

Carbohydrate Polymers 40 (1999) 261-269

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Chemical and physical properties of ginkgo (Ginkgo biloba) starch[☆]

K.E. Spence, J. Jane*

Department of Food Science and Human Nutrition, Center for Crops Utilization Research, 2312 Food Sciences Building, Iowa State University, Ames, IA 50011-1120, USA

Received 5 January 1999; received in revised form 2 April 1999; accepted 2 April 1999

Abstract

Starch isolated from mature *Ginkgo biloba* seeds and commercial normal maize starches were subjected to α-amylolysis and acid hydrolysis. Ginkgo starch was more resistant to pancreatic α-amylase hydrolysis than the normal maize starch. The chain length distribution of debranched amylopectin of the starches was analyzed by using high performance anion-exchange chromatography equipped with an amyloglucosidase reactor and a pulsed amperometric detector. The chain length distribution of ginkgo amylopectin showed higher amounts of both short and long chains compared to maize starch. Naegeli dextrins of the starches prepared by extensive acid hydrolysis over 12 days demonstrated that ginkgo starch was more susceptible than normal maize to acid hydrolysis. Ginkgo dextrins also demonstrate a lower concentration of singly branched chains than maize dextrins, and unlike maize dextrin, debranched ginkgo shows no multiple branched chains. The ginkgo starch displayed a C-type X-ray diffraction pattern, compared to an A-type pattern for maize. Ginkgo starch and maize starch contained 24.0 and 17.6% absolute amylose contents, respectively. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Ginkgo starch; α-amylolysis; Naegeli dextrins

1. Introduction

Ginkgo biloba is the oldest species of tree known, dating back to 300 million years, and is often called a "living fossil". The female trees produce a fruit with an orange flesh surrounding a hard, tan shell containing the kernel of the seed. The kernel is soft, green in color and edible. However, little is known about the structure of the starch within the seed.

Fuwa, Sugimoto, and Takaya (1979) reported that starches from ginkgo, potato, Chinese yam, banana, and lily were extremely resistant to the action of hog pancreatin. Starches of roots and tubers were, in general, more resistant than those of cereals (Fuwa et al., 1979). Ginkgo starch was one of the few non-root/tuber starches, which displayed resistance to the action of hog pancreatic α -amylase (Fuwa et al., 1979; Sugimoto, Fujita, Takaya, & Fuwa, 1980) and the only A-type starch to show resistance to glucoamylase hydrolysis (Fujimoto, Nakashima, Kubo, Saganuma & Nagahama, 1981).

Yamashita, Sugimoto, and Fuwa (1990) studied the

E-mail address: jjane@iastate.edu (J. Jane)

developmental changes in the properties of ginkgo starch. Ginkgo starch gave an A-type X-ray diffraction pattern during the early development of the starch but changed to C_A -type later towards maturity. The amylose content also tended to increase with development; and the starch, even when immature, was resistant to pancreatic α -amylase when compared to maize starch.

Enzyme digestibility studies have shown that native starches and starch crystallites of the A-type polymorph, display greater susceptibility to α -amylase hydrolysis than those of the B-type (Fuwa et al., 1979; Gallant, Bouchet, & Perez, 1992; Williamson, Belshaw, Self, Noel, Ring, Cairns, Morris, Clark, & Parker, 1992). It was intriguing to reveal what structural features of the ginkgo starch were responsible for its resistance to α -amylase hydrolysis. In this study we extracted starch from the *Ginkgo biloba* seed and investigated the fine chemical structure of the starch in comparison with maize starch.

2. Materials and methods

2.1. Materials

Protease from *Aspergillus sojae*, glucoamylase from *Rhizopus* niveus mold, and porcine pancreatic α -amylase were purchased from Sigma Chemical Co. (St. Louis,

[★] Journal paper No. J-18074 of Iowa Agriculture and Home Economics
Experiment Station, Ames, Iowa, Project No. 3258.

^{*} Corresponding author. Tel.: + 1-515-294-9892; fax: + 1-515-294-8181.

MO). The specific activity for *Aspergillus sojae* protease was given as 0.4 unit/mg solid (1 unit hydrolyzes casein to produce color equivalent to 1 μ mol tyrosine/min at pH 7 and 37°C by the Folin Ciocalteu reagent). The specific activities of glucoamylase and α -amylase were about 21,300 units/g solid and 1240 units/mg of protein, respectively. Crystalline *Pseudomonas* isoamylase (E.C. 3.2.1.68) was purchased from Hayashibara Shoji, Inc. (Okayama, Japan), with a specific activity of about 66,000 units/mg protein. The enzymes were used directly without any further purification. Other chemicals were reagent grade and used without other treatments.

