) and WCS (76.3%) (Fig. 1).

With the addition of SL, the NCS displayed a decrease in the pasting temperature, from 80.1 °C to 72.3 °C, and an increase in the peak viscosity, from 185.7 RVU to 231.0 RVU, and the HA7 displayed detectable viscosity (final viscosity = 26.9 RVU) (Fig. 2). These results were consistent with the mechanism that the SL removed the endogenous lipids from the NCS and HA7 as evidenced by the reduction in ΔH of the ALC dissociation peak (Table 1). The results also agreed with that the removal of endogenous lipids from starch using detergents facilitated the swelling of starch granules during heating (Debet & Gidley, 2006). With the addition of SL, the NCS displayed a pasting temperature and peak viscosity close to the TPS (Fig. 2).

The addition of FFAs, however, increased the pasting temperature of NCS and decreased its peak viscosity (except for LA),

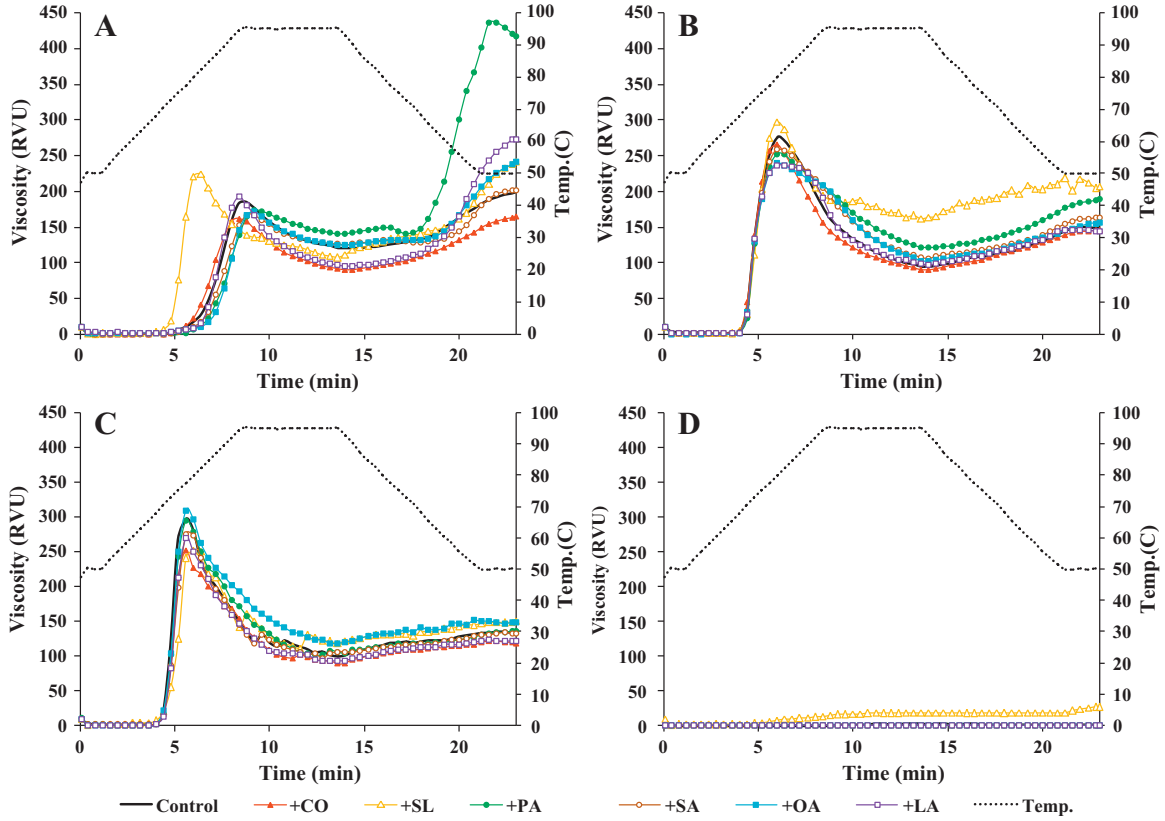

Fig. 2. Pasting profiles of starches and starch–lipid mixtures (starch:lipid = 10:1, w/w, dsb) measured using a Rapid Visco-Analyzer. Starch suspension (28.0 g total weight) with 8% starch (w/w, db) was used for the analysis. (A) Normal corn starch, (B) tapioca starch, (C) waxy corn starch, and (D) high-amylose corn starch. CO, corn oil; SL, soy lecithin; PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid.

resulting from ALC formation (Table 1). The addition of CO slightly decreased the pasting temperature of NCS and decreased the peak viscosity from 185.7 RVU to 161.3 RVU. The PA, LA, SL, and OA increased the final viscosity of NCS from 202.0 RVU to 420.9 RVU, 279.6 RVU, 253.5 RVU, and 252.1 RVU, respectively. The onset of a remarkably higher final-viscosity of the NCS at around 65 °C with the presence of PA could be related to the melting temperature of PA (62.1 °C). The amylose–PA complex in the swollen granule and the free PA could solidify and become rigid at around that temperature, and therefore drastically increased the final viscosity of the NCS–PA mixture.

