

Structures and physicochemical properties of starch from immature seeds of soybean varieties (*Glycine max* (L.) Merr.) exhibiting normal, low-linolenic or low-saturated fatty acid oil profiles at maturity [☆]

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Abstract

Soybean varieties bred for normal (NM), low-linolenic (LL) or low-saturate (LS) fatty acid composition were collected 20 d prior to harvest. Seed starch structure and functional properties were studied. Soybean starch had small granules (0.5–4.5 μm diameter), and C_B-type crystallinity. LL and LS soybean starch had significantly lower absolute amylose than NM soybean. Weight-average amylopectin molecular weight, measured by HPSEC, ranged from 5.91×10^8 g/mol for LL to 8.92×10^8 g/mol for NM. Soybean amylopectin had short average branch chain-length (DP 19.9–21.9) and LS amylopectin had significantly larger percentage of DP 6–12 than LL and NM soybeans. No significant differences among soybean varieties were observed for starch thermal and pasting properties, with onset gelatinization temperature (51.0–51.8 °C) and peak viscosity (79–100 RVU, 8% starch suspension). Further research is needed to understand if amylose content or amylopectin fine structure in developing soybean seeds relates to fatty acid composition of soybean oil at maturity.

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1. Introduction

Soybean is an extremely valuable crop to the United States of America (USA) economy. In 2004, USA planted 30.4 million hectares (75.2 million acres) of soybeans producing 85.49 million metric tons (3.14 billion bushels) worth US\$17.7 billion (American Soybean Association, 2005). Soybean oil is an important component, with its

market dominance exemplified by having 80% of all USA edible fats and oil consumption (American Soybean Association, 2005). In 2004, 8.5 million metric tons (18.8 billion pounds) of soybean oil was globally produced and comprised 57% of all world oilseed production and 30% of all world vegetable oil production (American Soybean Association, 2005). Consumption of soybean oil is for salad and cooking oil (44%), baking and frying (44%), margarine (7%) and industrial products (4%) (American Soybean Association, 2005). Green immature soybeans, referred to as edamame, are consumed during soybean development, especially just prior to seed hardening stage (≈ 20 d prior to harvest). Edamame, boiled with or without pods, is consumed as snacks, soups, salads or vegetable dishes primarily in Asia, and has a growing market in USA.

[☆] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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An emerging industrial product derived from soybean oil is soy biodiesel and 113.4 million liters (30.0 million gallons) was consumed by USA in 2004 (American Soybean Association, 2005). Soy biodiesel has been heavily researched in the last decade (Colucci & Borrero, 2005; Knothe, 1999) and is certain to increase with uncertainty over world crude petroleum oil prices. The composition of soybean oil influences its potential uses and it is unknown if changes in composition during seed development affect oil characteristics.

Soybeans cultivated in USA and Canada are composed of 43–48% protein, 18–21% oil, 4.9–6.8% sucrose, 0.8–1.2% raffinose and 3.5–4.3% stachyose (Hildebrand & Hymowitz, 1981; Pöysä & Woodrow, 2002; Wilcox & Shibles, 2001). Soybean seed protein and oil are nutritionally and economically the most important. Starch content of mature soybeans is very low (0.19–0.91%) (Wilson, Birmingham, Moon, & Snyder, 1978), which is similar to the high-oil containing peanut (Isleib, Pattee, & Giesbrecht, 2004), and both have the distinction of legumes that lack considerable levels of starch in mature seeds. However, starch does accumulate in soybean seeds during development with the highest levels of 10–15% of the dry matter reported 20 d prior to harvest (Yazdi-Samadi, Rinnie, & Seif, 1977). Soybeans and peanuts cultivated at elevated temperatures or carbon dioxide levels display reduced starch degradation during seed development and have 9–20% of the dry matter as starch at maturity (Golombek, Sridhar, & Singh, 1995; Thomas, Boote, Allen, Gallo-Meagher, & Davis, 2003). It remains unknown how the carbon derived from starch degradation in soybeans is utilized for biosynthesis of oil and protein and if starch structure, properties and degradation influence oil and protein structure. We recently studied the structure and functional properties of starch isolated from high-protein, lipoxygenase-free and low-linolenic acid soybean varieties (Stevenson, Doorenbos, Jane, & Inglett, 2006). Starch of low-linolenic acid soybean variety was found to have significantly higher apparent amylose content than high-protein soybeans and significantly higher absolute amylose content than lipoxygenase-free soybeans.

Starch is the main carbohydrate in plant storage organs. Starch has been extensively characterized in many cereal, root and tuber crops, as well as legumes, but there were no reports of starch characteristics from the leguminous soybean before the study of Stevenson et al. (2006). Earlier research focused on content (Wilson et al., 1978) or granule morphology (Nakamura, 1974) of soybean starch. In this study we characterize starch structure and properties from immature soybeans of one variety each that at maturity exhibit low-linolenic acid, low-saturated or normal fatty acid seed oil profiles. Characterization of immature soybean starches may provide some insight as to whether starch structure and properties relate to soybean oil composition in mature soybeans. Additionally, characterization of soybean starch will contribute to the understanding of structure/functional property relationships for starches from different botanical sources. Charac-

terization of soybean starch will also help us understand the texture of edamame. Sensory preferences of edamame have recently been studied among consumers (Wszelaki et al., 2005), and it is likely that starch characteristics contribute to overall edamame quality.