2.2. Starch isolation

Mature fruits of Ginkgo biloba trees located on the ISU campus were harvested on 18 October 1996. The fruit was removed and the seeds were washed thoroughly with cool tap water. Starch was isolated from the seeds in the laboratory following a modification of the methods of Radosavljevic, Jane, and Johnson (1998). The seeds were unshelled and the kernel (endosperm) was homogenized in a Hamilton Beach blender, Model 585-1 (Hamilton Beach Inc., Washington, DC) containing deionized water (1:2.5, w/v) at full speed for 4 min. The pH of the slurry was adjusted to 7.5 and subjected to a protease treatment (fungal protease from Aspergillus sojae). The slurry was incubated with 0.5% of the protease, based on the total amount of seeds, at 37°C and 95 rpm for 2 h in a shaking water bath. The slurry was filtered through a brass sieve and a nylon screen (160 and 53 µm, respectively) to remove the fiber fraction. The fiber fraction was washed and screened $(4\times)$. The starch was isolated by centrifugation at $6000 \times g$ for 20 min. The supernatant was discarded and the top green layer of protein was removed with a laboratory spatula. The starch was washed with distilled water $(3 \times)$ and dried in a laboratory convection oven at 40°C for 48 h. This produced a starch with a low protein content ($\leq 0.16\%$).

2.3. Fractionation

Amylose was separated from the amylopectin by following the methods of Schoch (1942) and Jane and Chen (1992). The recrystallization procedures for purifying amylopectin and amylose were repeated five times; until no amylose—butanol precipitated. The absence of amylose in the amylopectin fraction was confirmed by gel-permeation chromatography (Sepharose CL-2B, Pharmacia Inc., Piscataway, NJ).

2.4. Morphology of the starch

Starch (1%, w/w) was suspended in 100% methanol and mixed. A drop of the starch suspension was placed on the silver tape (non-sticky side) and attached to a brass disk. The specimens were sputter coated with gold:palladium (60:40), mounted and observed by using a JEOL 1850 scan-

ning electron microscope (SEM) (Tokyo, Japan) at the Bessey Microscopy Facility (ISU, Ames, IA). Micrographs were taken at $500 \times$ and $1500 \times$ magnification, and the diameter of the starch granules was determined.

2.5. Starch X-ray diffraction pattern

Starch samples were equilibrated in a saturated relative humidity chamber for 24 h at room temperature. Starch X-ray diffraction was performed on a Siemens D-500 X-ray diffractometer (Siemens, Madison, WI) with copper $K\alpha$ radiation. Signals of the reflection angle of 2θ , from 4 to 40° , were recorded.

2.6. Nitrogen content

Nitrogen contents were analyzed by the micro-Kjeldahl method with a Kjeltec digestor and distilling system (Tecator, Inc., Hoganas, Sweden). The protein contents of the samples were calculated by multiplying nitrogen content by 6.25.

2.7. Phosphorus determination

Total phosphorus contents were analyzed by dry ashing the whole starches, followed by molybdenum blue spectro-photometric analysis (Smith & Caruso, 1964). Phosphorus structures were analyzed by using P-31 NMR spectroscopy following the method of Lim, Kasemsuwan, and Jane (1994).

2.8. Lipid content determination

The lipid content of the starch was determined by using Goldfish solvent extractors (Laboratory Construction Co., Kansas City, MO). The solvent used was propanol/water (3:1, v/v) (Morrison & Coventry, 1985; Morrison, 1988).

2.9. Gel permeation chromatography (GPC)

Samples for the molecular size distribution analyses were prepared by following the procedures of Jane and Chen (1992). The total carbohydrate and amylose—iodine blue values were used to calculate the molecular size distributions and identify the amylose and amylopectin fractions, respectively.

2.10. Iodine affinities of starch and amylopectin

The iodine affinities of defatted ginkgo starch and fractionated amylopectin were determined by using a potentiometric autotitrator (702 SM Titrino, Brinkman Instrument, Westbury, NY) with Metrodata recording software. The analyses followed the methods of Schoch (1964) for amylose and Kasemsuwan, Jane, Schnable, Stinard, and Robertson (1995) for absolute amylose contents. The analyses were replicated at least three times. The absolute

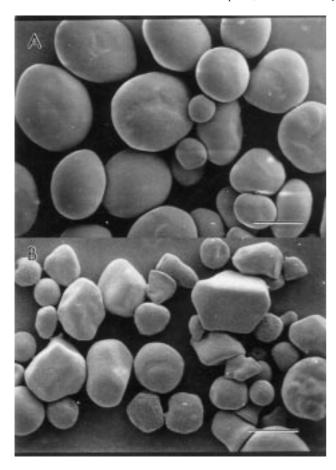


Fig. 1. Scanning electron micrographs of ginkgo starch granules (A) and maize starch granules (B). Bar = $10 \mu m$.

amylose content was calculated by the following equation:

$$A_{\text{amylose}} = IA_{\text{starch}} - IA_{\text{amylopectin}}/[0.20 - IA_{\text{amylopectin}}/100],$$

where A_{amylose} is the percentage of absolute amylose, IA_{starch} the iodine affinity of the whole defatted starch, and $IA_{\text{amylopectin}}$ is the iodine affinity of the amylopectin fraction.

