Structure of Tapioca Pearls Compared to Starch Noodles from Mung Beans[1]

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ABSTRACT

Commercial tapioca pearls contain approximately 60% gelatinized starch, as determined by differential scanning calorimetry and glucoamylase digestibility. Exhaustive digestions showed that 2, 5, and 6% of cooked tapioca pearls were resistant to α-amylase, acid (1*M* HCl at 35°C), and to a combination of isoamylase and β-amylase, respectively, whereas digestion of cooked starch noodles from mung bean gave 12, 16, and 26%, respectively, of resistant residues. All the resistant residues gave the B-polymorphic X-ray pattern typical of retrograded starch. At 75% moisture, the α-amylase-resistant residue did not melt below 147°C, whereas the acid-resistant residue melted at 128°C (T_p), and the isoamylase and β-amylase-resistant residue melted at 92°C (T_p). Size-exclusion chromatography showed that the α-amylase-resistant residues contained unit chains with a peak at a number-average degree of polymerization (DP_n) of 33–37, and the acid-resistant residues contained chains with a peak at DP_n 25–26. The isoamylase and β-amylase-resistant fractions were composed mainly of long linear chains, supporting the hypothesis that, in cooked mung bean starch noodles and tapioca pearls, micelles of retrograded amylose formed a structural network that resisted disintegration during cooking.

Tapioca starch is renowned for its bland flavor and clear, thick pastes. However, the paste has a stringy texture. This stringiness can be overcome by chemically cross-linking the starch or by converting the starch to pearls.

Starch pearls are partially cooked small spheres (1–6 mm diameter) (Grace 1977) formed from agglomerated starch granules. Tapioca pearls, when cooked in puddings and fillings, thicken by absorbing water and form clear, shiny gel particles with an elastic mouthfeel. To date, only tapioca and sago starches are reported to give starch pearls (Ruddle et al 1978). Hirao et al (1989) studied the cooking conditions and cooking quality of tapioca pearls.

Tapioca pearls are produced mainly in Thailand, Indonesia, and Malaysia and are shipped to the European Economic Community, United States, and Japan (Jones 1983). The manufacturing of tapioca pearls is labor-intensive (Grace 1977, Ruddle et al 1978). The spherical agglomeration of tapioca starch appears to follow the mechanisms of coalescing, layering, and crushing-layering demonstrated for other materials (Levine et al 1987).

An attempt was made, in our laboratory, to make pearls from wheat starch (Xu 1992). The quality of the resulting pearls was markedly inferior to that of commercial and laboratory-made tapioca pearls after cooking. The cooked wheat starch pearls had a tender texture and an opaque appearance, whereas cooked tapioca pearls were strongly elastic and appeared clear with a glossy surface. Pearls made from cross-linked, hydroxypropylated or acetylated wheat starch, either alone or with 50% unmodified starch, had improved clarity, but they were too weak to withstand cooking. Results from these first attempts to prepare wheat starch pearls indicated that an understanding of the structure of tapioca pearls was needed before undertaking additional efforts to make pearls from other starches.

Starch noodles, like tapioca pearls, are composed almost entirely of starch. They are of interest in this investigation because their structure has been probed by several groups (Lii and Chang 1981, Takahashi et al 1987, Mestres et al 1988, Singh et al 1989).

Starch noodles are made from legume starches that have apparent amylose levels of 35–45%. The structure of mung bean starch noodles has been described (Mestres et al 1988) as a ramified three-dimensional network held together by short segments of strongly retrograded amylose. Those strongly retrograded zones melt at temperatures above the boiling point of water. In this study, we compared the structures of commercial samples of tapioca pearls and mung bean starch noodles. The methods used to measure retrograded starch often give data with high variance, so we included the starch noodles in this investigation to allow a comparison of literature data with ours.

MATERIALS AND METHODS

Materials

Tapioca pearls (3 mm diameter, Reese Finer Foods, Inc., Dayton, OH) and mung bean starch noodles (~0.5 mm diameter) imported from China were purchased in local food stores. Tapioca starch was obtained from A. E. Staley Manufacturing Co. (Decatur, IL). Decorticated mung beans, products of Thailand, were obtained from a local food store. Mung bean starch was isolated from the decorticated beans according to Schoch and Maywald (1968), except for washing the starch with 0.1% NaOH to remove protein and other contaminants (Lii and Chang 1981).

Crystalline α-amylase (Type II-A, 2,080 units per milligram of solids) from a *Bacillus* species and crystalline β-amylase (Type

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1-B, 980 units per milligram of protein) from sweet potato were obtained from Sigma Chemical Co. (St. Louis, MO). One unit of α-amylase (or β-amylase) liberated 1.0 mg of maltose from soluble starch in 3 min at pH 6.9 (or pH 4.8) at 20°C. Crystalline isoamylase (5,900 units per milligram of protein) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The specific activity of isoamylase was about 5,900 units per milligram of protein as defined by Yokobayashi et al (1970).

Dextran T-500 and Sephadex G-100 were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden).

General Methods

Moisture was measured by weight loss upon heating in an oven at 130°C for 1 hr; lipid was measured by extraction with ethyl ether, and protein by Kjeldahl N × 5.7 (AACC 1983). Total carbohydrate was determined by the phenol-sulfuric acid method (Dubois et al 1956), and reducing power was determined by the modified Park-Johnson method (Park and Johnson 1949, Hizukuri et al 1981). Glucose and maltose were used as reference standards in determining total carbohydrate and reducing power, respectively. All data except moisture are reported on a dry weight basis. Samples of mung bean starch noodles and tapioca pearls were ground in a coffee grinder into particles of less than 500 μm before analysis.

Degree of gelatinization was estimated by either the reduction in gelatinization enthalpy in the differential scanning calorimeter (Holm et al 1985) or by increased susceptibility to glucoamylase (Chiang and Johnson 1977). The level of glucoamylase was reduced by 95% in this work. Duplicate determinations were made; the data given are mean values.

