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Characterization of Nubet and Franubet barley starches

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Abstract

Franubet, a mutant of Nubet, had grains of smaller weight, less starch, but greater protein content than did Nubet. Nubet starch had simple granules with bimodal granule size distribution (diameters of 9–22 and 1.2–4 μm for the A and B granules, respectively), whereas Franubet starch had smaller (diameters of 1–4 μm) and irregular-shaped granules, and some displayed compound starch granules. There were no significant differences in X-ray diffraction pattern and amylose content, but Nubet starch had a greater phosphorous (0.045%) and bound lipid (0.61%) content than Franubet starch (0.026 and 0.47%, respectively). Franubet amylopectin (AP) had similar proportions (4.0%) of chains with DP 6–9 and larger proportions (21.5%) of chains with DP >37 than did Nubet AP (4.2 and 19.7%, respectively). Franubet starch showed lower onset gelatinization temperature (52.8 °C) and smaller enthalpy change in melting of amylose–lipid complex (0.5 J/g) than did Nubet starch (54.6 °C and 1.0 J/g, respectively). Franubet starch displayed a lower pasting temperature, less final and setback viscosity but greater peak and breakdown viscosity, which could be attributed to its lower lipid and phospholipid content.

Keywords: Barley starches; Hull-less barley; Compound granule; Amylopectin; Lipid

1. Introduction

Barley is one of the major cereal crops. Barley grain is mainly used in the brewing and malting industries and for animal feeds. There has been a great interest focused on the hull-less barley (HB) because of its high β-glucan content that has been reported to reduce serum cholesterol and glucose levels (Bhatty, 1999). Starch is the most abundant component of HB, ranging from 60–75% on a dry weight basis (Bhatty, 1997). Many studies have reported structures and properties of starch isolated from HB (Bhatty & Rossnagel, 1998; Song & Jane, 2000; Zheng, Han, & Bhatty, 1998). Several new HB genotypes with different starch structures and granule morphologies have been developed, and their properties have been reported (Li, Vasanthan, Rossnalgel, & Hoover, 2001a,b).

Starch granules are synthesized in amyloplasts and can be classified as simple, compound, and semi-compound granules, depending on the number of starch granules initiated in each amyloplast (Shannon & Garwood, 1984). A simple granule is formed when one granule is initiated in an amyloplast, whereas compound granules are produced when two or more granules are initiated in one amyloplast. A starch granule consisting of smooth surface but having compound granules in the internal structure is referred to as a semi-compound granule (Shannon & Garwood, 1984). The morphology of starch granules also depends on the initiation and developmental process of the granules. Compound starch granules have polyhedral shapes, resulting from tight packing in a constraint space during the development of starch granules.

Nubet is a hull-less variety of Betzes barley. Franubet is a mutant produced by subjecting Nubet to chemical mutagenesis (DeHaas & Goering, 1983). Franubet starch shows severely altered granule morphology. Nubet starch consists of simple granules and has a typical bimodal granule-size distribution, whereas Franubet starch consists of a mixture of simple, compound, and semi-compound granules (Verhoeven, 2002).

In this study, we investigated and compared the physicochemical properties and lipid contents and fatty acid compositions of Nubet and Franubet starches and determined the compositions of grains of the two cultivars. The aim of the study was to investigate factors related to and

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responsible for the altered granule morphology of Franubet starch.

2. Material and methods

2.1. Materials and starch isolation

Nubet and Franubet grains were grown in John Innes Centre (Norwich, UK) and their starches were isolated by the diluted alkaline-protease method (Song & Jane, 2000).

2.2. Chemical composition and weight of grain

The weight of 100 randomly-picked whole, unfractured grains was determined in triplicate to compare the average grain weight of Nubet and Franubet barley. The starch and β-glucan contents were determined by using total starch and β-glucan assay kits (Megazyme international Ireland Ltd., Co. Wicklow, Ireland), respectively. The protein content was determined by using a RapidN III (Elementar Americas, Inc., Mt Laurel, NJ). A conversion factor of 6.25 was used to calculate the protein content from the nitrogen content. The lipid content was analyzed by using a gravimetric method after extraction with 2-propanol:petroleum ether (2:3) on Goldfish apparatus.

2.3. Scanning electron microscopy

The morphology of starch granules was studied by using a scanning electron microscope (JEOL model 1850, Tokyo, Japan). Scanning electron microscopy was performed following the method reported by McPherson & Jane (1999).