2.11. Analysis of amylopectin branch chain length distribution

Amylopectins were debranched by using iso-amylase following the procedure of Jane and Chen (1992). The chain length distribution analysis of amylopectin and Naegeli dextrins was performed by using high-performance anion-exchange chromatography with an enzyme reactor and pulsed amperometric detection (HPAEC-ENZ-PAD) (Dionex, Sunnyvale, CA) following the procedures of Wong and Jane (1997). Sample separation was performed by employing a Carbopac PA 100 anion-exchange (4 × 250 mm) column, and a Carbopac PA 100 guard column (3 × 25 mm). An immobilized postcolumn enzyme reactor (2 × 23 mm) with amyloglucosidase was inserted between the anion exchange column and the PAD detector. The enzyme reactor converted each fraction separated by the

anion exchange column and allowed for quantitative detection of each fraction by the PAD. The eluents were 100 mM sodium hydroxide (A) and 100 mM sodium hydroxide in 300 mM sodium nitrate solution (B). The separation gradient was: 0–5 min, 99% A and 1% B; 5–30 min, linear gradient to 8% B; 30–150 min, linear gradient to 30% B; 150–200 min, linear gradient to 45% B. The concentration of the debranched amylopectin solution for the injection was 1 mg/ml.

2.12. Enzymatic hydrolysis

The starches were analyzed for resistance to porcine pancreatic α-amylase hydrolysis following procedures modified from Hoover, Rorke, and Martin (1991). One hundred milligrams of starch was suspended in 25 ml of water with vigorous mixing by using a Fisher Vortex Genie 2 (Fisher Scientific, Pittsburgh, PA) and separated into 5 ml aliquots. To each starch suspension, 4 ml of phosphate buffer (pH 6.9) and 4 μl of α -amylase was added. The suspensions were incubated at 37°C and 120 rpm in a water bath shaker. The samples were removed and analyzed after 3, 24, 48, and 72 h. The supernatant was analyzed for total carbohydrate content by using the phenol-sulfuric analysis (Dubois, Giles, Hamilton, Rebers, & Smith, 1956) and the total reducing sugars by the Somogy-Nelson method (Somogy, 1945). Samples were duplicated and controls without enzymes were subjected to the same experimental conditions.

2.13. Naegeli dextrins

The rapid method for the Naegeli dextrin preparation was employed by following the procedures reported by Umeki and Kainuma (1981). The supernatant was siphoned off on day 3, 6, 9 and 12. The preparation and analysis of the Naegeli dextrins followed the methods reported by Jane, Wong, and McPherson (1997).

2.14. Starch thermal properties

The analysis about the thermal properties of the starches was performed on a Perkin-Elmer DSC 7 equipped with an Intra-Cooler II System and a thermal analysis data station operating with a Pyris software package (Perkin-Elmer, Norwalk, CT) (Wang, White, Pollak, & Jane, 1993).

2.15. Pasting properties

Pasting curves of isolated starch (8%, w/w dry starch basis, dsb; 28 g total weight) were determined by using a Rapid Visco Analyzer RVA 4 (Newport Scientific Pty. Ltd, Warriewood, NSW, Australia). The pasting procedure used the following profile: 1 min at 50°C, heat to 95°C at 6°C/min, hold for 5 min, cool to 50°C at 6°C/min. The rotating speed of the paddle was kept at 160 rpm throughout the run.

Table 1 Physical and chemical properties of ginkgo starch compared to maize starch

| Attribute | Ginkgo (Content (%) or Measurement) ^a | Maize (Content (%) or Measurement) ^a | |
|-------------------------------|--|---|--|
| X-ray diffraction pattern | С | A | |
| Iodine affinity (IA) | | | |
| Starch | $6.64 (0.001)^{b}$ | 4.37 (0.05) | |
| Amylopectin | 2.42 (0.09) | 0.85 (0.04) | |
| Amylose content | | | |
| Apparent | 33.2 | 21.8 | |
| Absolute ^c | 24.0 | 18.4 | |
| Total lipid | 0.64 (0.04) | 0.82 (0.08) | |
| Total phosphorus ^d | 0.006 | 0.019 | |

^a The data are averages of at least three replicates.