In contrast to the NCS, the TPS showed no difference in its pasting temperature with the presence of lipids. The differences could be attributed to that the TPS granules lacked endogenous lipids and swelled promptly to reach its peak viscosity and then dispersed. At the temperature above the pasting temperature (69.1 °C), amylose of TPS could complex with the added lipids in the aqueous suspension. The complex formation with FFAs decreased the peak viscosity of TPS, resulting from restricted swelling. The SL slightly increased the peak viscosity of TPS, but CO decreased it. The PA slightly increased the final viscosity of TPS, suggesting little amylose–PA complex present in swollen granules. Addition of lipids showed less impacts on the pasting properties of WCS (Table 1).

Results of starch-gel strength with or without added lipids are shown in Table 3. Without added lipids, the NCS was the only starch to form a gel at 8% (w/w, dsb) concentration after storage at 4 °C for 72 h. Gel formation is a result of the interaction between amylose and amylopectin in the granule to develop networks and hold water in swollen granules (Ott & Hester, 1965). The swollen starch-granules maintaining integrity greatly contribute to the gel strength of starch (Miles, Morris, Orford, & Ring, 1985; Ring, 1985). The ALC present in the native NCS interacted with amylopectin to form networks, held water, and maintained integrity of swollen starch-granules and, therefore, facilitated its gel formation. The native TPS and WCS granules did not have ALC (Table 1) and, thus, were easily dispersed during cooking (Fig. 2) and could not form a gel.

The gel strength of NCS increased with the addition of CO, but decreased with the addition of other lipids (Table 3). As the results in Table 2 suggested, after cooking the NCS with the SL and FFAs, there were increasing ALC formed. ALC are known to develop lamellar crystallites at a high concentration and lose water-binding and gel-forming ability (Putseys et al., 2010; Takahashi & Seib, 1988). The decreases in the gel strength of NCS with the addition of SL and FFAs positively correlated ($r = 0.92$, $p = 0.03$) with T_o of the ALC dissociation peaks shown in Table 2.

After the TPS was thoroughly mixed with the SL, OA, and LA as dry mixtures and then cooked with water to form pastes, the pastes developed gel, but not with the PA and SA. The differences could be attributed to that the liquid-form OA and LA and water-soluble SL could readily penetrate into the TPS granules, form complexes with amylose, and develop networks with amylopectin in the granules during cooking. PA and SA, however, had high melting-temperatures, 62.1 °C and 70.6 °C, respectively, which were close to or above the pasting temperature of the TPS (69.1 °C). The complex formation with these two FFAs likely occurred after TPS granules were already swollen or dispersed. Thus, ALC with SA and PA failed to interact with amylopectin within the granule to maintain the integrity of swollen granules for the subsequent gel formation. The explanation was further supported by the results of

Table 3
Gel strength of starch gels (8%, w/w, dsb) prepared with or without added lipids (10%, w/w, dsb).[a,b,c]

Sample[d]	Gel strength (g)						
	Control	+CO	+SL	+PA	+SA	+OA	+LA
NCS	403.4 ± 11.2 b	523.7 ± 14.4 a	283.6 ± 8.8 c	165.0 ± 5.6 f	172.6 ± 5.5 f	192.2 ± 5.6 e	246.1 ± 8.0 d
TPS	N.A.[e]	N.A.	92.2 ± 4.8	N.A.	N.A.	85.6 ± 4.0	92.6 ± 4.6
WCS	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
HA7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

[a] The samples were prepared by cooking starch suspensions using an amylograph followed by storage at 4 °C for 72 h.
[b] The gel strength was analyzed using a Texture Analyzer TA-XT2i.
[c] Values of the NCS with the same letter are not significantly different at $p < 0.05$.
[d] NCS, normal corn starch; TPS, tapioca starch; WCS, waxy corn starch; HA7, high-amylose corn starch; CO, corn oil; SL, soy lecithin; PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid.
[e] N.A., not available because the sample did not form starch gel.