2. Materials and methods

2.1. Plant material

Soybean varieties (*Glycine max* (L.) Merr.) that exhibit normal (A02-338013), low-linolenic acid (A03-946005) and low-saturated (A03-844066) fatty acid seed oil profiles at maturity were cultivated at Iowa State University Agricultural Engineering & Agronomy Research Center in western outskirts of Ames, IA. Soybean seeds were obtained from the soybean breeding project at Department of Agronomy, Iowa State University, Ames, IA. Soybean plots consisted of eight rows wide and about 100 m long for each soybean line. Normal crop husbandry was conducted. Soybean pods estimated, based on local grower knowledge, to be approximately 20 d prior to commercial harvest were harvested on September 8, 2004, for the three soybean lines. Three replicates were collected corresponding to soybean pods harvested from three adjacent rows. Soybean pods were transported and stored at refrigerated temperatures prior to starch extraction (<2 d).

2.2. Starch isolation, starch content and water content of soybean seeds

Starch was isolated from soybean pods using a method reported by Kasemsuwan, Jane, Schnable, Stinar, and Robertson (1995) with further modification by Stevenson (2003). One day after harvest, soybean pods were blended in 0.3% (w/v) sodium metabisulfite using a Waring commercial blender (Waring Corp., New Hartford, CT, high mode used). Initially soybean seeds and pods were extracted separately, but no significant starch content was found in pods and therefore pods were not removed for starch extraction. Soybean seed puree was then filtered through a screen of 106 μ m mesh and filtrate was spun at 10,500g for 40 min to deposit starch. To remove protein, lipids and chlorophyll, starch pellet was washed with 10% toluene in 0.1 M sodium chloride and left standing for at least 4 h. This step was repeated 15–25 times. The toluene/salt solution treated starch was then washed three times with deionized water, twice with ethanol and then recovered by filtration using Whatman No. 4 filter paper. Purified starch cake was dried in a convection oven at 35 °C for 48 h. Water content of soybean seeds was determined by freeze-drying finely diced seeds. Total starch content of freeze-dried soybean seed powders, measured in duplicate, was determined using total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), based on AOAC method 996.11, AACC method 76.13 and ICC standard method No. 168, in which soybean powders

are hydrolyzed with α -amylase and amyloglucosidase, and subsequent glucose content determined using glucose oxidase-peroxidase. An internal standard of corn starch was added to the samples to check quantitative recovery of starch. Oil content of freeze-dried, ground soybean seeds collected 20 d prior to harvest and at harvest for each replicate was determined using a Soxtec System HT 1043 extraction unit (Tecator Inc., Herndon, VA). Protein content of freeze-dried, ground soybean seeds collected 20 d prior to harvest and at harvest for each replicate was determined using the Leco[®] CHN-2000 series Elemental Analyzer (Leco Corp., St. Joseph, MI). Fatty acid composition of extracted oil from seeds at harvest was analyzed by injecting fatty acid methyl esters (House, Larson, Johnson, DeVries, & Martin, 1994) into a Hewlett–Packard 6890 gas chromatograph (Hewlett–Packard, Santa Clara, CA) equipped with a flame ionization detector and an SP-2380 column (60 m \times 0.25 mm i.d., 0.20 μ m film thickness, Supelco, Bellefonte, PA) using helium as the carrier gas at a linear flow velocity of 18 cm/s (Eller & King, 2000).

2.3. Starch granule morphology

Starch granules of each cultivar were spread on silver tape and mounted on a brass disk, then coated with gold/palladium (60/40). Sample images were observed at 1500, 2500 and 5000 \times magnification under a scanning electron microscope (JEOL model 1850, Tokyo, Japan).

2.4. Starch crystallinity

Crystallinity of starch granules was determined using X-ray diffractometry. X-ray diffraction patterns were obtained with copper, K_{α} radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following the procedure reported by Song and Jane (2000). X-ray diffraction patterns of starch were classified into A-, B- or C-type polymorphism as established by Katz and Itallie van (1930). Percentage crystallinity was calculated following the method of Hayakawa, Tanaka, Nakamura, Endo, and Hoshino (1997). The following equation was used to determine percentage crystallinity:

$$\text{Crystallinity (\%)} = A_c / (A_c + A_a) \times 100$$

where A_c = crystalline area and A_a = amorphous area, on the X-ray diffractogram.

2.5. Molecular weight and gyration radius of amylopectin

Weight-average molecular weight and z -average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch analysis, duplicate measurements of each replicate for all soybean lines, was conducted as described by Yoo and Jane (2002a). The HPSEC system consisted of a HP 1050 series isocratic

pump (Hewlett–Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett–Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak SB-G guard column and SB-806 and SB-804 analytical columns (Showa Denko K.K., JM Science, Grand Island, NY) were used following the method of Yoo and Jane (2002b), except flow rate being 0.6 mL/min and sample concentration 0.5 mg/mL.

2.6. Apparent and absolute amylose contents

Apparent and absolute amylose contents of starch were determined by measuring iodine affinities of defatted whole starch and of amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY) (Kasemsuwan et al., 1995). Starch samples were dissolved and defatted in 90% dimethyl sulfoxide (DMSO) solution and followed by alcohol precipitation. The absolute amylose content was determined by subtracting the iodine affinity of amylopectin from that of the defatted whole starch (Takeda & Hizukuri, 1987). The analysis was duplicated for each replicate of each soybean line.