2.4. X-ray diffraction patterns

Starch samples were equilibrated in a chamber of 100% relative humidity for 24 h at 25 °C. X-ray patterns of starch samples were obtained with copper, Kα radiation using a diffractometer (D-500, Siemens, Madison, WI). The diffractometer was operated at 27 mA and 50 KV. The scanning region of the two-theta angle (2θ) was from 4–37° with a 0.05° step size and a count time of 2 s.

2.5. Amylose contents of starch samples determined by iodine affinity

Iodine affinity (IA) of defatted starch and isolated amylopectin (AP) were determined by using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). Starch was defatted with 90% DMSO solution, and AP was separated following a modified Schoch method (Jane & Chen, 1992). Apparent amylose (AM) content was calculated by dividing the IA of starch by 20% (Takeda and Hizukuri, 1987). Absolute AM content was calculated following the method of McPherson & Jane (1999) using the equation of $C = (IA_s\text{-}IA_{AP+IC}) \times 100/(20 - IA_{AP+IC})$, where C, IAs and IA_{AP+IC} represent the percentage of absolute AM content, IA of starch, and IA of AP plus intermediate components, respectively.

2.6. Phosphorus contents of starch

The phosphorus content of starch was determined by using a colorimetric chemical method (Smith & Caruso, 1964).

2.7. Branch chain length distribution of amylopectin

Fractionated AP samples were debranched using isoamylase following the procedure of Jane & Chen (1992). The chain length distribution of AP was analyzed by using high-performance anion exchange chromatography (Dionex DX-300 system, Sunnyvale, CA) equipped with a post-column amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) following the method reported by Wong & Jane (1997). An anion-exchange analytical column (CarboPac PA-100) and guard column (PA-100) were used for separating debranched samples.

2.8. Molecular weight distribution of starch by gel permeation chromatography (GPC) and high-performance size-exclusion chromatography (HPSEC)

The molecular weight (Mw) distributions of AM and AP were determined by using a GPC column (2.6 ID × 70.0 cm) packed with Sepharose CL-2B gel (Pharmacia, Inc., Piscataway, NJ), following the procedure of Song & Jane (2000). Fractions of 4.8 ml each were collected. Total carbohydrate content using phenol sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and AM content using iodine blue value (Juliano, 1971) were measured at 490 and 630 nm, respectively.

The Mw of AP was determined by using high-performance size-exclusion chromatography (HP 1050 series isocratic pump) equipped with multi-angle laser light scattering (MALLS, model Dawn-F, Wyatt Tech., Santa Barbara, CA) and refractive index detectors (HP 1047A, Hewlett Packard, Wilmington, DE). Two sequentially connected analytical columns (Shodex KB-806 and KB-804, Showa Denko K.K., Tokyo, Japan) with a Shodex OH pack KB-G guard column were used for separation. The starch sample preparation and operating conditions were done following the method of Yoo & Jane (2002a).

2.9. Thermal properties

The thermal properties of starch were analyzed by using a differential scanning calorimeter (DSC-7, Perkin–Elmer, Norwalk, CT) equipped with an intracooling II system. Starch (3 mg, dry basis [db]) and water (9 μl) were sealed in an aluminum pan and equilibrated at 25 °C for 2 h.

The samples were scanned from 25 to 120 °C at a heating rate of 10 °C/min using an empty pan as the reference. The determination of retrogradation property was conducted following the same method using the same gelatinized sample after being stored at 4 °C for 7 days.

2.10. Pasting properties

Pasting properties of starch were determined by using a Rapid Visco-Analyzer (RVA-4, Newport Scientific, Sydney, Australia). Starch suspensions (8%, db, w/w) were equilibrated at 50 °C for 1 min, heated to 95 °C at 6 °C/min, held at 95 °C for 5 min, and cooled to 50 °C at a rate of 6 °C/min while stirring the sample at 160 rpm.

2.11. α-Amylase hydrolysis

Enzyme hydrolysis was done following the method of Vasanthan and Bhatty (1996). Starch (100 mg) was suspended in 25 ml of deionized water, and then 20 ml of phosphate buffer (0.1 M, pH 6.9) containing porcine pancreatic α-amylase (6 units/mg starch, Sigma Chemical Co.) was added. The mixture was incubated at 37 °C and 120 rpm in a water-bath shaker. Aliquots (2 ml) were withdrawn at 0, 3, 6, 24, 48, and 72 h and centrifuged at 3000 rpm for 10 min. The supernatant was analyzed for total carbohydrate (Dubois et al., 1956). The degree of hydrolysis was expressed as the percentage of reducing sugar released from starch (db).