Differential scanning calorimetry (DSC) was performed as described by Yost and Hoseney (1986). Samples (~2.5 mg) were weighed into aluminum pans using a microbalance and water (~7.5 mg) was added. The pans were sealed and allowed to stand for 1 hr, then heated at 10°C/min over the temperature range of 7–147°C. Indium served as the reference standard. The onset temperature (T_o) and final temperature (T_f) of a peak were determined by the intercept of the baseline and the extrapolated leading and trailing edges of the peak. Enthalpies of transitions, computed using a thermal analysis data station (Perkin-Elmer Inc., Hayward, CA), were the means of at least three tests. The standard error was less than 5%.

The Brabender Viskograph-E (Brabender OHG, Duisburg, Germany) was used to compare the pasting properties. Samples (25 g, db) of ground tapioca pearls or tapioca starch were mixed with 375 ml of water. A sample was heated in the amylograph from 30 to 95°C at 1.5°C/min, held at 95°C for 30 min, and then cooled to 30°C at 1.5°C/min.

X-ray diffraction patterns were recorded on a Philips APD-3520 X-ray diffractometer (Philips Electronic Instruments, Inc., Mahwah, NJ). The X-rays were the K_α radiation achieved by bombardment of the copper target at 35 kV and 20 mA. Diffractograms were registered at $2\theta = 2$–$40°$ at a scan rate of 2°/min.

Size-exclusion chromatography was done on a Sephadex G-100 column (25 × 600 mm). The excluded and total volumes of the column were calibrated using Dextran T-500 and D-glucose, respectively. Recovery of the carbohydrate applied to the column was over 95%. Duplicate chromatograms were essentially the same.

Microscopy

Starch pearls (0.2 g) were soaked in water (10 ml) overnight, and the mixture was stirred with a spatula to disperse the solids. The dispersion was examined under a Zeiss microscope with normal and polarized light, and photographs were taken with an SX-70 AutoFocus Land camera and an SX-70 microscope adapter (Polaroid Corp., Cambridge, MA). A 2% suspension of tapioca starch was also photographed.

Intact starch pearls and split pearls were examined by scanning electron microscopy (SEM) (ETEC Autoscan, Perkin-Elmer). After the pearls were split in half with a sharp blade, the halves were mounted on specimen holders with colloidal graphite (Ted Pella Inc., Tustin, CA) and then sputter-coated with gold (Conductavac I, Seevac Inc., Deltona, FL).

The samples were also partially digested before SEM. Tapioca pearls or mung bean starch noodles (1.6 g) were soaked overnight in water (25 ml). After the mixture was boiled for 1 hr and then cooled, it was added to 0.2M phosphate (25 ml, pH 6) buffer. Bacterial α-amylase (900 units) was added, and the digestion proceeded for 5 hr at 25°C, with occasional swirling. In other experiments, suspensions of cooked and cooled pearls or noodles (1.6 g in 25 ml) were buffered with 0.02M acetate (25 ml, pH 3.8) buffer to which 900 units of isoamylase was added, and the mixture was digested for 24 hr at 25°C, with occasional swirling. A control sample of pearls or noodles was cooked but not treated with enzymes. After digestion, the residues were rinsed four times with distilled water and solvent-exchanged successively with 25, 50, 75, 90, and 100% ethanol by soaking for 2 hr at 25°C. The residues were then subjected to critical-point drying, mounted on specimen stubs with colloidal graphite or double-sided sticky tape, sputter-coated with gold, and examined as described above. To examine the internal structure of the resistant residues, the cores of the tapioca pearls were cut with a sharp blade before mounting and drying on the specimen stubs; the starch noodle residues were fractured after cooling on dry ice.

Acid- and Enzyme-Catalyzed Hydrolysis of Cooked Tapioca Pearls and Mung Bean Starch Noodles

Tapioca pearls (18 g) and mung bean starch noodles (~1 cm long, 18 g) were soaked separately overnight in 0.8% aqueous sodium azide (250 ml) at 25°C. Both mixtures were heated in a boiling water bath for 1 hr, cooled to 25°C, and then sheared in a Waring Blendor (20 sec for tapioca pearls and 5 min for mung bean starch noodles). The dispersions were transferred quantitatively and made to volume (500 ml) with water. After an equal volume of 2M aqueous HCl was added, each sample was maintained at 35°C in a water bath and gently shaken once a day for up to 20 days. An aliquot (0.1 ml) of a digest was taken at various times and made to 10 ml volume with water. After centrifuging, triplicate aliquots (1 ml each) of the supernatant were assayed for total carbohydrate.

In a similar manner, the dispersions of cooked pearls and noodles were made to volume (500 ml) and mixed with 0.2M phosphate buffer (500 ml, pH 6) containing α-amylase (9,000 units). Each mixture was maintained at 25°C for up to 20 days. In another digestion, a mixture of isoamylase (9,000 units) and β-amylase (4,500 units) was used to digest the dispersions in 0.01M acetate buffer (pH 3.8) at 25°C for 60 hr.

At the end of either the acid or enzyme digestion, the mixture was centrifuged, the supernatant discarded, and the residue rinsed with water. The washing and centrifugation steps were repeated four times, and the residue was dried over phosphorous pentoxide under vacuum. The dried residues were analyzed by DSC, X-ray diffractometry, and gel-permeation chromatography.

Gel-Permeation Chromatography

The residues (10 mg) isolated after acid and enzyme digestion of cooked pearls and starch noodles were dissolved in 0.5 ml of 2M aqueous potassium hydroxide at 25°C. Each solution was diluted with water (9.5 ml), centrifuged, and filtered through a 0.45-μm syringe filter (Gelman Sciences, Ann Arbor, MI). An aliquot (5 ml) of the mixture was immediately injected onto a Sephadex G-100 column (25 × 600 mm). Components were eluted with 0.1M aqueous potassium hydroxide at a flow rate of 0.5 ml/min in the ascending direction. Fractions (5 ml per tube) were collected and analyzed for total carbohydrate and reducing power, from which degrees of polymerization (DP) were calculated.