2.7. Amylopectin branch chain-length distribution

Amylopectin was fractionated by selective precipitation of amylose using n -butanol as a complexing agent (Schoch, 1942). Amylopectin (2 mg/mL) was defatted in 90% DMSO at 100 °C for 1 h, followed by stirring at 25 °C for 24 h and then dispersed in 0.1 M sodium acetate, pH 4.5, and debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloclavata*) (EN 102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) as described by Jane and Chen (1992). Branch chain-length distribution of amylopectin was determined by using an HPAEC system (Dionex-300 and Dionex-GP50 gradient pump, Sunnyvale, CA) equipped with an amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma Chemical Co., St. Louis, MO) post-column, on-line reactor and a pulsed amperometric detector (Dionex-ED50, Sunnyvale, CA) (HPAEC-ENZ-PAD) (Wong & Jane, 1997). PA-100 anion exchange analytical column (250 \times 4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously (McPherson & Jane, 1999) except Chromeleon[®] version 6.50 software was used. HPAEC-ENZ-PAD analysis was duplicated for each replicate of each soybean variety.

2.8. Thermal properties of starch

Thermal properties of starch were determined by using a differential scanning calorimeter (DSC 2920 modulated, TA Instruments, New Castle, DE) (Jane et al., 1999). Starch (2 mg, dry starch basis (dsb)) was accurately

weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. The sample was allowed to equilibrate for 2 h and scanned at a rate of 5 °C/min over a temperature range of 0–120 °C. An empty pan was used as the reference. The rate of starch retrogradation was determined using the same gelatinized samples, stored at 4 °C for 7 d, and analyzed using the same process described for gelatinization (White, Abbas, & Johnson, 1989). All thermal analyses were conducted in triplicate for each replicate of each soybean line.

2.9. Pasting properties of starch

Starch pasting properties were analyzed using a Rapid Visco-Analyser (RVA-4, Foss North America, Eden Prairie, MN) (Jane et al., 1999). Starch suspension (8%, w/w, dsb), in duplicate for each replicate of each soybean line, was prepared by weighing starch (2.24 g, dsb) into a RVA canister and making up the total weight to 28 g with deionized water. Starch suspension was equilibrated at 30 °C for 1 min, heated at a rate of 6.0 °C/min to 95 °C, maintained at 95 °C for 5.5 min, cooled to 50 °C at a rate of 6.0 °C/min and then maintained at 50 °C for 5 min. Constant paddle rotating speed (160 rpm) was used throughout entire analysis, except for rapid stirring at 960 rpm for the first 10 s to disperse starch sample.

2.10. Statistical analysis

All statistical significance tests were calculated using SAS (1999) one-way ANOVA and multiple comparison by applying Tukey difference test (Tukey, 1993) at the 5% level of significance.

3. Results and discussion

3.1. Soybean composition

Composition of soybeans collected 20 d prior to, and at commercial harvest, is shown in Table 1. Starch content of

soybeans 20 d prior to harvest ranged from 10.4% to 11.7% (dry weight basis), which was consistent with previous reports at same developmental stage (Stevenson et al., 2006; Yazdi-Samadi et al., 1977). Composition of soybeans at commercial harvest maturity showed that a greater proportion of carbon from starch degradation was assimilated for protein synthesis rather than lipids. However, little is known about physiological changes in developing soybeans. The 0.6–1.3% increase in oil content may involve synthesis of specific fatty acids, which could be important in determining the overall fatty acid composition. Additionally, the possibility that a greater amount of lipids could be synthesized in the last 20 d prior to harvest if some lipids are metabolized, should not be ignored. A sigmoidal accumulation of palmitic, linoleic and linolenic acids during advancement of filling stage has been observed for soybeans, but stearic acid accumulation varied depending on cultivar and oleic acid accumulated rapidly at late maturity stages for some cultivars (Ishikawa, Hasegawa, Takagi, & Tanisaka, 2001). Therefore the small increase in oil content of three soybean lines could involve changes in fatty acid composition. At commercial harvest maturity, fatty acid composition of three soybean varieties is as expected (Table 2).

3.2. Starch granule morphology

Scanning electron micrographs of starch isolated from all three soybean varieties showed similar granule morphology and small size distribution, with the diameters ranging between 0.5 and 4.5 µm (Fig. 1). Starch granule distribution was similar to previous study (Stevenson et al., 2006), and like that study, starch granules were very irregular, which could result from partial amylolytic hydrolysis. Soybeans contain β-amylase (Ren, Thompson, & Madison, 1993) that might have hydrolyzed starch granules, but starch metabolism was reported to be independent of β-amylase activity in developing soybean seeds (Adams, Broman, & Rinne, 1981a). Occurrence of α-amylase in developing soybeans has been reported (Adams, Broman,

Table 1
Soybean composition 20 d prior to harvest and at commercial harvest

Variety	Water content (%) ^a	Starch content (% dry weight) ^b	Protein content (% dry weight)	Lipid content (% dry weight)
20 d prior to harvest				
Low-linolenic acid	65.0a ^c	11.6	36.0b	19.4
Low-saturates	62.4b	10.4	38.0a	19.3
Normal soybean	63.3b	11.7	38.6a	19.6
	<i>P</i> = 0.002 ^d	<i>P</i> = 0.20	<i>P</i> = 0.01	<i>P</i> = 0.79
Harvest ^c				
Low-linolenic acid	7.6		44.0	20.7
Low-saturates	7.7		45.1	20.3
Normal soybean	7.9		46.4	20.2

^a Average of three replicates.

^b Starch contents were averaged of two analyses from each of three replicates.