2.12. Surface and bound lipid content and Fatty acid composition

The surface lipid content of starch granules was analyzed by the method of Kaukovirta-Norja, Peinikainen, Olkku, & Laakso (1997). The surface lipid was extracted by shaking starch (3 g, db) in a 25 ml chloroform–methanol mixture (CM, 2:1, v/v). The extraction process was repeated three times, and the extracts were combined and dried under N_2 gas. The bound lipid content present inside of the starch granules was analyzed by a combination of enzyme hydrolysis and solvent extraction methods (Robbinson, Weinert, & Solms 1983; Strange & Schaich, 2000) with slight modification. The dried, CM-treated starch (2 g, db) was suspended in 7 ml deionized water and treated with thermo-stable α-amylase (Type XII-A, 300 units/g starch, Sigma Chemical Co.). The suspension was incubated at 80 °C for 30 min in a shaking water bath at 200 rpm. After cooling, the sample was transferred into a separation funnel and extracted with 25 ml CM mixture. The funnel was shaken and left overnight to allow for phase separation. The lower layer of chloroform containing the lipid was collected and dried under a stream of N_2 gas. The lipid content was determined gravimetrically.

The fatty acid composition of the lipid extract was determined by using gas chromatography (HP 6890, Hewlett Packard Co., Valley Forge, PA) on a capillary column (HP-5; 0.25 mm id × 30 m with 0.25 μm film thickness, Hewlett Packard Co.). The fatty acids were detected using a flame ionization detector. The sample preparation and operational procedure was reported by Nam, Du, & Ahn (2001).

3. Results and discussion

The chemical composition and weight of Nubet and Franubet grains are presented in Table 1. Franubet grains were substantially smaller (78% of weight of Nubet grains) than Nubet. Franubet grains had proportionally more protein (16.8%) than did Nubet grains (13.6%), but the protein content per grain was similar between Franubet (0.54 g/100 grains) and Nubet (0.56 g/100 grains). β-glucan and lipid content of the barley grains were similar between Nubet and Franubet on weight basis. The greatest difference was in starch content. Franubet grains had proportionally less starch (54.1%) than did Nubet grains (60.6%) on the dry weight basis. The starch content per grain of Franubet grain (1.7 g/100 grain) was 32% smaller than that of Nubet grain (2.5 g/100 grain).

Scanning electron micrographs of Nubet and Franubet starches are shown in Fig. 1. Significant differences were observed in granule size distribution and granule shape between the two starches. Nubet starch showed a distinct bimodal size distribution and smooth surface as expected for typical simple granules of barley starch. Large (A) granules exhibited a disk-shape with diameters of 9–22 μm, and small (B) granules had a spherical shape with diameters of 1.2–4 μm. Franubet starch did not show the distinct bimodal size distribution. Most of Franubet starch granules

Table 1
Chemical composition and grain weight of Nubet and Franubet barley grains

Flours	Starch (%, db)	Protein[a] (%, db)	Lipid[b] (%, db)	β-glucan (%, db)	Grain weight (g/100 grains)	Starch/grain (g/100 grains)	Protein/grain (g/100 grains)
Nubet	60.6 ± 0.6	13.6 ± 0.2	4.1 ± 0.0	5.7 ± 0.2	4.1 ± 0.1	2.5	0.56
Franubet	54.1 ± 0.2	16.8 ± 0.2	4.8 ± 0.3	5.5 ± 0.2	3.2 ± 0.1	1.7	0.54

Averaged from three replicates ± standard deviation of each sample.
[a] N content × 6.25.
[b] Lipid extracted by 2-propanaol-petroleum ether (2:3).