Table 4
Downfield changes in ^{13}C-chemical shifts of amylodextrin.[a]

Sample[b]	Chemical shift change (ppm)[c]					
	C 1[d]	C 4	C 2	C 3	C 5	C 6
+CO	0.01	0.02	0.00	0.00	0.00	0.00
+SL	0.03	0.06	0.00	0.00	0.00	0.00
+OA	0.03	0.05	0.00	0.00	0.00	0.00

[a] ^{13}C NMR spectra were obtained after 2500 scans of amylodextrin (average DP 25) with or without added lipids (25%, w/w, dry amylodextrin basis) in aqueous solutions at 25 °C.
[b] CO, corn oil; SL, soy lecithin; OA, oleic acid.
[c] Chemical shift change = chemical shift of amylodextrin with lipid − chemical shift of amylodextrin control.
[d] Different carbons in the anhydroglucose unit of the amylodextrin.

no gel formation when the SL, OA, and LA were added after the TPS was cooked (data not shown). The WCS did not develop a gel with or without added lipids because of lacking amylose (Tables 1 and 3). HA7 was only partially gelatinized after cooking in the amylograph, and the starch granules hardly swelled (Table 1 and Fig. 2) (Hasjim et al., 2010; Jiang, Campbell, Blanco, & Jane, 2010). Therefore, it failed to form a gel.

The addition of CO reduced the enzymatic-hydrolysis rates and viscosities of the NCS, TPS, and HA7 (Figs. 1 and 2) and increased the gel strength of the NCS (Table 3). DSC thermograms, however, did not show an ALC dissociation peak with the added CO (Tables 1 and 2). To reveal whether CO formed helical complexes with amylose, ^{13}C NMR was used to examine the conformational change of amylodextrin (short-chain amylose, average DP 25) with the presence of CO.

To form a helical complex with lipids, the conformation of the amylose molecule is changed from a random coil to a helix, which alters the torsion angles (φ and ψ) of the glycosidic bonds (French & Murpny, 1977). The changes in the torsion angles affect the patterns of electron distribution on the carbons 1 and 4 of the bonds and, thus, cause downfield changes in the chemical shifts of carbons 1 and 4 of the anhydroglucose unit in the NMR spectrum (Gidley & Bociek, 1988; Jane et al., 1985). The downfield changes in ^{13}C-chemical shifts of carbons 1 and 4 of amylodextrin with the presence of CO, SL, and OA demonstrated that the three lipids formed helical complexes with linear starch molecules with the extent of SL > OA > CO (Table 4) (Jane et al., 1985). It was plausible that one or more fatty acids of the CO formed complexes with amylose, which restricted granule swelling and, consequently, decreased the enzymatic-hydrolysis rates and viscosities of the NCS, TPS, and HA7. Because of steric hindrance for the three fatty acids of the triglyceride molecule to form helical complex with amylose, interactions between the fatty acids of CO and amylose could be heterogeneous and weak. Consequently, the ALC with CO did not show a dissociation peak in the DSC thermograms (Tables 1 and 2).

4. Conclusions

Lipids of different structures showed different effects on the enzymatic-hydrolysis rates and physical properties of starches. The SL and FFAs formed ALC with NCS, TPS, and HA7 and showed well-defined ALC dissociation peaks in the DSC thermograms. The ALC with FFAs decreased the peak viscosities of NCS and TPS during cooking. The addition of SL to the NCS and HA7, however, facilitated the swelling of the starches, resulting from that the SL removed the endogenous lipids from the starch granules. Adding SL, OA, and LA to the TPS as dry mixtures prior to cooking introduced ALC formation within the granules, which allowed gel formation. Adding SL and FFAs to the NCS, however, decreased the gel strength of NCS, resulting from lamellar-crystallite formation and loss of water-binding capacity.

Adding CO to starches decreased their viscosities and enhanced the gel strength of NCS, but did not show an ALC dissociation peak in the DSC thermograms. ^{13}C NMR spectra of amylodextrin showed downfield changes in the chemical shifts of carbons 1 and 4 of anhydroglucose unit with the presence of CO, indicating that the CO did form helical complexes with amylose, but to a lesser extent.

All the lipids, after cooking with the starches, significantly decreased the starch-hydrolysis rates except WCS because it lacked amylose. The interactions between starch and lipids could be useful to produce starchy foods with reduced glycemic-index.

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References

Ai, Y., Medic, J., Jiang, H., Wang, D., & Jane, J. (2011). Starch characterization and ethanol production of sorghum. *Journal of Agricultural and Food Chemistry*, *59*, 7385–7392.