3. Results and discussion

The ginkgo starch granules were comparable in size to

maize starch when viewed by the SEM (5–20 µm diameter) (Fig. 1). The ginkgo starch granules were spherical or oval in shape with a smooth surface. Some ginkgo starch granules showed irregular indentations on the surface, however, no pores were observed. The maize starch granules were polygonal shaped with a diameter range of 5–20 µm. the maize starch granules are known to have pores or channels on the surface (Fannon, Shull, & BeMiller, 1993).

The chemical components of the starch are presented in Table 1. Careful isolation and washing procedures resulted in clean ginkgo starch (yield = 22% of seed weight). The purity of the starch was checked by chemical analysis and the SEM. The protein content was 0.16% for ginkgo starch. The total lipid content was 0.64% for ginkgo starch and 0.82% for maize starch. The total phosphorus contents of ginkgo and maize starches were 0.006 and 0.019%, respectively (Table 1). 31 P NMR spectrum of ginkgo starch (not shown) gave signals at chemical shifts of ~ 4.00 ppm, indicating the presence of phosphomonoester and no phospholipids. Maize starch has been shown to contain phospholipids and a small amount of inorganic phosphate (Kasemsuwan & Jane, 1996).

The ginkgo starch gave a C-type X-ray diffraction pattern

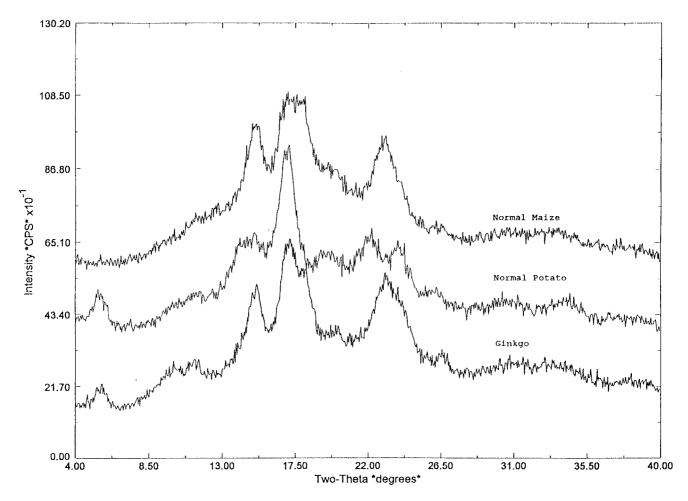


Fig. 2. X-ray diffraction patterns of maize starch, ginkgo starch, and potato starch.

^b() = Standard deviation.

^c Absolute amylose content was calculated from the following formula (Kasemsuwan & Jane, 1996): [(IA defatted starch – IA amylopectin)/IA amylose – IA amylopectin/100].

^d Percentage of phosphorus in starches (dsb) (w/w).

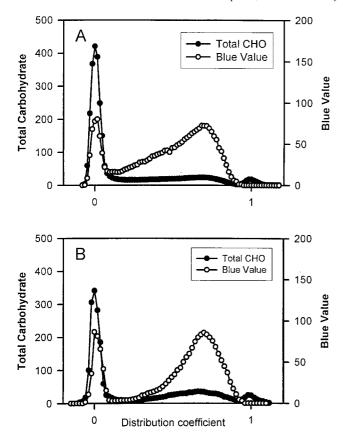


Fig. 3. Sepharose CL-2B column profiles of ginkgo starch and maize starch. Fractions were analyzed for total carbohydrate $(\bullet - \bullet)$ and blue value $(\bigcirc -\bigcirc)$; A = ginkgo, B = maize.

(Fig. 2). Maize and potato starches, used as references, displayed A- and B-type patterns, respectively (Fig. 2) (Zobel, 1964). In general, legume starches and some tropical tuber starches, as well that of banana, give the C-type pattern which represents a mixture of A- and B-type crystallinity within the granule (Sarko & Wu, 1978; Gernat, Radosta, Damaschun, & Schierbaum, 1990; Bogracheva, Morris, Ring & Hedley, 1998). Both A-type (Fuwa et al., 1979; Fujimoto et al., 1981; Saganuma, Fujimoto, Kitahara,

& Nafahama, 1996) and C_A-type (Yamashita et al., 1990) X-ray diffraction patterns have been reported for ginkgo starch. The X-ray diffraction pattern of the ginkgo endosperm starch changed from A-type to C_A-type during maturation of the seed (Yamashita et al., 1990). The C-type diffraction pattern reported here indicates the seeds used in this study which were harvested in late October, were mature.

The iodine titrations of the defatted starches showed that the ginkgo starch contained more apparent amylose (33.2%) than maize starch (21.8%) (Table 1). The reported values for ginkgo apparent amylose content range from 26 to 34% (Yamashita et al., 1990; Fujimoto et al., 1981). To determine the absolute amylose content of the starches, the iodine affinity (IA) of the amylopectin was measured. This was necessary because long branch chains of amylopectin in the starch may form a helical complex with iodine and result in a larger IA and an overestimate of amylose content (Takeda and Hizukuri, 1987; Kasemsuwan et al., 1995). After subtracting the IA of amylopectin, the absolute amylose content in the ginkgo starch was 24.0% and that of the maize starch was 18.4%.