A resistant residue (10 mg) was dissolved in 2M aqueous potassium hydroxide (0.5 ml), and the solution was neutralized with 2M hydrochloric acid. Dimethyl sulfoxide (2 ml), water (2 ml), and 10 mM acetate buffer (5 ml, pH 3.8) were added, followed immediately by isoamylase (328 units). The mixture was incubated at 45°C for 24 hr. The reaction was stopped by heating in a boiling water bath for 10 min. After centrifugation and filtration

through a 0.45-μm syringe filter, an aliquot of the clear solution (5 ml) was injected onto a Sephadex G-100 column. The column was developed using 0.1M aqueous potassium hydroxide.

RESULTS AND DISCUSSION

Protein, ash, and lipid in a commercial sample of tapioca pearls were much lower than in tapioca flour (Table I). Thus, the pearls examined in this work were composed of refined tapioca starch.

Thermograms of Tapioca Pearls

The DSC thermogram of ground tapioca pearls measured at 75% moisture level between 20–120°C showed a single endotherm at 63–69–76°C (T_o, T_p, T_f) with ΔH 6.8 J/g. Tapioca starch showed an endotherm with the same temperature range and ΔH 15.7 J/g, indicating that the pearls contained 57% gelatinized starch. An endotherm at ~50°C, characteristic of crystalline retrograded amylopectin (Zeleznak and Hoseney 1986, Ring et al 1987, Slade and Levine 1987), was not observed in the thermogram of tapioca pearls.

The thermogram of ground mung bean starch noodles showed no crystals that melt at 67–72–78°C, which are endogenous to mung bean starch (ΔH 13.5 J/g), but it did show a broad peak for retrograded amylopectin crystals at 40–56–68°C with ΔH 6.4 J/g. These data on mung bean starch noodles agree with previous findings of Mestres et al (1988), who reported the noodles are fully gelatinized, but that they contained retrograded amylopectin crystals with T_p 50°C and ΔH 6.1 J/g.

The lack of retrograded amylopectin crystals in tapioca pearls may be a consequence of the low levels of water used to produce the pearls. The pearls' structure is "set" by heating the spherical particles containing 40–50% moisture. When the product is heated to set the structure, drying occurs, the amylopectin molecules probably lose their mobility, and recrystallization is inhibited. On the other hand, starch noodles are produced from a thin stream of a starch slurry (65% moisture) that is added to a large excess of boiling water. Subsequent cooling and drying of the noodle, which contains fully gelatinized starch, are conducive to amylopectin recrystallization.

The extent of gelatinization in tapioca pearls was found to be 60% using glucoamylase digestibility (Chiang and Johnson 1977). This figure agreed well with the DSC data on tapioca pearls but not with that on mung bean noodles. The Chiang and Johnson method was modified in this work by using 5% of the level of enzyme originally used. In order to apply the Chiang and Johnson method to starch noodles, a second modification also was made. In the step to determine total starch in the noodles, they were dissolved in 2M potassium hydroxide instead of 1M sodium hydroxide. With those two modifications, the extent of gelatinization in starch noodles was only 12%. That low percentage contrasted markedly with the DSC data that indicated complete gelatinization of the mung bean starch noodles and formation of some retrograded starch. Apparently, the junction zones of the retrograded amylose and amylopectin formed barriers to glucoamylase digestion of the starch noodles.

Pasting Properties of Tapioca Pearls

The amylogram of ground tapioca pearls (Fig. 1) showed a somewhat higher consistency than tapioca starch did during the initial stages of heating and during setback. No distinctive pasting peak was observed for tapioca pearls. The reduced pasting peak may have resulted from increased association between starch molecules that formed during the hydrothermal treatment to manufacture the pearls. The increased physical association would be similar to the effects introduced by chemically cross-linking starch. The initial low-temperature consistency may be attributed to the pregelatinized starch in the pearls, and the increased setback may be attributed to the shear stability of granules with increased molecular association.

Microscopic Structure of Starch Pearls

The tapioca pearls stirred in water overnight at 25°C disintegrated to form a dispersion of particulate matter. Under light microscopy, the dispersion of particles displayed a mixture of intact starch granules and swollen granules (Fig. 2c and d). Some of the swollen granules appeared as ghosts, indicating complete gelatinization (Fig. 2c). Under polarized light (Fig. 2d), some starch granules in the pearls showed no birefringence, whereas other granules showed birefringence patterns with enlarged dark zones extending radially from the hilum. Tapioca starch gave the normal maltese cross under polarized light (Fig. 2b). Because

TABLE I
Proximate Analyses (%) of Tapioca Products[a]

Sample	Protein	Ash	Lipids
Tapioca flour[b]	3.70	1.80	1.4
Tapioca starch	0.39	0.11	0
Tapioca pearls	0.52	0.16	0

[a]Values calculated on a dry-weight basis.
[b]Data from Grace (1977).

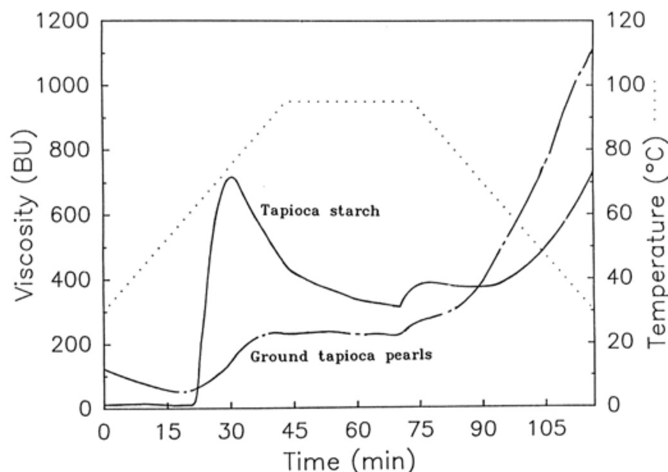

Fig. 1. Amylograms of tapioca starch and tapioca pearls at a sample concentration of 6.25%. The dotted line shows the temperature profile.