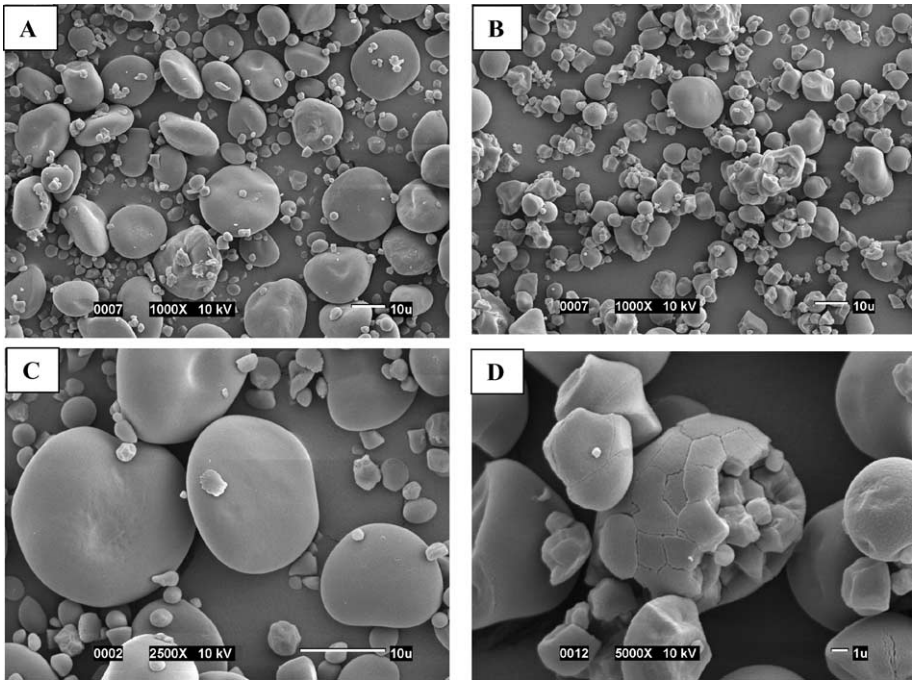

Fig. 1. Scanning electron micrographs of Nubet (A and C) and Franubet starches (B and D) at various magnifications (A, B, × 1000; C, × 2500; D, × 5000).

were irregular or polygonal shaped with diameters of 1–4 μm. Franubet starch also consisted of some large, disk-shaped granules and some irregular-shaped, compound starch granules (diameters 15–19 μm). The result was in agreement with those reported by Chung (1982) and Verhoeven (2002).

X-ray diffraction patterns of Nubet and Franubet starches are shown in Fig. 2. Both Nubet and Franubet starches displayed the A-type X-ray pattern. However a small peak at 6° 2-θ and a greater intensity of the peak at 17° 2-θ observed in the Franubet starch indicated the presence of B-type polymorphism. There was no significant difference in the percentage cystallinity of Nubet (37%) and Franubet (36%) starches.

Amylose (AM) content and IA of the starches are shown in Table 2. Franubet starch had apparent and absolute AM contents (26.7 and 23.1%, respectively) similar to Nubet starch (27.2 and 23.6%, respectively).

Phosphorus present in normal cereal starch is mainly in the form of phospholipids. Phospholipids are known to develop strong helical complexes with amylose. Although at low concentration, phospholipids play an important role in governing starch pasting, gelatinization, and retrogradation properties (Jane, Kasemsuwan & Chen, 1996;

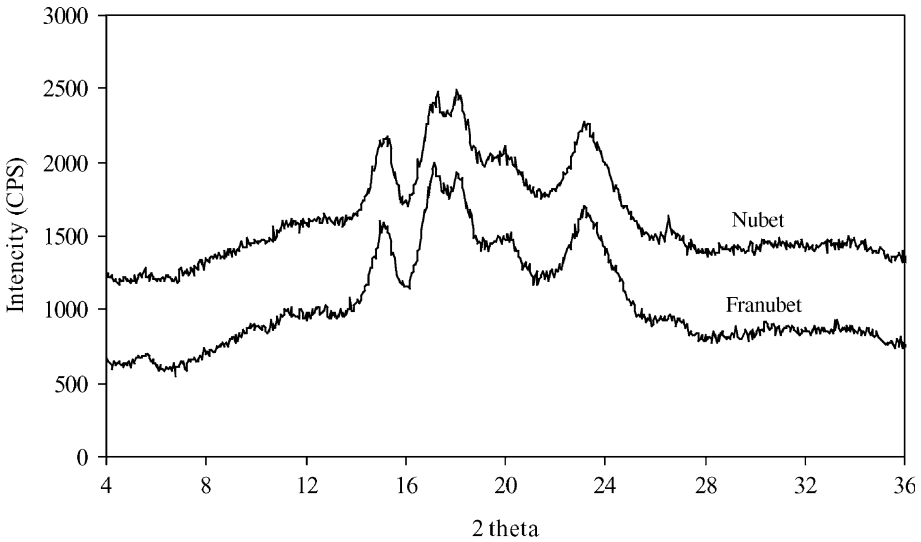

Fig. 2. X-ray diffraction patterns of Nubet and Franubet starches.