^c Values with different letters denote differences at the 5% level of significance for each comparison among varieties in the respective column.

^d *P* represents the probability of *F*-statistic exceeding expected for each comparison among varieties in the respective column.

^e Field replication was not possible for sampling at commercial harvest, therefore no statistical analysis was conducted.

Table 2
Fatty acid composition of oil from soybeans at commercial harvest maturity

Variety	C16:0 ^a	C18:0	C18:1	C18:2	C18:3
Low-linolenic acid	12.4	4.4	28.2	53.8	1.1
Low-saturates	5.3	2.6	26.2	57.2	8.8
Normal soybean	13.0	3.4	21.7	53.3	8.6

^a C16:0, C18:0, C18:1, C18:2 and C18:3 represent palmitic, stearic, oleic, linoleic and linolenic fatty acids, respectively.

Norby, & Rinne, 1981b), but activity does decline towards maturity, levels are generally low at all development stages, and the lack of future studies relative to β -amylase casts doubt on its contribution to starch metabolism in soybeans. Soybean starch small granules were similar in diameter to Chinese taro, small pigweed and parsnip starch, not

quite as small as Iliuaua dasheen, amaranth and cow cockle starch, but smaller than observations reported for all other starches (Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994).

3.3. Starch crystalline structure

Soybean starches all exhibited the C_B-type X-ray diffraction patterns (Fig. 2), that is composed of mixture of A- and B-type polymorphs with the latter being predominant. A strong peak was observed at $2\theta = 17.2^\circ$, characteristic of the B-type starches, and a single peak was observed at $2\theta = 22\text{--}24^\circ$ with another peak at $2\theta = 15^\circ$, characteristic of the A-type starch. There was an unexpected peak at $2\theta = 26.2^\circ$ for both normal and low-saturate soybeans. The C_B-type X-ray diffraction pattern has been observed previously for soybean starches from seeds 20 d prior to harvest (Stevenson et al., 2006). Although we mention in previous

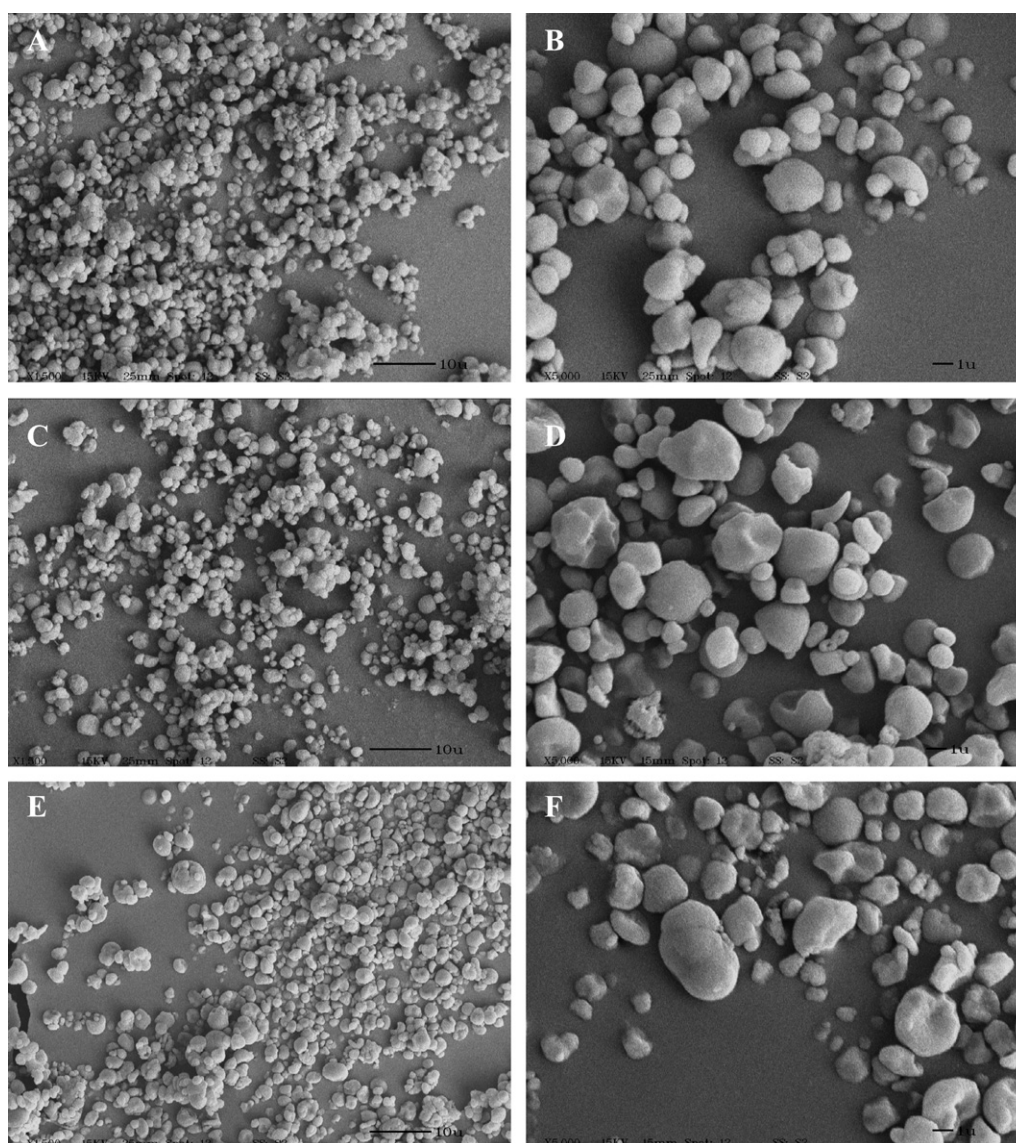


Fig. 1. Scanning electron micrographs of low-linolenic acid (A and B), low-saturates (C and D) and normal soybean (E and F) soybean starches (scale bar = 10 μ m for A, C and E, and 1 μ m for B, D and F).