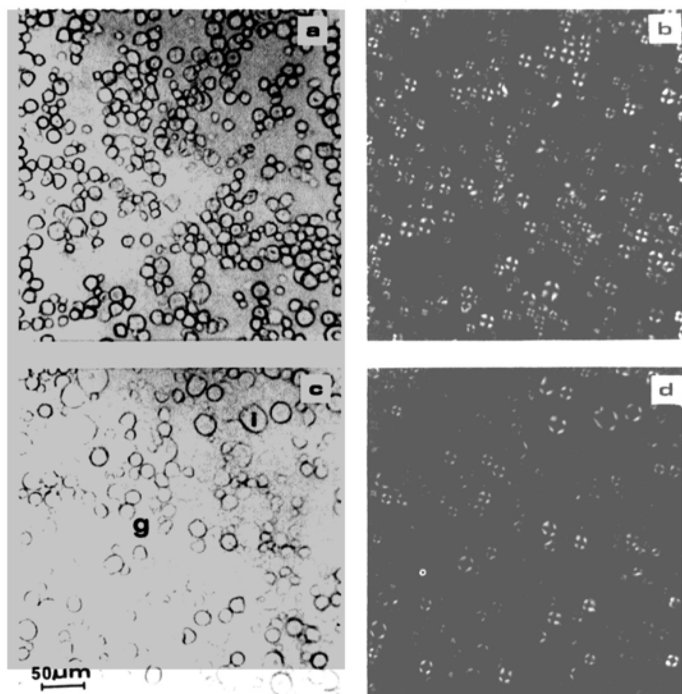

Fig. 2. Light micrographs (200×) of native tapioca starch (**a** and **b**) and of tapioca pearls (**c** and **d**) dispersed in water and illuminated with normal light (**a** and **c**) and polarized light (**b** and **d**), g = ghost and i = intact granule.

gelatinization in excess water begins at the hilum and occurs over a 1–2°C temperature range for individual granules (French 1984), the incomplete gelatinization of individual granules is consistent with the heating of the tapioca starch in a limited amount of water (less than 65% moisture content) during preparation of the pearls.

Mestres et al (1988) reported that mung bean starch noodles contained no birefringent granules but instead contained birefringent filaments that were attributed to retrograded starch. We did not observe birefringent filaments in dispersed pearls, which indicated less highly retrograded starch in tapioca pearls than in the noodles. Our data on glucoamylase digestibility and DSC properties agreed with that conclusion.

SEM showed intact as well as fused granules on the surface of tapioca pearls (Fig. 3b). When a pearl was split, the inside surface (Fig. 3c) revealed concentric shells of differing densities. The spherical shells near the surface of a pearl were more dense (Fig. 3c and d), perhaps for two reasons. During formation of the pearl, the moist starch compacted more tightly as the mass and diameter of the particles increased during rolling and forming. Subsequent heating of the raw, wet pearl resulted in gelatinization of starch in the outer shells, which may have inhibited the escape of air or water vapor from the interior. As the inner zones increased in temperature and the remaining moist starch gelatinized and swelled, voids were created in the hot starch paste toward the center of the pearl.

Generally, the starch granules inside the pearls appeared to have undergone more swelling and solubilization than those on the outer surface (contrast Fig. 3b and e). Figure 3f is a high magnification of the surface of an inner void showing granule remnants embedded in a continuous network of starch molecules, most of which are probably amylose.

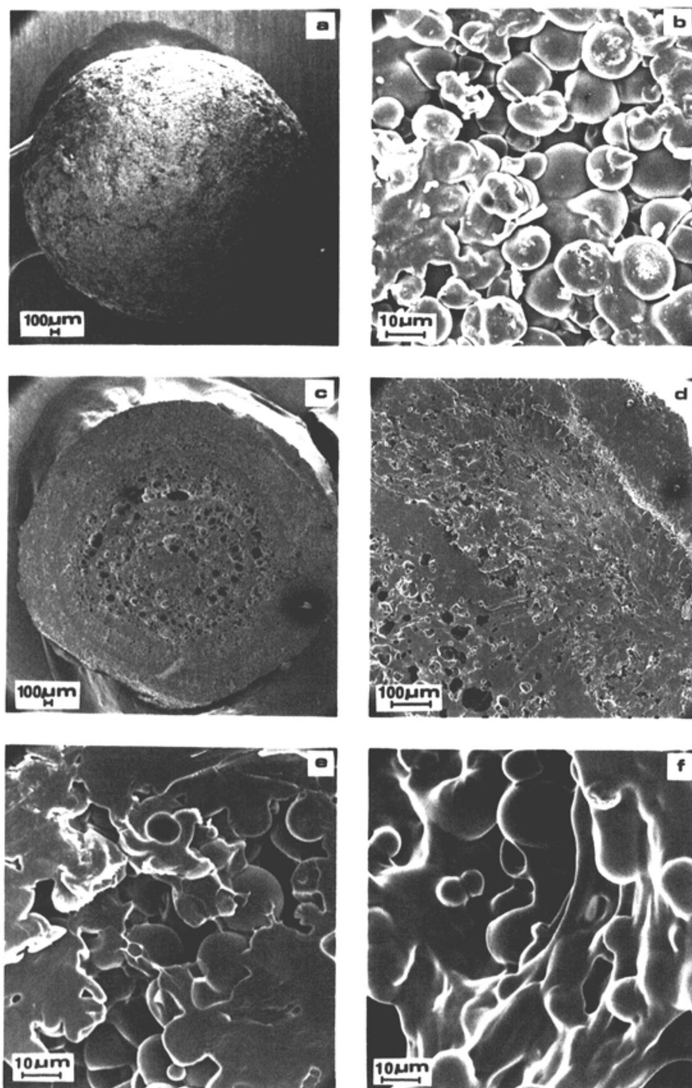

Fig. 3. Scanning electron micrographs of the surface of a tapioca pearl at 20× (**a**) and 1,000× (**b**); the inner surface of a split pearl at 20× (**c**), 100× (**d**), and 1,000× (**e**); and an internal void (**f**) in the inner surface at 1,000×.