Barclay, A. W., Petocz, P., McMillan-Price, J., Flood, V. M., Prvan, T., Mitchell, P., et al. (2008). Glycemic index, glycemic load, and chronic disease risk – A metaanalysis of observational studies. *American Journal of Clinical Nutrition*, *87*, 627–637.

Biliaderis, C. G., & Galloway, G. (1989). Crystallization behavior of amylose-V complexes: Structure–property relationships. *Carbohydrate Research*, *189*, 31–48.

Björck, I., Asp, N.-G., Birkhed, D., Eliasson, A.-C., Sjöberg, L.-B., & Lundquist, I. (1984). Effects of processing on starch availability *in vitro* and *in vivo*. II: Drum-drying of wheat flour. *Journal of Cereal Science*, *2*, 165–178.

Cui, R., & Oates, C. G. (1999). The effect of amylose–lipid complex formation on enzyme susceptibility of sago starch. *Food Chemistry*, *65*, 417–425.

Debet, M. R., & Gidley, M. J. (2006). Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydrate Polymers*, *64*, 452–465.

Duncan, S. E. (2000). Lipids: Basic concepts. In G. L. Christen, & J. Smith (Eds.), *Food Chemistry: Principles and Applications* (pp. 79–96). West Sacramento, CA: Science Technology System.

Eliasson, A.-C., & Krog, N. (1985). Physical properties of amylose–monoglyceride complexes. *Journal of Cereal Science*, *3*, 239–248.

Fanta, G. F., Shogren, R. L., & Salch, J. H. (1999). Steam jet cooking of high-amylose starch–fatty acid mixtures. An investigation of complex formation. *Carbohydrate Polymers*, *38*, 1–6.

French, A. D., & Murpny, V. G. (1977). Computer modeling in the study of starch. *Cereal Foods World*, *22*, 61–70.

Gidley, M. J., & Bociek, S. M. (1988). ^{13}C CP/MAS NMR studies of amylose inclusion complexes, cyclodextrins, and the amorphous phase of starch granules: Relationships between glycosidic linkage conformation and solid-state ^{13}C chemical shifts. *Journal of the American Chemical Society*, *110*, 3820–3829.

Godet, M. C., Buleon, A., Tran, V., & Colonna, P. (1993). Structural features of fatty acid–amylose complexes. *Carbohydrate Polymers*, *21*, 91–95.

Hasjim, J., Lee, S.-O., Hendrich, S., Setiawan, S., Ai, Y., & Jane, J. (2010). Characterization of a novel resistant-starch and its effects on postprandial plasma-glucose and insulin responses. *Cereal Chemistry*, *87*, 257–262.

Hizukuri, S., Takeda, Y., & Yasuda, M. (1981). Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydrate Research*, *94*, 205–213.

Jane, J., & Robyt, J. F. (1984). Structure studies of amylose-V complexes and retrograded amylose by action of alpha amylases, and a new method for preparing amylodextrins. *Carbohydrate Research*, *132*, 105–118.

Jane, J., Robyt, J. F., & Huang, D.-H. (1985). ^{13}C NMR, study of the conformation of helical complexes of amylodextrin and of amylose in solution. *Carbohydrate Research*, *140*, 21–35.

Jenkins, D. J. A., Wolever, T. M. S., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., et al. (1981). Glycemic index of foods: A physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition*, *34*, 362–366.

Jiang, H., Campbell, M., Blanco, M., & Jane, J. (2010). Characterization of maize amylose-extender (*ae*) mutant starches. Part II: Structures and properties of starch residues remaining after enzymatic hydrolysis at boiling-water temperature. *Carbohydrate Polymers*, *80*, 1–12.

Jiang, H., Lio, J., Blanco, M., Campbell, M., & Jane, J. (2010). Resistant-starch formation in high-amylose maize starch during kernel development. *Journal of Agricultural and Food Chemistry*, *58*, 8043–8047.

Karkalas, J., Ma, S., Morrison, W. R., & Pethrick, R. A. (1995). Some factors determining the thermal-properties of amylose inclusion complexes with fatty-acids. *Carbohydrate Research*, *268*, 233–247.

Kasemsuwan, T., & Jane, J. (1996). Quantitative method for the survey of starch phosphate derivatives and starch phospholipids by ^{31}P Nuclear Magnetic Resonance Spectroscopy. *Cereal Chemistry*, *73*, 702–707.

Kowblansky, M. (1985). Calorimetric investigation of inclusion complexes of amylose with long-chain aliphatic-compounds containing different functional groups. *Macromolecules*, *18*, 1776–1779.

Krog, N. (1971). Amylose complexing effect of food grade emulsifiers. *Starch*, *23*, 206–210.