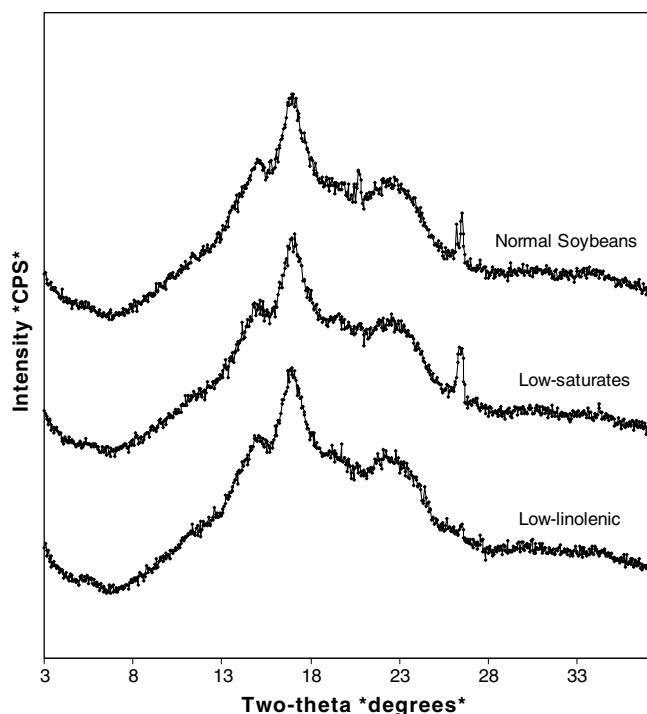


Fig. 2. X-ray diffraction patterns of low-linolenic acid, low-saturates and normal soybean starches.

section that starch metabolism has been reported to be independent of β -amylase activity, we can not dismiss the possibility that β - or α -amylases partially hydrolyzed starch at this developmental stage resulting in B-type polymorphs, because subsequent structural analyses we report are more consistent with A-type native starches. Starch damaged by enzymatic attack has been reported to retrograde and form B-type polymorphs (Gérard, Colonna, Buléon, & Planchot, 2001; Gidley et al., 1995), but it is unclear whether the exoacting β -amylase, hydrolyzing starch from only non-reducing ends, would alter polymorphism. As mentioned in previous section, low levels of α -amylase are present in developing soybeans (Adams et al., 1981b), so hydrolysis could be occurring from α - or β -amylase, or possibly from combined activity.

3.4. Iodine affinity and amylose content

The iodine affinities of the defatted whole starches and the corresponding apparent amylose contents are shown in Table 3, with the absolute amylose content of normal soybean variety significantly higher than low-linolenic acid and low-saturate varieties. The lower absolute amylose content of low-linolenic acid soybean starch reflected the significantly higher iodine affinity of amylopectin fraction relative to the two other soybean varieties studied. Amylopectin fraction iodine affinities of the soybean varieties studied were towards the low-end of the range for all starches studied (Jane et al., 1999), indicating that soybean amylopectin has short branch-chains. Absolute amylose

Table 3

Iodine affinities, apparent amylose and absolute amylose contents of defatted soybean starches

Variety	Iodine affinity		Apparent amylose content (%) ^a	Absolute amylose content (%) ^b
	Whole starch	Amylopectin fraction		
Low-linolenic acid	4.51 ^c	1.46a	22.7	16.5b
Low-saturates	4.36	0.79b	21.9	18.7b
Normal soybean	4.56	0.77b	22.9	19.8a
	$P = 0.28^d$	$P = 0.01$	$P = 0.28$	$P = 0.05$

^a Apparent amylose contents were averaged from two analyses for each of three replicates. Values were calculated from dividing iodine affinity by a factor of 0.199.

^b Absolute amylose contents were averaged from two analyses for each of three replicates. Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199 based on formula by Takeda et al. (1987).

^c Values with different letters denote differences at the 5% level of significance for each comparison among soybean varieties in the respective column.

^d P represents the probability of F -statistic exceeding expected for each comparison among soybean varieties in the respective column.

content of low-linolenic acid soybean variety in this study 20 d prior to harvest (16.5%) was very similar to a different low-linolenic acid soybean variety (16.2%) collected at same developmental stage but cultivated 200 miles away (Stevenson et al., 2006). In both studies the low-linolenic acid soybean varieties possessed the *fan1*(A5), *fan2* and *fan3* genes that are thought to have partial deletion of ω -3 desaturase gene, providing the ultra-low-linolenic acid trait (Fehr, 2006). In the same previous study, low-linolenic acid soybean variety had higher absolute amylose content than a high-protein and lipoxygenase-free soybean variety (Stevenson et al., 2006). However, in this study the low-linolenic acid variety was found to have lower absolute amylose content than a soybean variety with oil that has normal or low-saturate fatty acid composition. Therefore, since the soybean variety with normal fatty acid composition had highest absolute amylose content, this study indicates a possibility that differences in amylose content could be influencing soybean oil fatty acid profile at maturity. Since no obvious relationship has ever been establish between starch structure during development and oil composition at maturity for any oilseed crop, further studies are needed involving a greater number of soybean varieties with varying oil characteristics.