Acid and Enzyme Hydrolysis of Cooked Tapioca Pearls

Freshly cooked (and sheared) tapioca pearls and mung bean starch noodles, when treated with 1*M* hydrochloric acid at 35°C, showed a relatively rapid digestion of accessible starch (Fig. 4). After three days, approximately 90 and 70% of the cooked pearls and starch noodles, respectively, were dissolved in the acid. Then, the rate of acid digestion dramatically decreased and approached zero after approximately 15 days (Fig. 4).

Digestion of cooked tapioca pearls with bacterial α-amylase rapidly removed the accessible starch fraction (Fig. 4) and gave 2% of isolated resistant material compared to 5% resistant to acid digestion (Table II). α-Amylase digestion of cooked starch noodles also gave a lower yield (12%) of isolated resistant material than did acid digestion at 35°C (16%). Those results indicate that additional reorganization and retrogradation of starch probably occurred during acid treatment but not during α-amylase digestion.

Kainuma et al (1981) developed a β-amylase-pullulanase system to study rice starch retrogradation after cooking. In our investigation, we first used isoamylase alone to digest the cooked tapioca pearls and mung bean starch noodles, but we observed apparent reassociation and precipitation of debranched chains. A combination of isoamylase and β-amylase, therefore, was adopted to digest the cooked tapioca pearls and mung bean starch noodles. Figure 5 shows that the digestions of cooked pearls and starch noodles with a mixture of those two amylases leveled off within 12 hr. Thus, β-amylase appeared to prevent the recrystallization of chains debranched by isoamylase. The yields of residues resistant to isoamylase and β-amylase combined were higher than the yields from acid or α-amylase digestions (Table II).

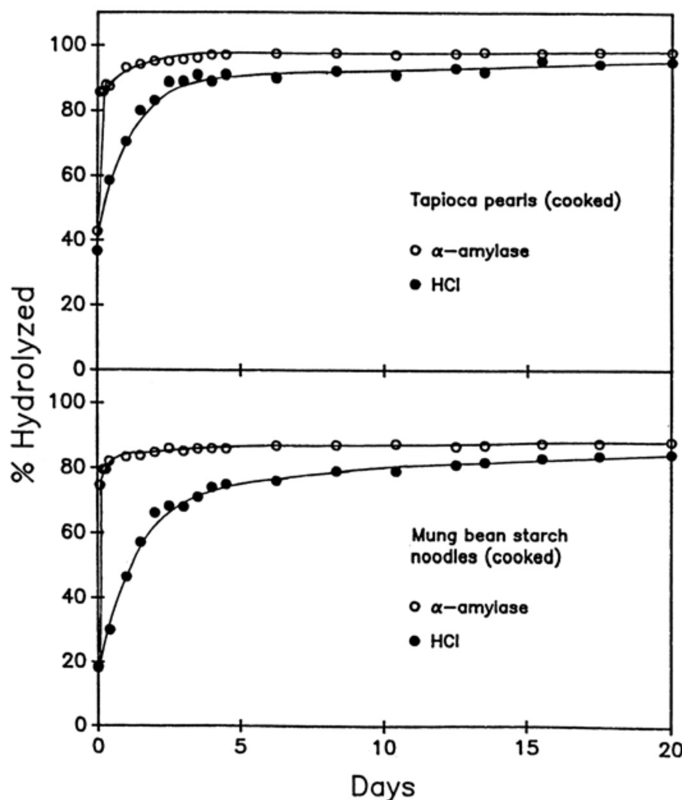

Fig. 4. Hydrolysis of cooked tapioca pearls and cooked mung bean starch noodles using 1*M* HCl at 35°C or α-amylase.

TABLE II
Yield and Differential Scanning Calorimetry (DSC) Data on Resistant Residues after Digestion of Cooked Starch Pearls and Mung Bean Starch Noodles with Acid and Enzymes

	Digestion Conditions[a]				DSC of Residue[b]			
Food	Catalyst	Temp. °C	Time	Yield[c] (%)	T_o (°C)	T_p (°C)	T_f (°C)	ΔH (J/g)
Tapioca pearl	HCl	35	20 days	5	101	128	139	20
	α-amylase	25	20 days	2	...	...	...	...
	Isoamylase/β-amylase	25	60 hr	6	74	92	110	9
Starch noodle	HCl	35	20 days	16	90	128	141	18
	α-amylase	25	20 days	12	...	...	...	...
	Isoamylase/β-amylase	25	60 hr	26	74	90	104	12

[a]Starch pearls and noodles (0.072 g/ml) were soaked overnight, cooked for 1 hr, cooled, sheared in a high-speed blender, and immediately subjected to digestion with 1M HCl, α-amylase (500 unit per gram), or isoamylase/β-amylase (500/250 units per gram) or isoamylase/β-amylase (500/250 units per gram) after addition of an equal volume of acid or buffer containing an enzyme.
[b]T_o = temperature at onset, T_p = temperature at peak, T_f = temperature at final, ΔH = enthalpy of cooked starch, J/g = joules per gram.
[c]Calculated based on yield of residue.