Table 2
Amylose and phosphorous contents of Nubet and Franubet starches

Starches	Iodine affinity		Amylose content (%)[a]		Phosphorous content (%)
	IA_S[b]	IA_{AP+IC}[c]	Apparent[d]	Absolute[e]	
Nubet	5.43 ± 0.03	0.94 ± 0.02	27.15 ± 0.14	23.57 ± 0.16	0.045 ± 0.000
Franubet	5.34 ± 0.04	0.97 ± 0.05	26.72 ± 0.18	23.07 ± 0.12	0.026 ± 0.000

Averaged from three replicates ± standard deviation of each sample.
[a] Iodine affinity for pure amylose was 0.2 (Takeda and Hizukuri, 1987).
[b] IA_S is the iodine affinity of the whole defatted starch.
[c] IA_{AP+IC} is the iodine affinity of the amylopectin and the intermediate component mixture.
[d] Calculated as $IA_S \times 100/20$.
[e] Calculated as $(IA_S - IA_{AP+IC}) \times 100/(20 - IA_{AP+IC})$.

Yoo & Jane, 2002b). The phosphorous contents of Nubet and Franubet starches were 0.045 and 0.026%, respectively, indicating significantly larger phospholipid content in Nubet starch (Table 2).

Chain length distributions of debranched AP of Nubet and Franubet starch determined by using HPAEC-ENZ-PAD are shown in Fig. 3, and the results are summarized in Table 3. Nubet and Franubet starches showed the same peak chain lengths for the short and long branch chains at degree of polymerization (DP) 12 and 43, respectively. Franubet starch displayed fewer branch chains of DP 9–16 than did Nubet starch (Fig. 3) and a larger proportion (21.5%) of long branch chains (DP > 37) than did Nubet starch (19.7%). Franubet AP showed a longer average chain length (DP 24.9) than did Nubet AP (DP 24.0). There was no detectable difference in molecular weight distribution between Nubet and Franubet starches measured by GPC (data not shown). The Franubet starch had a larger weight average Mw (2.0×10^9) than did Nubet starch (1.50×10^9). The lack of significant difference in structure between the two starches is in agreement with the study of Verhoeven (2002), which showed that there were no significant difference between Franubet and Nubet in any of the enzymes involved in the synthesis and degradation of starch that were tested.

The gelatinization properties of Nubet and Franubet starches are given in Table 4. Franubet starch showed

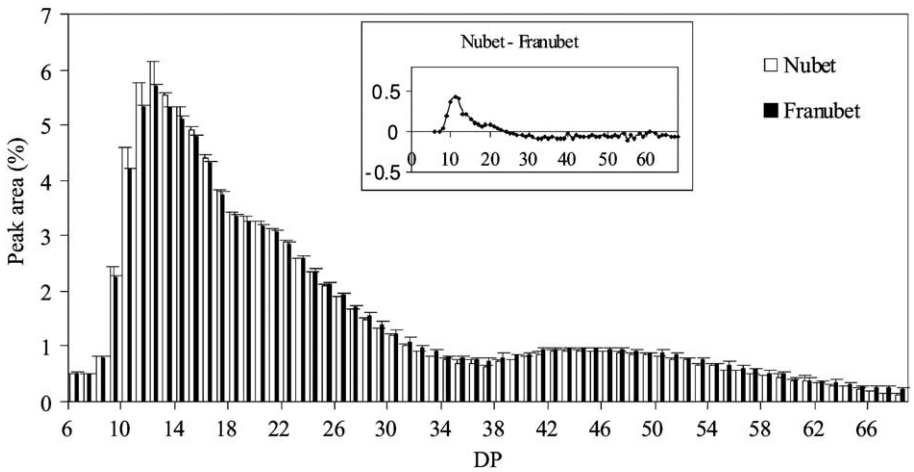

Fig. 3. Peak area distributions of debranched Nubet and Franubet starches analyzed by using HPAEC-ENZ-PAD. DP, Degree of polymerization.

Table 3
Branch chain-length distributions of Nubet and Franubet amylopectins

Starches	Peak DP		Average chain length	Percent distribution					Highest detectable DP
	I	II		DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP ≥ 37	
Nubet	12	43	24.0	4.2 ± 0.0	20.7 ± 0.0	45.0 ± 0.2	14.6 ± 0.2	19.7 ± 0.4	68
Franubet	12	43	24.9	4.0 ± 0.1	19.3 ± 0.2	43.9 ± 0.0	15.3 ± 0.6	21.5 ± 0.4	68

Averaged from three replicates ± standard deviation of each analysis.