Although it is difficult to conceive a relationship between starch structure and oil composition based on current knowledge, there is also the counterargument of why do soybeans with different seed oil fatty acid composition at maturity have different starch structures during development? Therefore we must consider possible explanations for this observation. Susceptibility of amylose and amylopectin to amylases could be a possible mechanism of how amylose content may influence oil fatty acid composition. Several studies report preference of amylases for hydrolyzing either amylose or amylopectin (Freer, 1993; Kamasaka,

Sugimoto, Takata, Nishimura, & Kuriki, 2002; Lauro, Forssell, Suortti, Hulleman, & Poutanen, 1999; Planchot & Colonna, 1995). Amylose could have greater resistant to enzyme hydrolysis due to formation of lipid complex (Lauro et al., 1999) or its low solubility (Robyt, 1998). Soybean starch is largely thought to be degraded by β -amylase, an exo-acting class of enzymes with a substrate preference that is unambiguous. All studies report that β -amylase preferentially degrades amylopectin faster than amylose (Boyce & Volenec, 1992; Dicko et al., 1999; Lizotte, Henson, & Duke, 1990), but we do not know if this occurs *in vivo* for soybeans. Some fatty acids, such as oleic acid, have been reported to rapidly accumulate late in soybean development (Ishikawa et al., 2001). While no information is available about whether the availability of carbon close to soybean seed maturity can influence the extent of fatty acid synthesis, studies in rape and sunflower have shown that availability of carbon due to starch synthesis and degradation during seed development influences fatty acid synthesis (Eastmond & Rawsthorne, 2000; Pleite, Pike, Garcés, Martínez-Force, & Rawsthorne, 2005; Vigeolas, Möhlmann, Martini, Neuhaus, & Geigenberger, 2004). Considerably more research is needed to establish if accumulation of fatty acids during development of the soybean varieties in the study by Ishikawa et al. (2001) is observed in other soybean varieties. Additionally, determining what regulates fatty acid synthesis during soybean developmental stages would also be worthwhile. Further research also needs to be conducted on *in vivo* β - and α -amylase activities of soybeans during development, especially the latter which has been largely ignored, and also the substrate affinities of each enzyme for amylose and amylopectin. There is no good explanation for our observations based on current knowledge, but the best explanation that could be surmised to support a relationship would be if the low-linolenic acid and low-saturate fatty acid soybean varieties, have amylopectin, which is higher 20 d prior to harvest compared to normal soybeans, preferentially degraded by β - or α -amylase, providing greater amount of carbon for synthesis of fatty acids such as oleic acid that would be reducing the concentration of both linolenic and unsaturated fatty acids.

3.5. Amylopectin molecular weight and gyration radius

Weight-average molecular weight (M_w), polydispersity and gyration radius (R_z) of soybean starch amylopectin are shown in Table 4. The M_w of soybean amylopectin ranged from 5.91×10^8 to 8.92×10^8 g/mol for the three varieties, which was within typical range for A-type starches (Yoo & Jane, 2002b). Similar to absolute amylose content, amylopectin M_w and gyration radius at 20 d prior to harvest of the low-linolenic acid variety (5.91×10^8 and 420 nm, respectively) in this study were similar to a low-linolenic acid variety (5.11×10^8 and 406 nm, respectively) cultivated 200 miles away (Stevenson et al., 2006). Because of high variation among field replicates for each soybean variety for measurements of amylopectin molecular weight,

Table 4

Average amylopectin molecular weight, polydispersity, gyration radius and density of dispersed soybean amylopectin^a

Variety ^b	$M_w \times 10^8$ (g/mol) ^c	Polydispersity (M_w)	R_z (nm) ^d	ρ (g/mol/nm ³) ^e
Low-linolenic acid	5.91	3.73	420	7.4
Low-saturates	7.04	2.55	439	9.0
Normal soybean	8.92	4.59	479	12.3
	$P = 0.73^f$	$P = 0.59$	$P = 0.91$	$P = 0.74$

^a Data were obtained from two analyses of three replicates.

^b Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; freshly prepared starch aqueous solution (100 μ L; 0.5 mg/mL) was injected to HPSEC system.

^c Weight-average molecular weight.

^d z-average radius of gyration.

^e Dispersed amylopectin density is equal to M_w/R_z^3 . Values for density may not correspond directly to data in table due to rounding of M_w and R_z .

^f P represents the probability of F -statistic exceeding expected for each comparison among soybean varieties in the respective column.

polydispersity and gyration radius, there were no significant differences observed. Amylopectin polydispersity (M_w/M_n) was similar to many other native starches (Stevenson, 2003).