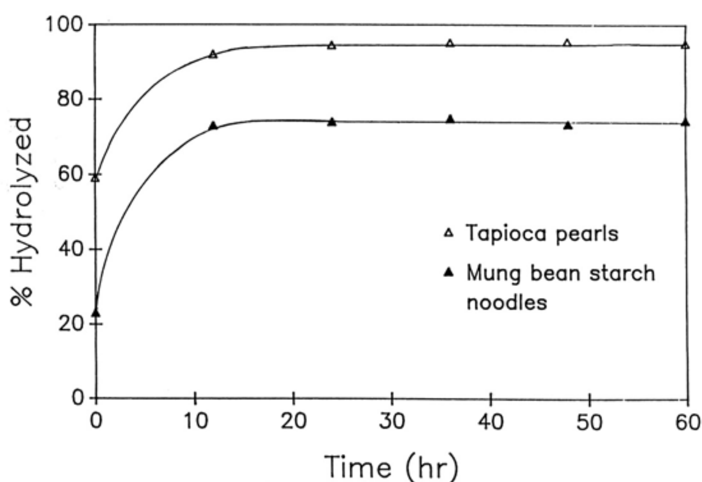

Fig. 5. Hydrolysis of cooked tapioca pearls and cooked mung bean starch noodles using a mixture of isoamylase and β-amylase.

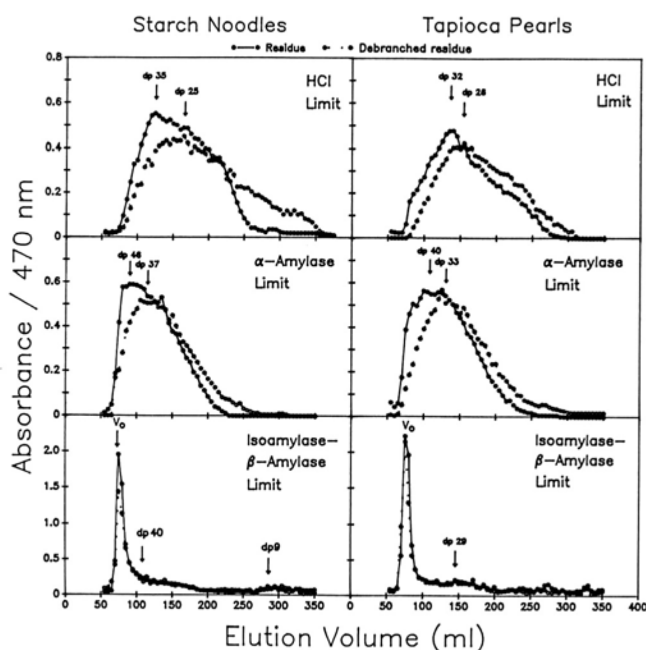

Fig. 6. Gel-permeation chromatography of residues of cooked tapioca pearls and cooked mung bean starch noodles resistant to 1*M* HCl at 35°C, α-amylase, and a mixture of isoamylase and β-amylase, before and after debranching by isoamylase.

Gel-Permeation Chromatography of Residues Resistant to Acid and Enzyme Hydrolysis.

Gel-permeation chromatography of the acid-resistant molecules in cooked tapioca pearls and mung bean starch noodles showed peaks with DP_n 32 and 35, respectively, whereas α-amylase-resistant residues showed peaks at DP 40 and 46, respectively (Fig. 6). After debranching, the chain lengths (CL) of the acid- and α-amylase-resistant residues peaked at CL 25–26 and 33–37, respectively (Fig. 6). The differences between DP and CL of the residues indicated some branch points in the resistant residues.

The residues resistant to the isoamylase and β-amylase combination contained mainly large molecules that appeared at the void volume of the column (Fig. 6). Upon debranching, the peak at the void volume decreased only slightly, indicating the presence of long linear chains. Because the size-exclusion limit (V_o) of the gel was ~100,000 Daltons, the molecules resistant to the isoamylase and β-amylase combination must have been amylose.

DSC and X-Ray Analysis of Resistant Residues

The acid-resistant residues of cooked mung bean starch noodles gave a melting endotherm with ΔH 18 J/g at T_p 128°C (Table II). Mestres et al (1988) reported ΔH 7.9 J/g at T_p 119°C for the acid-resistant residue from uncooked mung bean starch noodles. The acid-resistant residues of cooked tapioca pearls gave an endotherm with ΔH 20 J/g at T_p 128°C (Table II). The residues resistant to the combination of isoamylase and β-amylase from cooked tapioca pearls and mung bean starch noodles gave endotherms with ΔH 9J/g at T_p 92°C, and ΔH 12 J/g at T_p 90°C, respectively (Table II). On the other hand, the α-amylase-resistant residues gave no peak in the DSC between 7 and 147°C. For comparison, tapioca starch and mung bean starch had gelatinization peaks beginning at 63 and 67°C under the same DSC conditions, with enthalpies of melting at 15.7 and 13.5 J/g, respectively.

The high melting temperature (T_p 128°C) of the acid-resistant residues from the two foods may be explained, in part, by the lack of cooperative melting in the acid-resistant fractions because of a low number of amorphous chains (Donovan and Mapes 1980, Slade and Levine 1987). Naegeli dextrins from potato starch have T_p 70–73°C in excess water compared to T_p 64°C for the original starch. Moreover, the average chain lengths (DP_n = 25–26, Fig. 6) of the acid-resistant fractions were longer than those of a highly crystalline, short chain amylose (DP 15) isolated in the B-polymorphic form (Whittam et al 1990). The increased chain lengths of the acid-resistant fractions were associated with a high T_p of 128°C compared to 77°C for the short-chain amylose, both determined in excess water (>75% moisture).

The α-amylase-resistant residues did not show a peak in the temperature range tested (7–147°C), but they did show the same degree of X-ray crystallinity as the acid-resistant fractions (Figs. 7 and 8). The melting endotherms must be 150°C, or above, because of the increased chain lengths (peaks at DP_n 33 and 37, Fig. 6). The melting temperature of recrystallized amylose is about 160°C (Biliaderis et al 1985, Ring et al 1987).

The low melting temperatures (T_p 90–92°C) of the residues resistant to the combination of isoamylase and β-amylase are difficult to understand. Those residues are composed mostly of amylose molecules (Fig. 6), so they would be expected to have a high melting temperature. X-ray diffractograms showed that the acid- and enzyme-hydrolysis residues were the expected B-polymorph from both starch pearls and noodles (Figs. 7 and 8).