Li, L., Jiang, H., Campbell, M., Blanco, M., & Jane, J. (2008). Characterization of maize amylose-extender (ae) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. *Carbohydrate Polymers*, *74*, 396–404.

Lim, S.-T., Kasemsuwan, T., & Jane, J. (1994). Characterization of phosphorus in starch by ^{31}P-Nuclear Magnetic Resonance Spectroscopy. *Cereal Chemistry*, *71*, 488–493.

Livesey, G., Taylor, R., Hulshof, T., & Howlett, J. (2008). Glycemic response and health – A systematic review and meta-analysis: Relations between dietary glycemic properties and health outcomes. *American Journal of Clinical Nutrition*, *87*, 258–268.

McClements, D. J., & Decker, E. A. (2008). Lipids. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Fennema's food chemistry* (pp. 155–216). Boca Raton, FL: CRC Press.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, *135*, 271–281.

Morgan, K. R. (2003). *Starch products involving a starch–lipid complex, their preparation and uses*. World Patent, WO 2003102072.

Nishiyama, Y., Mazeau, K., Morin, M., Cardoso, M. B., Chanzy, H., & Putaux, J.-L. (2010). Molecular and crystal structure of 7-fold V-amylose complexed with 2-propanol. *Macromolecules*, *43*, 8628–8636.

Ott, M., & Hester, E. E. (1965). Gel formation as related to concentration of amylose and degree of starch swelling. *Cereal Chemistry*, *42*, 476–484.

Putseys, J. A., Lamberts, L., & Delcour, J. A. (2010). Amylose-inclusion complexes: Formation, identity and physico-chemical properties. *Journal of Cereal Science*, *51*, 238–247.

Raphaelides, S., & Karkalas, J. (1988). Thermal dissociation of amylose–fatty acid complexes. *Carbohydrate Research*, *172*, 65–82.

Rappenecker, G., & Zugenmaier, P. (1981). Detailed refinement of the crystal structure of V_h-amylose. *Carbohydrate Research*, *89*, 11–19.

Ring, S. G. (1985). Some studies on starch gelation. *Starch*, *37*, 80–83.

Seneviratne, H. D., & Biliaderis, C. G. (1991). Action of α-amylase on amylose–lipid complex superstructures. *Journal of Cereal Science*, *13*, 129–143.

Setiawan, S., Widjaja, H., Rakphongphairoj, V., & Jane, J. (2010). Effects of drying conditions of corn kernels and storage at an elevated humidity on starch structures and properties. *Journal of Agricultural and Food Chemistry*, *58*, 12260–12267.

Simpson, T. D., Dintzis, F. R., & Taylor, N. W. (1972). A V_7 conformation of dimethyl sulfoxide–amylose complex. *Biopolymers*, *11*, 2591–2600.

Song, Y., & Jane, J. (2000). Characterization of barley starches of waxy, normal, and high amylose varieties. *Carbohydrate Polymers*, *41*, 365–377.

Swinkels, J. J. M. (1985). Composition and properties of commercial native starches. *Starch*, *37*, 1–5.

Takahashi, S., & Seib, P. A. (1988). Paste and gel properties of prime corn and wheat starches with and without native lipids. *Cereal Chemistry*, *65*, 474–483.

Takeda, Y., Hizukuri, S., & Juliano, B. O. (1987). Structure of rice amylopectins with low and high affinities for iodine. *Carbohydrate Research*, *168*, 79–88.

Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I: Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, *67*, 551–557.

Tufvesson, F., & Eliasson, A.-C. (2000). Formation and crystallization of amylose–monoglyceride complex in a starch matrix. *Carbohydrate Polymers*, *43*, 359–365.

Tufvesson, F., Wahlgren, M., & Eliasson, A.-C. (2003a). Formation of amylose–lipid complexes and effects of temperature treatment. Part 1: Monoglycerides. *Starch*, *55*, 61–71.

Tufvesson, F., Wahlgren, M., & Eliasson, A.-C. (2003b). Formation of amylose–lipid complexes and effects of temperature treatment. Part 2: Fatty acids. *Starch*, *55*, 138–149.

Yamashita, Y., & Monobe, K. (1971). Single crystals of amylose V complexes. III: Crystals with 8_1 helical configuration. *Journal of Polymer Science Part A-2: Polymer Physics*, *9*, 1471–1481.

Yuan, C. R. (2000). *Starch-emulsifier composition and methods for making*. US Patent, US006017388A.

Yuan, C. R. (2001). *High-amylose starch-emulsifier composition and methods of making*. US Patent, US006228419B1.