Table 4
Thermal properties of Franubet and Nubet starches

Starches	Peak I[a]				Peak II[b]			
	T_o	T_p	T_c	ΔH (J/g)	T_o	T_p	T_c	ΔH (J/g)
Nubet	54.6 ± 0.3	58.1 ± 0.3	62.7 ± 0.5	12.0 ± 0.7	92.5 ± 1.3	97.4 ± 0.5	101.9 ± 0.5	1.0 ± 0.2
Franubet	52.8 ± 0.5	57.1 ± 0.6	63.2 ± 0.6	11.9 ± 0.2	90.8 ± 1.4	96.3 ± 0.4	100.4 ± 1.6	0.5 ± 0.2

T_o, T_p and T_c, onset, peak, and conclusion temperatures (°C) of endotherm; ΔH, enthalpy change of gelatinization and melting of amylose–lipid complex. Averaged from three replicates ± standard deviation of each analysis.
[a] Gelatinization.
[b] Melting of amylose–lipid complex.

a lower onset (T_o, 52.8 °C) but slightly higher complete gelatinization temperature (T_c, 63.2 °C) compared with Nubet starch (54.6 and 62.7 °C, respectively). The range of gelatinization temperature of Franubet (10.4 °C) was larger than that of Nubet (8.1 °C). Many studies have shown that lower T_o and T_c are related to larger proportions of short branch chains and smaller proportions of long branch chains (Jane et al., 1999; Yuan, Thompson, & Boyer, 1993). The lower T_o and broader range of gelatinization temperature of Franubet starch might be attributed to its smaller size and heterogeneously packed, fractured granules. Small granules have larger ratios of surface area to unit weight of starch, and thus hydrate and swell more efficiently than large granules (Li et al., 2001a,b; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001; Vasanthan & Bhatty, 1996). In addition, the presence of B-polymorphs in Franubet starch also contributed to its lower T_o value. Whittan, Noel, & Ring (1990) reported starch of B polymorphs had a lower *T*o than that of the A polymorph when the chain length was the same.

The enthalpy change (ΔH) of gelatinization of Nubet and Franubet starches did not show a significant difference, which was consistent with their similar AP contents and percentage crystallinity. The ΔH of melting of amylose–lipid complex in Franubet starch was less than that of Nubet starch, which was consistent with the lower phosphorous (phospholipid) content of Franubet starch (0.026%) compared with Nubet starch (0.045%).

Thermal properties of retrograded starches are summarized in Table 5. Retrograded Franubet and Nubet starches showed a similar thermal transition temperature, whereas ΔH for the melting of retrograded Franubet starches (3.7 J/g) was greater than that of Nubet (3.4 J/g). Higher percentage

Table 5
Properties of retrograded Nubet and Franubet starches

Starches	T_o	T_p	T_c	ΔH (J/g)	Retrogradation (%)[a]
Nubet	35.4 ± 1.0	47.6 ± 0.1	56.6 ± 0.7	3.4 ± 0.1	28.7 ± 1.2
Franubet	35.2 ± 1.5	47.5 ± 0.0	56.8 ± 0.2	3.7 ± 0.2	31.0 ± 1.8

T_o, T_p and T_c, onset, peak and conclusion temperatures (°C) of endotherm; ΔH, enthalpy of dissociation of retrograded starch. Averaged from three replicates ± standard deviation of each analysis.
[a] % Retrogradation = (enthalpy of retrograded starch/enthalpy of native starch) × 100.

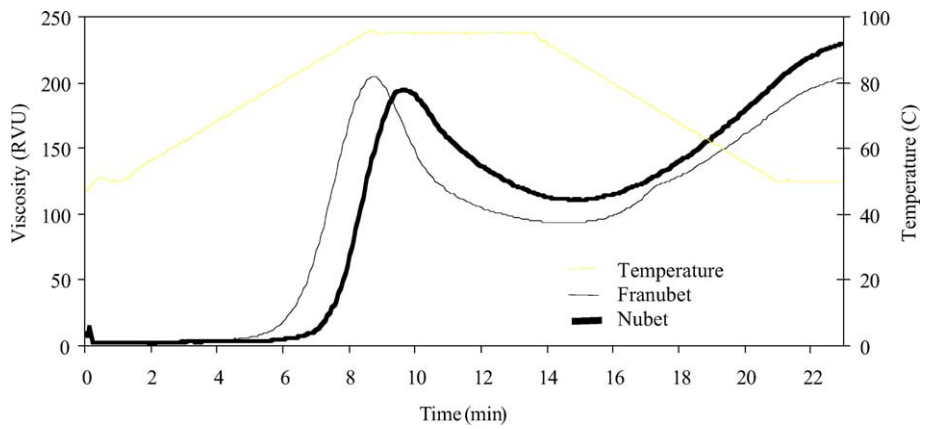

Fig. 4. Pasting profiles of Nubet and Franubet starches (8% db, w/w) measured by Rapid Visco Analyser.