Microscopic Structure of α-Amylase and Isoamylase Hydrolysis Residues of Cooked Tapioca Pearls

Figure 9 shows SEMs of the residues isolated after digestion of cooked (not sheared) mung bean starch noodles with α-amylase for 5 hr or with isoamylase for 24 hr. The resistant residues (Fig. 9c and d) contained long, thick filaments that may be the birefringent strands reported by Mestres et al (1988). The filaments in the cooked noodles appeared to outline remnants of starch granules (Fig. 9c).

Digestion of cooked tapioca pearls with isoamylase caused the release of shells (S in Fig. 10b). Those shells originated from the concentric spheres (rings), as evidenced by the rings on the surface of split uncooked pearls (Fig. 3). The shells, released by isoamylase, were composed of a three-dimensional network of filaments (Fig. 10c and d) that were smaller in diameter than those observed in the starch noodles (Fig. 9c and d). A second sample of cooked pearls was digested with α-amylase or isoamylase; SEM (Fig. 11c–f) revealed an overall pattern of highly digested starch granules held together by filaments.

Structural Models

Mestres et al (1988), based on their findings of acid hydrolysis of uncooked mung bean starch noodles at 35°C, proposed that junction zones anchor the three-dimensional structure. In our

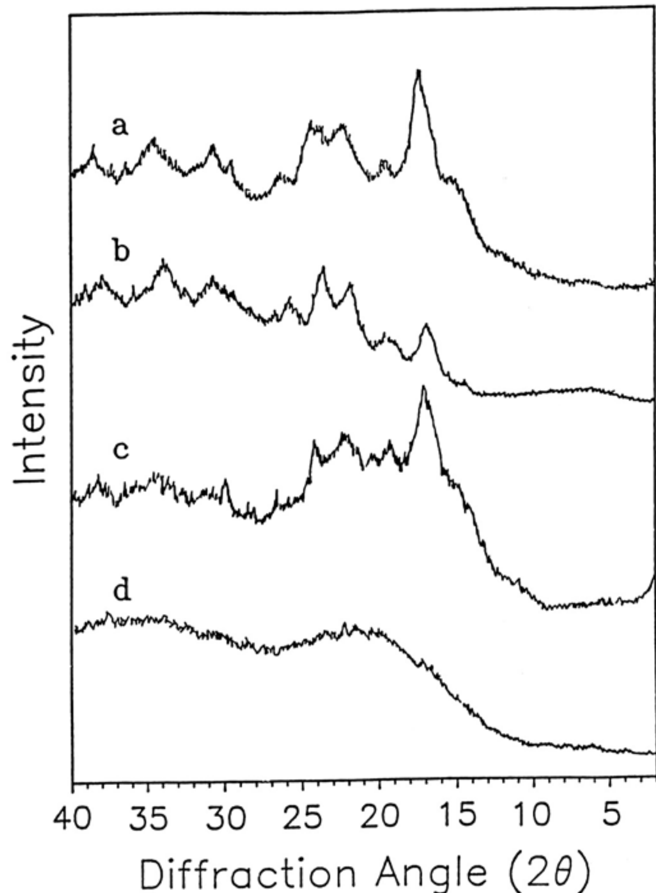

Fig. 7. X-ray diffraction patterns of residues of cooked tapioca pearls resistant to 1 M HCl at 35°C (**a**), α-amylase (**b**), and a mixture of isoamylase and β-amylase (**c**). **d** = undigested sample of tapioca pearls. All samples held under ambient environment before X-ray measurement.

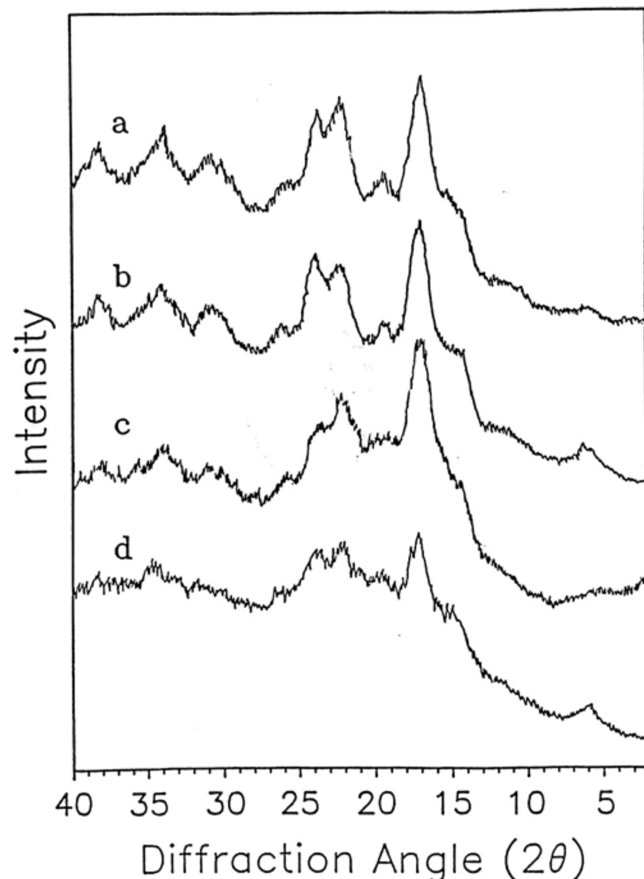

Fig. 8. X-ray diffraction patterns of residues of cooked starch noodles resistant to 1 M HCl at 35°C (**a**), α-amylase (**b**), and a mixture of isoamylase and β-amylase (**c**). **d** = undigested sample of starch noodles. All samples held under ambient environment before X-ray measurement.