3.6. Amylopectin branch chain-length distribution

Amylopectin branch chain-length distributions for the starches of soybean varieties are shown in Fig. 3 and summarized in Table 5. The most distinctive characteristic of all soybean amylopectins was the short average branch chain-length (Table 5 and Fig. 3) that is towards the short-end of all A-type starches and far shorter than all other B- and C-type starches (Jane et al., 1999). While some variation was observed for amylopectin branch chain-length distribution between the low-linolenic acid variety in this study and a different low-linolenic variety cultivated 200 miles away in a previous study (Stevenson et al., 2006), there was similarity in percentage of long branch-chains with DP of ≥ 25 (25.6% and 26.1%, respectively). All three soybean variety amylopectins exhibited a slight dip in percentage peak area at DP 8 that was also observed in a previous study for low-linolenic acid and lipoxigenase-free, but not for high-protein soybean variety (Stevenson et al., 2006). A dip in percentage peak area for DP 8 has been observed in potato, mungbean and Chinese taro amylopectin (Jane et al., 1999). Low-saturate soybean variety had amylopectins with lower average branch chain-length and significantly greater proportion of DP 6–12. Further research evaluating many soybean varieties with varying oil fatty acid composition would be worthwhile to establish if fine structure of amylopectin influences oil characteristics. Additionally, minimizing variation from within field replicates of the same variety may reveal further relationships between amylopectin fine structure and oil fatty acid composition. All three soybean varieties had considerable amounts of very short branch-chains (DP 3–5), which

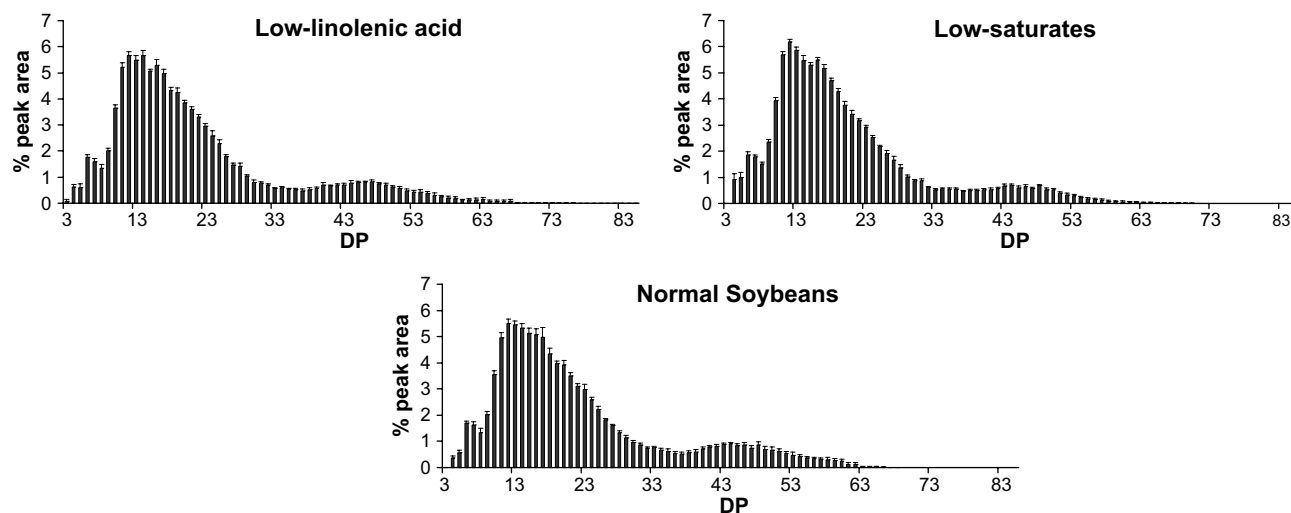


Fig. 3. Relative peak area distributions of low-linolenic acid, low-saturates and normal soybean amylopectin branch chain-lengths analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of three replicates. DP, degree of polymerization.

Table 5
Branch chain-length distributions of soybean seed amylopectins^a

Soybean variety	Peak DP		Average CL	Percent distribution ^b					
	I	II		DP 3–5	DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP ≥ 37
Low-linolenic acid	13.0	46.3	21.5	1.3	6.7	21.2b ^c	51.3	12.4	13.2
Low-saturates	12.2	45.5	19.9	1.9	7.5	23.4a	52.1	12.6	9.9
Normal soybean	13.5	46.2	21.9	0.9	6.7	20.7b	50.4	13.3	14.7
			$P = 0.09^d$	$P = 0.31$	$P = 0.17$	$P = 0.02$	$P = 0.76$	$P = 0.46$	$P = 0.16$

^a Values were calculated from two injections for each of three replicates.

^b Grouping of degree of polymerization (DP) numbers followed that of Hanashiro et al. (1996).

^c Values with different letters denote differences at the 5% level of significance for each comparison among soybean varieties in the respective column.

^d P represents the probability of F -statistic exceeding expected for each comparison among soybean varieties in the respective column.

may indicate β - or α -amylase partial degradation of starch occurred. A considerable amount of DP 3–5 amylopectin branch-chains has been previously observed in winter squash starches from fruit at harvest, in which starch is undergoing an initial phase of rapid starch degradation (Stevenson, Yoo, Hurst, & Jane, 2005). Similar to findings on amylose content, there are no previous reports for any oilseed crop that could help suggest how amylopectin fine structure could influence fatty acid composition. The low-saturate soybean variety had higher percentage of amylopectin branch chain-lengths of DP ≤ 12 compared with the normal soybean variety (32.8% and 27.3%, respectively), and had lower proportion of long branch chains with DP ≥ 37 (9.9% and 14.7%, respectively). The best explanation that could be surmised would be that the structure of amylopectin from the low-saturate soybean variety is conducive to faster amylolytic degradation in the final 20 d prior to harvest, based on the vast studies characterizing amylases. Faster starch degradation would allow greater amount of carbon available at late maturity. As discussed earlier, it is unknown if availability of carbon can influence fatty acid composition. For low-saturate soybeans, greater synthesis of oleic and other unsaturated fatty

acids close to maturity, which has been previously reported (Ishikawa et al., 2001), would thereby reduce the percentage of saturated fatty acids. Greater enzymic susceptibility of amylopectin molecules with greater proportion of short branch chains has been reported (Gallant, Bouchet, Buléon, & Pérez, 1992; Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001).