Table 6
Pasting properties of Nubet and Franubet starches

Starches	Viscosity (RVU)[a]				Peak time (min)	Pasting temperature (°C)
	Peak 1	Breakdown	Final	Setback		
Nubet	194.4 ± 3.4	83.8 ± 0.5	229.6 ± 5.0	119.0 ± 2.1	9.7 ± 0.0	85.5 ± 1.7
Franubet	204.6 ± 1.8	111.1 ± 1.4	203.5 ± 0.9	110.0 ± 1.3	8.8 ± 0.0	78.9 ± 0.8

8% (w/w, db) starch suspension. Averaged from duplicates ± standard deviation of each analysis.
[a] Measured in Rapid Visco-Analyser unit.

retrogradation of Franubet starch might come from its smaller lipid content and larger proportion of AP long branch-chains of DP > 25 (Yuan et al., 1993).

Pasting profiles of Nubet and Franubet starches are shown in Fig. 4 and are summarized in Table 6. Franubet starch displayed a lower pasting temperature (78.6 °C), less final viscosity (203.5 RVU) and setback viscosity (110 RVU), but larger peak viscosity (204.6 RVU) and breakdown viscosity (111 RVU) than did Nubet starch (85.5 °C, 229.6, 119, 194.4, and 83.8 RVU, respectively). Differences in the pasting properties between Nubet and Franubet starches could be attributed to their different phospholipids content, and the small, fractured structure of Franubet starch granules. The larger amylose–lipid complex content of Nubet starch restricted the swelling of the starch granules and amylose leaching and, thus, resulted in a higher pasting temperature, less peak viscosity, and less shear-thinning compared with Franubet starch (Jane et al 1999; Tester & Morrison, 1990). Furthermore, the larger proportion of long branch-chain, the higher Mw of Franubet AP, and the less lipid and phospholipid contents also contributed to the greater peak viscosity of Franubet starch (Shibanuma, Takeda, & Hizukuri, 1996; Takeda, Takeda, Suzuki, & Hizukuri, 1989). The fractured granular structure and lower lipid content of Franubet starch could have been responsible for the smaller final viscosity and setback viscosity.

The study of starch hydrolysis by amylase is a means to reveal starch granule structure. The susceptibility of starch granules to amylolysis depends on granule size, crystallinity, polymorphisms, amylose content, and AP branch chain length (Gérard, Colonna, Buléon, & Planchot, 2001; Jane et al., 2003). Franubet starch showed a greater hydrolysis rate during the entire incubation period of 72 h than did Nubet starch (Fig. 5). The faster hydrolysis rate of Franubet starch can be attributed to its smaller granule size. In addition, the lower lipid content of Franubet starch might also have contributed to its faster hydrolysis rate. Seneviratine & Biliaderis (1991) reported that amylose complexed with lipid showed less susceptibility to α-amylase hydrolysis than amylose alone. Our result contradicted that of DeHaas and Goering (1983), who reported that Franubet starch was less susceptible to α-amylase than Nubet starch. This discrepancy might be due to the different methods of analysis. The previous work of Dehaas and Goering was done by using an amylograph to determine the hydrolysis rate.

Since an amylose-lipid complex develops instantly and gives crystalline structure, it could serve as a nucleus for starch granule development. Several studies have proposed that lipids are involved in starch synthesis (Fekete & Vieweg, 1978; Morrison & Gadan, 1987). We analyzed the lipid content and fatty acid composition of Nubet and Franubet starch to investigate whether changes in lipids or fatty acid composition were associated with the different granule morphologies. As shown in Table 7, Nubet starch had a lower surface lipid content (0.017%) but higher bound lipid content (0.609%) than did Franubet starch (0.019 and 0.466%, respectively). The higher surface lipid content of Franubet starch could be attributed to its smaller granules