Molecular size distributions of the ginkgo and maize starches determined by gel permeation chromatography showed that the ginkgo starch consisted of large molecular weight amylose as indicated by the high blue value following the amylopectin peak (Fig. 3). The large amylose molecules are not present in maize starch.

HPAEC-ENZ-PAD has been demonstrated to be a sensitive and quantitative analytical method for amylodextrins and has been employed to examine the branch chain length distribution of debranched amylopectins (Wong and Jane, 1997). The normalized branch chain length distribution profiles for ginkgo and maize are shown in Fig. 4. Both amylopectins had a peak dp number of 13 and chains up to dp number 82 and 80 were detected for ginkgo and maize, respectively. The average chain lengths for the two starches were similar: 24.2 for ginkgo and 24.4 for maize (Table 2). The increase from dp 6 to 7 followed by a lower peak at dp 8 for ginkgo differed from the stair-step increase from dp 6 to

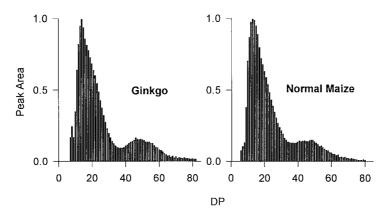


Fig. 4. Normalized peak area chromatograms of isoamylase debranched amylopectins of ginkgo and maize starches produced by using high performance anion exchange chromatography with an on-line enzyme reactor and pulsed amperometric detector.

Table 2 Branch chain length distributions (results are the mean of three replicates)

| | | | % Distribution | | | | | Highest detectable DP |
|-----------------|----------|--------------|----------------|---------------------------------|--------------|-----------------|--------------|-----------------------|
| | | fa | | fb ₁ fb ₂ | | fb ₃ | | |
| | Peak DP | Average CL | DP 6-9 | DP 10-12 | DP 13-24 | DP 25-36 | DP ≥ 37 | |
| Ginkgo Maize | 13 13 | 24.2 24.4 | 5.3 1.9 | 13.3 16.0 | 47.9 47.9 | 13.7 14.9 | 19.8 19.3 | 82 80 |

9 in maize starch. The distribution shape in the range of dp 6-9 for ginkgo was similar to that of lotus and arrowhead which are both C-type starches (Hanshiro, Abe, & Hizukuri, 1996). The profiles also showed that ginkgo contained more short chains (fa, dp 6-12) and long chains (fb₃, dp > 37) than maize (Table 2). The proportion of dp 6-9, in particular, is higher in ginkgo (peak area = 5.3%) than in maize amylopectin (peak area = 1.9%). Maize amylopectin showed a higher proportion of chains, dp 25-36, than ginkgo. The chain length distribution for maize differed slightly from that reported by Hanshiro et al. (1996).

The degrees of hydrolysis and the mode of attack by porcine pancreatic α -amylase on ginkgo starch and maize starch are presented in Fig. 5. The two starches exhibited

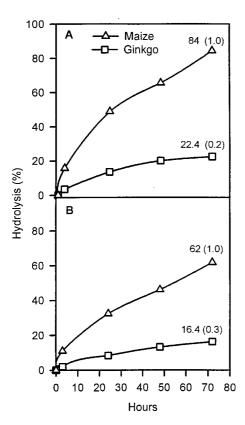


Fig. 5. Enzyme hydrolysis at 37°C by α -amylase (0.2 μ l enzyme/mg starch) (Porcine pancreatic, specific activity 1240 units/mg protein; 25 mg protein/1.1 ml) of ginkgo and maize starches. Amount of hydrolysis determined by: A = total carbohydrate, B = reducing value. Numbers in () represent one standard deviation.