3.7. Thermal properties

Thermal properties of the soybean starches are shown in Table 6. Onset gelatinization temperatures (T_o) for soybean starches are lower than the majority of native starches reported in literature, whereas change in enthalpy of gelatinization (ΔH) was typical (Stevenson, 2003). Low T_o of soybean starch could be because of high proportion of short and intermediate amylopectin branch-chains (DP ≤ 24), a relationship that has been previously reported for other starches (Jane et al., 1999; Ji et al., 2003). High variation within field replicates of the same variety resulted in no significant differences observed in starch thermal properties among the soybean varieties. No significant differences were also observed in thermal properties of retro-

Table 6
Thermal properties of native soybean starches

Soybean variety ^a	Starch gelatinization			Amylose–lipid thermal transition		
	T_o (°C) ^b	T_p (°C)	ΔH (J/g)	T_o (°C) ^b	T_p (°C)	ΔH (J/g)
Low-linolenic acid	51.0	55.3	12.7	87.4	91.4	1.5
Low-saturates	51.2	55.2	10.7	87.2	91.6	1.6
Normal soybean	51.8	55.8	12.3	87.5	91.9	2.4
	$P = 0.86^c$	$P = 0.87$	$P = 0.30$	$P = 0.90$	$P = 0.61$	$P = 0.66$

^a Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o , T_p and ΔH represent onset and peak gelatinization temperature, and enthalpy change of gelatinization, respectively.

^b Values were calculated from three analyses for each of three replicates.

^c P represents the probability of F -statistic exceeding expected for each comparison among soybean varieties in the respective column.

graded soybean starches (Table 7). T_o and ΔH of starch from low-linolenic acid soybean variety in this study (51.0 °C and 12.7 J/g, respectively) were similar to low-linolenic acid soybean variety (52.0 °C and 12.3 J/g, respectively) in previous study cultivated 200 miles away (Stevenson et al., 2006).

3.8. Pasting properties

Pasting properties of soybean starches are summarized in Table 8. Low-linolenic acid soybean starch paste tended to have higher viscosity throughout RVA test conditions, but was not significantly more viscous than the other two soybean varieties. Peak and final viscosity of starch pastes from all three soybean varieties studied were low compared with most non-genetically modified starches for an 8% (w/w) starch suspension (Jane et al., 1999; Stevenson, 2003). Large variation was observed within field replicates of the same variety, resulting in no significant differences for any pasting parameter measured.

To conclude, this study showed that both a low-linolenic acid and low-saturate fatty acid soybean variety had starch with significantly lower absolute amylose content than a normal soybean variety. Low-saturate soybean variety also had amylopectin molecules with significantly higher percentage of DP 6–12 branch chain-lengths compared with low-linolenic acid and normal soybean amylopectin. Field replicates of the same soybean variety had high variation, with further differences among soybean varieties likely to be found once variation is minimized. This study along with a previous study (Stevenson et al., 2006) that found low-linolenic acid soybeans had significantly higher absolute amylose than a high-protein and lipoxygenase-free soybean variety, suggest that further research is needed to establish whether amylose content and the fine structure of amylopectin of developing soybean seeds can affect the fatty acid composition of soybean oil in mature seeds. The 7–8% increase in protein content during the 20 d prior to harvest, suggests that studies investigating whether carbon from starch degradation is utilized for protein

Table 7
Thermal properties of retrograded soybean starch

Soybean variety ^a	Starch gelatinization			Amylose–lipid thermal transition		
	T_o (°C)	T_p (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	ΔH (J/g)
Low-linolenic acid	40.7	50.8	6.5	87.1	91.0	1.7
Low-saturates	39.5	51.1	7.2	87.2	91.1	1.3
Normal soybean	38.6	50.6	5.9	87.6	91.8	1.4
	$P = 0.32^b$	$P = 0.77$	$P = 0.61$	$P = 0.56$	$P = 0.14$	$P = 0.17$

^a Same starch samples after gelatinization (see Table 5) were stored for 7 days at 4 °C and rescan using DSC.

^b P represents the probability of F -statistic exceeding expected for each comparison among soybean varieties in the respective column.

Table 8
Pasting properties of soybean starches measured by Rapid Visco-Analyser^{a,b}

Soybean variety	Peak viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Pasting temperature (°C)
Low-linolenic acid	100.2	24.9	119.2	43.9	74.6
Low-saturates	78.5	24.6	90.8	37.0	68.9
Normal soybean	96.8	38.1	98.1	39.5	82.4
	$P = 0.90^c$	$P = 0.86$	$P = 0.77$	$P = 0.93$	$P = 0.51$

^a 8% (w/w) starch suspension measured in duplicate for all three replicates.

^b Viscosity measured in Rapid Visco-Analyser units (RVU), 1 RVU = 12 centipoise.

^c P represents the probability of F -statistic exceeding expected for each comparison among soybean varieties in the respective column.

synthesis would also be worthwhile. It appears unlikely that starch is degraded for synthesis of sucrose, raffinose or stachyose as these sugars have been found to accumulate at a steady rate during soybean development (Blackman, Obendorf, & Léopold, 1992; Lowell & Kuo, 1989).

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