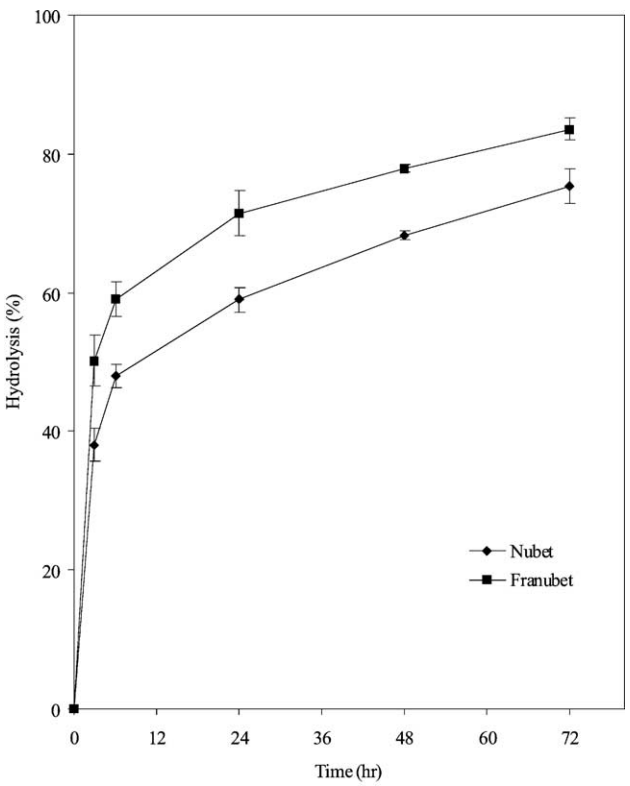

Fig. 5. Enzyme hydrolysis rates of Nubet and Franubet starches by α-amylase.

Table 7
Lipid content (%) and fatty acid composition (as% total fatty acid) of Nubet and Franubet starches

Starch	Lipid type	Lipid content (%)	Fatty acid composition (%)				
			C16:0	C18:0	C18:1	C18:2	C18:3
Nubet	Surface	0.017 ± 0.001	58.5 ± 0.3	30.2 ± 1.6	–	11.4 ± 1.9	–
	Bound	0.609 ± 0.017	44.4 ± 6.1	2.7 ± 1.8	3.3 ± 0.8	46.6 ± 4.7	3.0 ± 0.5
Franubet	Surface	0.019 ± 0.001	64.0 ± 5.0	28.7 ± 4.1	–	7.3 ± 0.8	–
	Bound	0.466 ± 0.018	58.5 ± 5.3	3.0 ± 0.1	5.1 ± 0.2	31.5 ± 4.9	2.0 ± 0.4

Averaged from three replicates ± standard deviation of each analysis.

that had a larger relative surface area to the weight of starch. Nubet and Franubet starches showed similar fatty acid composition in their surface lipid but were different in their bound lipid (Table 7). The major fatty acid in Nubet starch was linoleic acid (C18:2), whereas that in Franubet starch was palmitic acid (C16:0). Nubet starch had a larger proportion of polyunsaturated fatty acid (53%) but a smaller proportion of saturated fatty acid (47%) than did Franubet starch (39 and 61%, respectively). Although saturated fatty acids are better complexing agents, the total content of the saturated fatty acids present in Franubet and Nubet starch on the basis of the dry weight of starch are about the same (28.7%). Thus, lipids and fatty acids composition are not likely to play the major role in the compound granule development in Franubet starch.

Several starch mutants have been reported to produce starch with compound granule-like structures, which are not observed in the wild type. Examples are Notch-2 and Risø 17 mutants in barley (Burton et al., 2002), *sugary 1* (Boyer, Daniels, & Shannon, 1977), *sugary 2* (Perera, Lu, Sell, & Jane, 2001) and *brittle-1* (Shannon, Pien, Cad, & Liu, 1998) mutants in maize, starch synthase III antisensed potato starch (Edwards et al., 1999; Marshall et al., 1996), and *rr* pea starch (Verhoeven, 2002) etc. These studies show that many genetic alterations can result in compound starch granules.

Franubet grain was substantially smaller in size and had a lower starch content than did Nubet grain. Other mutants displaying the altered starch granule structure, such as Risø 17 and Norch-2, *sugary 2* maize, and *rr* pea (Verhoeven, 2002) etc., also showed a reduced starch content. In the case of *sugary 2* maize starch and *rr* pea starch, large fissures occur in these granules, which give them a compound-like appearance. Franubet starch also showed large fissures inside the starch granules when viewed with confocal microscope (unpublished data). It is plausible that insufficient starch synthesized during the granule development results in fissures and starch molecules crystallize to separate particles in the amyloplast to develop compound granule-like structures. Further study is needed to reveal the mechanism of compound starch development.

4. Conclusion

Considering the severely altered starch granule morphology of Franubet starch, the lack of significant differences in amylose content, X-ray diffraction pattern, chain-length distribution and Mw of amylopectin was surprising. A significant difference was observed in phospholipids and starch content between Franubet and Nubet starches. The greater bound-lipid content of Nubet starch resulted in a higher pasting temperature and less peak and breakdown viscosity. Further studies are needed to determine whether the difference in phospholipid content, insufficient starch synthesis, or some other factor is responsible for the altered starch granule morphology.

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