widely different susceptibilities to enzymatic hydrolysis. Maize starch conversion reached 84% in 72 h as measured by supernatant total carbohydrate and 62% as measured by reducing value. The ginkgo starch granules were shown to be highly resistant to the action of α -amylase. After 72 h of enzymatic treatment, 22.4 and 16.4% were hydrolyzed as measured by total carbohydrate and reducing value, respectively. The SEM of the starches showed a slight increase in surface erosion for ginkgo starch over the time course of αamylase attack up to 72 h (Fig. 6a-c). Maize starch was highly pitted and eroded after only 24 h (Fig. 6d-f). Fuwa et al. (1979) demonstrated that, in addition to some root and tuber starches, ginkgo, high amylose maize, banana, and chestnut starches were also significantly more resistant than cereal starches to in vitro digestion by porcine pancreatic α -amylase. The erosion of specific areas giving rise to circular pits in maize starch has been noted by several authors (Leach & Schoch, 1961; Fuwa et al, 1979; Knutson, Khoo, Cluskey & Inglett, 1982; Hoover et al., 1991; Gallant et al., 1992). Knutson et al. (1982) showed the α -amylolysis of dent and waxy maize starches varied directly with the surface area of the granules. High amylose varieties of starches have inhibited enzymatic hydrolysis compared to their normal counterparts (Leach & Schoch, 1961; Knutson et al., 1982; Cone & Wolters, 1990). In addition to amylose content, other factors such as starch crystalline structure and arrangement must also play a role since potato and banana starches are highly resistant starches with normal amylose contents (Fuwa et al., 1979, Sugimoto et al., 1980). A-type spherocrystals have been shown to be 3.5 times more degraded by α -amylase than the B-type (Planchot, Colonna, and Buleon, 1997).

The acid hydrolysis rates for the two starches differed. Ginkgo starch had a higher final percentage hydrolysis than normal maize after an initially lower rate up to 3 days (Fig. 7). The Naegeli dextrins from 19.8, 60.0, and 64.9% hydrolysis of ginkgo starch displayed a peak chain length at dp 13, the same as the debranched amylopectin (Fig. 8 a-c). However, after 12 days hydrolysis (76.6%) the peak shifted to dp 12 (Fig. 8d). A second peak in each chromatogram, corresponding to singly branched molecules, occurred at dp 24 or 25. There were no multiple branched chains evident at dp > 37 as was observed in normal maize starch (Jane et al., 1997). Following isoamylase debranching, the singly branched molecules were hydrolyzed to two linear

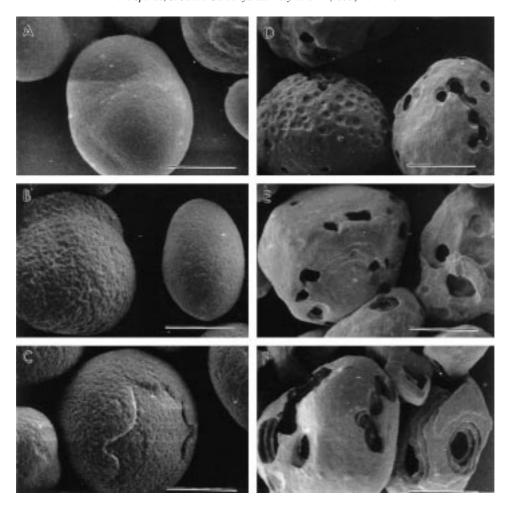


Fig. 6. The action of porcine pancreatic α -amylase on ginkgo and maize starch. Incubation periods and percentage hydrolysis (total carbohydrate value of supernatant) for ginkgo: (a) 24 h, 13.5%; (b) 48 h, 20.0%; (c) 72 h, 22.4%. Maize starch incubation period and percentage hydrolysis: (d) 24 h, 48.9%; (e) 48 h, 65.5%; (f) 72 h, 84.4%.

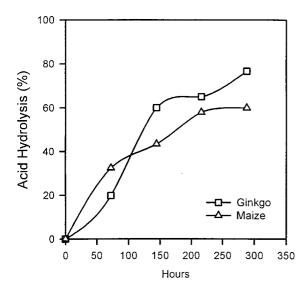


Fig. 7. Acid hydrolysis (15.3% $\rm H_2SO_4,\ v/v)$ of ginkgo and normal maize starch at 38°C.

molecules and the second peak disappeared (Fig. 9). These results are similar to those for maize Naegeli dextrins, however ginkgo starch displayed a lower proportion of singly branched chains in the Naegeli dextrin compared to maize starch (Jane et al., 1997). Jane et al. (1997) have shown that substantial amounts of branch linkages of Atype starches are present within the crystalline region, whereas, branch linkages for B-type starches are clustered within the amorphous region and are susceptible to acid hydrolysis. Jane et al. (1997) also proposed a model to describe the branching patterns of A- and B-type starches. The scattered branch points within an A-type crystalline region were suggested to present an inferior crystal with "weak points" for enzymatic hydrolysis. With the differences in the structural features between ginkgo and maize starches, we may attribute the enzyme resistance of ginkgo starch to its higher amylose content and fewer amylopectin branch linkages located in the crystalline region than maize starch.

Thermal analysis by using the DSC showed gelatinization temperature onsets (T_0) of 60.8 and 65.6°C for ginkgo and

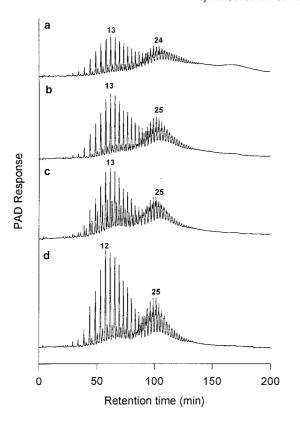


Fig. 8. HPAEC-ENZ-PAD chromatograms of ginkgo Naegeli dextrins with 19.8% (a), 59.6% (b), 64.9% (c), and 76.6% (d) of total acid hydrolysis.

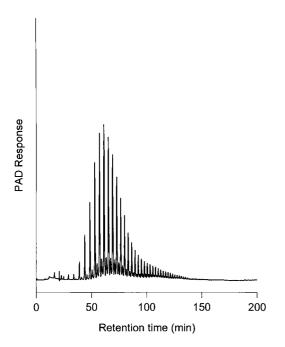


Fig. 9. HPAEC-ENZ-PAD chromatogram following isoamylase debranching of the Naegeli dextrin from 76.6% acid hydrolysis.

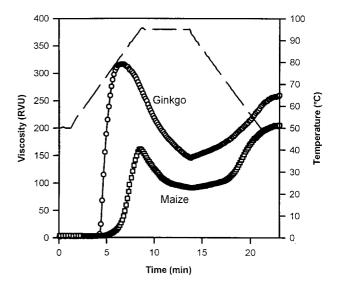


Fig. 10. Rapid viscoamylograph of ginkgo starch $(\bigcirc -\bigcirc)$ and maize starch $(\bigcirc -\bigcirc)$ pastes (8%, w/v). Temperature profile (---).

maize starch, respectively (Table 3). The slow onset of gelatinization for ginkgo starch may be attributed to the higher proportion of short branch chain lengths of the amylopectin and the presence of monophosphate derivatives. The gelatinization temperature range $(T_c - T_o)$ was 17.9°C for ginkgo, giving a broader endotherm than maize (this study), sweet potato (Takeda, Tokunga, Takeda, & Hizukuri, 1986), lima bean or potato (Hoover et al., 1991). The gelatinization enthalpy change (ΔH) of 14.6 J/g was higher for ginkgo than for maize starch. Maize starch demonstrated a slightly higher percentage of retrogradation (R) (Table 3). The lower degree of retrogradation of ginkgo starch might be attributed to the higher content of short branches of amylopectin (Shi & Seib, 1992), the monophosphate derivatives, and the longer amylose molecules.

The RVA pasting properties of ginkgo and maize starch are shown in Fig. 10. At the same solid concentration level (8%; w/w, dsb), ginkgo starch displayed a greater viscosity than maize starch. The difference between DSC gelatinization onset (T_0) and the pasting temperature was greater for maize starch than ginkgo because of inhibited granule swelling by amylose–lipid complexes present in the starch. The amylose–lipid complexes also reduce the peak viscosity of the maize starch. The high peak viscosity of ginkgo starch may be attributed to its phosphate derivatives.

4. Conclusions

Ginkgo starch displays a C-type X-ray diffraction pattern and the amylopectin has a higher proportion of short chains (dp 6–9) compared to maize starch. Ginkgo has a higher absolute amylose content (24.0%) and larger amylose molecules than maize starch (18.4%). The surface of ginkgo starch displays no pores and was extremely resistant to

Table 3
Thermal properties of ginkgo starch and maize starch (results are the mean of three replicates)

| | $T_{\rm o}$ (°C) | $T_{\rm p}$ (°C) | $T_{\rm c}$ (°C) | $T_{\rm c}-T_{\rm o}(^{\circ}{ m C})$ | ΔH (J/g) | <i>R</i> ^b (%) |
|--------------------|------------------|------------------|------------------|---------------------------------------|------------------|---------------------------|
| Ginkgo | 60.8 (0.2) | 67.1 (0.3) | 78.7 (0.3) | 17.9 | 14.6 (0.3) | _ |
| Maize | 65.6 (0.05) | 70.5 (0.1) | 78.5 (0.5) | 12.8 | 13.8 (0.1) | _ |
| Retrograded ginkgo | 36.1 (0.6) | 50.8 (0.4) | 65.7 (0.2) | 29.5 | 7.0 (0.08) | 48.0 (0.5) |
| Retrograded maize | 36.6 (0.9) | 49.0 (1.0) | 70.3 (0.9) | 33.7 | 7.6 (0.2) | 54.8 (0.5) |

^a Retrogradation after 7 days at 4°C.

enzymatic attack. The SEM showed surface erosion for ginkgo starches incubated with porcine pancreatic α -amylase whereas maize starch granules were pitted and highly degraded. Naegeli dextrins of the starches revealed that fewer branch points were present within the crystalline region of ginkgo amylopectin, protected from acid hydrolysis, and unlike maize starch, no multiple branched chains were found in gingko Naegeli dextrins.

Acknowledgements

The authors would like to thank Dr M. Radosaljevic, L. Taylor, and Dr A. McPherson for their assistance with collecting and isolating the ginkgo starch.

References

Bogracheva, T., Morris, V. J., Ring, S. G., & Hedley, C. L. (1998). Biopolymers, 45, 323.

Cone, J. W., & Wolters, M. G. E. (1990). Starch/Starke, 42 (8), 298.

Dubois, M., Giles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956).
Anal. Chem., 28, 350.

Fannon, J. E., Shull, J. M., & BeMiller, J. N. (1993). Cereal Chem., 70, 611.
Fujimoto, S., Nakashima, S., Kubo, Y., Saganuma, T., & Nagahama, T. (1981). J. Jap. Soc. Starch Sci., 28, 180.

Fuwa, H., Sugimoto, Y., & Takaya, T. (1979). J. Jap. Soc. Starch Sci., 26, 105.

Gallant, D. J., Bouchet, B., Perez, S., & Eur, J. (1992). *Clinical Nutr.*, 46, S3–S16.

Gernat, C., Radosta, S., Damaschun, G., & Schierbaum, F. (1990). *Starch/Staerke*, 42 (5), 175.

Hanshiro, I., Abe, J., & Hizukuri, S. (1996). Carbohydrate Res., 283, 151.
Hoover, R., Rorke, S. C., & Martin, A. M. (1991). J. Food Biochem., 15, 111

Jane, J., & Chen, J. (1992). Cereal Chem., 69, 60.

Jane, J., Wong, K. S., & McPherson, A. E. (1997). Carbohydr. Res., 300, 219

Kasemsuwan, T., & Jane, J. (1996). Cereal Chem., 73, 702.

Kasemsuwan, T., Jane, J., Schnable, P., Stinard, P., & Robertson, D. (1995). Cereal Chem., 72, 457.

Knutson, C. A., Khoo, U., Cluskey, J. E., & Inglett, G. E. (1982). Cereal Chem., 59, 512.

Leach, H. W., & Schoch, T. J. (1961). Cereal Chem., 38, 34.

Lim, S.-T., Kasemsuwan, T., & Jane, J. (1994). *Cereal Chem.*, 70, 145. Morrison, W. R. (1988). *J. Cereal Sci.*, 8, 1.

Morrison, W. R., & Coventry, A. M. (1985). Starch/Staerke, 37, 83.

Planchot, V., Colonna, P., & Buleon, A. (1997). Carbohydr. Res., 298, 319.Radosavljevic, M., Jane, J., & Johnson, L. A. (1998). Cereal Chem., 75, 212.

Sarko, A., & Wu, H. C. H. (1978). Starch/Staerke, 30 (3), 73.

Schoch, T. J. (1942). J. Am. Chem. Soc., 64, 2954.

Schoch, T. J. (1964). In R. L. Whistler (Ed.), (pp. 157). Methods in carbohydrate chemistry, 4. New York: Academic Press.

Shi, Y. C., & Seib, P. A. (1992). Carbohydr. Res., 227, 131.

Smith, R. J., & Caruso, J. -L. (1964). In R. L. Whistler (Ed.), (pp. 42).
Methods in carbohydrate chemistry, 4. New York: Academic Press.

Sugimoto, Y., Fujita, S., Takaya, T., & Fuwa, H. (1980). Starch, 32 (9), 290.

Somogy, M. (1945). J. Biol. Chem., 160, 61.

Saganuma, T., Fujimoto, S., Kitahara, K., & Nafahama, T. (1996). Oyo Toshitsu Kagaku, 43, 525.

Takeda, Y., & Hizukuri, S. (1987). Carbohydr. Res., 168, 79.

Takeda, Y., Tokunaga, N., Takeda, C., & Hizukuri, S. (1986). Starch/ Staerke, 38, 345.

Umeki, K., & Kainuma, K. (1981). Carbohydr. Res., 96, 143.

Wang, Y. J., White, P., Pollak, L., & Jane, J. (1993). Cereal Chem., 70, 171.
Williamson, G., Belshaw, N. J., Self, D. J., Noel, T. R., Ring, S. G., Cairns,
P., Morris, V. J., Clark, S. A., & Parker, M. L. (1992). Carbohydrate Polymers, 18, 179.

Wong, K. S., & Jane, J. (1997). J. Liq. Chromatogr., 20, 297.

Yamashita, Y., Sugimoto, Y., & Fuwa, H. (1990). Nippon Kasei Gakkaishi, 41, 723.

Zobel, H. F. (1964). In R. L. Whistler (Ed.), (pp. 109). Methods in carbohydrate chemistry, 4. New York: Academic Press.