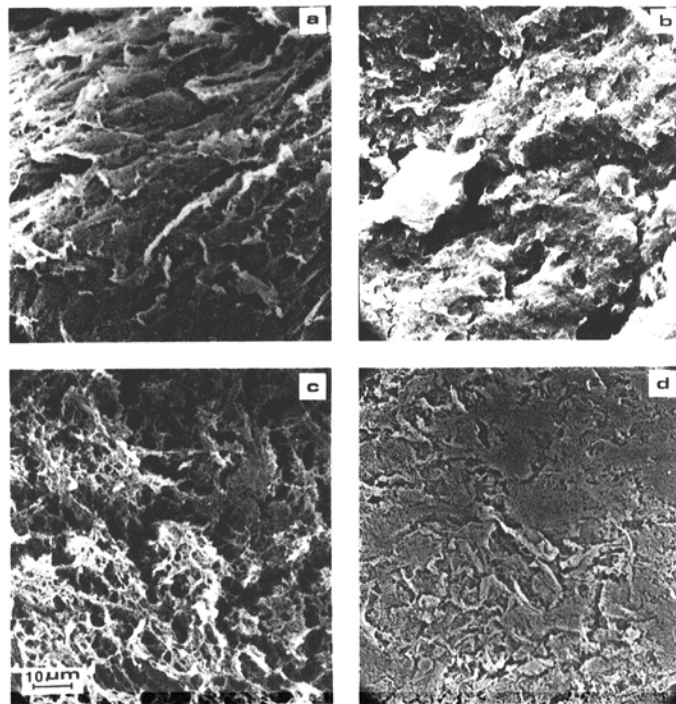

Fig. 9. Scanning electron micrographs (1,000×) of the surface of starch noodles after cooking (**a**), a fractured cross section (**b**), the surface of the residue after cooking followed by α-amylase digestion (**c**), and the surface of the residue after cooking followed by isoamylase digestion (**d**). Digestion with α-amylase (567 units per gram of dry noodles) was carried out at 25°C for 5 hr at pH 6. Digestion with isoamylase (567 units per gram) was done at 25°C for 24 hr at pH 3.8. Samples subjected to critical-point drying before viewing.

investigation, cooked, rather than uncooked, noodles and pearls were subjected to enzymatic and acid digestions. Our results generally agree with the structural model proposed by those authors, but additional information allows further speculation.

We conjecture that the structure of cooked starch noodles has three phases: the micelle, the paracrystalline fringe, and the filler mass (amorphous zone). The micelle (zone A, Fig. 12) contains retrograded segments of amylose molecules and is resistant to acid and enzymes. It is the most highly organized zone and contains crystallites. In cooked starch noodles or pearls, the length of a micelle would be shorter than 32–35 anhydroglucose units, which is the DP_n of the acid-resistant residues (Fig. 6).

Attached to the micelle is the paracrystalline fringe (zone B, Fig. 12), composed of less organized material. The paracrystalline fringe can be attacked by acid and α-amylase but not by isoamylase. The molecules in the paracrystalline zone are practically all linear; that zone does not swell sufficiently to satisfy the large combining site of isoamylase. On the other hand, the paracrystalline fringe can be trimmed by acid or α-amylase, and the acid-resistant residues would be expected (Jane and Robyt 1984) to be shorter than the α-amylase-resistant residues, as shown in Figure 6. The somewhat longer molecules in the α-amylase-resistant residue caused it to have a higher melting temperature than that of the acid-resistant residue (Table II). Moreover, digestion of cooked pearls and noodles with α-amylase gave lower yields of resistant material than did digestion with acid. The chains in the paracrystalline fringe underwent crystallization as they were depolymerized by acid because of the tiny size of the proton catalyst.

The third, and most prominent, zone in the cooked noodle is the filler mass or amorphous zone. This zone is poorly organized and is accessible to all hydrolytic enzymes and, of course, to acid. Besides occupying a large volume in a noodle, the filler mass helps bind together the fringes of two micelles. Some of the amylose molecules in the filler mass could span the gap between two micelles and form a junction zone in a double helical, nonparallel conformation (Matsukura et al 1983, Hinrichs et al 1987). Other amylose molecules might transverse two or more micelles, with the remainder of their lengths remaining within the filler mass. Practically all the filler mass would be hydrolyzed by acid

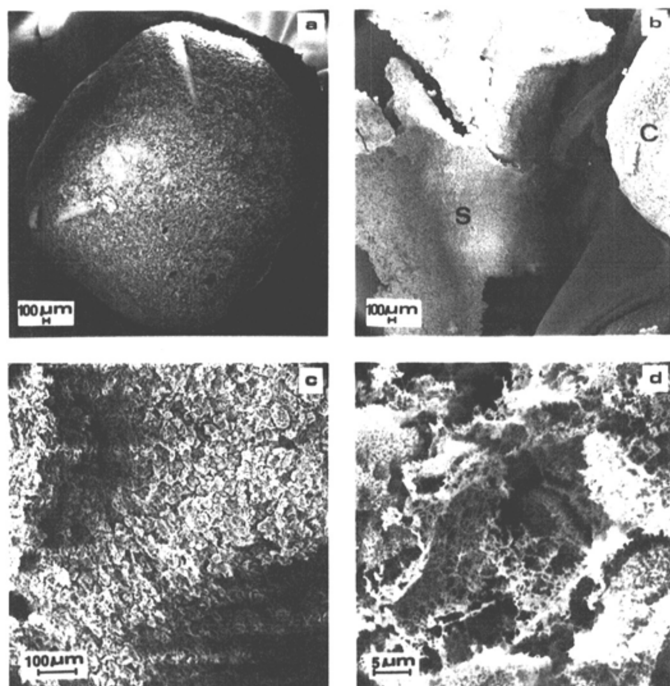

Fig. 10. Scanning electron micrographs of the residue of a tapioca pearl after cooking and isoamylase digestion showing the core at 20× (**a**) and the shell at 20× (**b**), 100× (**c**), and 2,000× (**d**). Digestion conditions as given for Fig. 9. C = core and S = shell.

and α-amylase, but some molecules in that zone would remain after treatment with the isoamylase and β-amylase combination. Amylose molecules running through micelle structures would be more or less spared digestion with the isoamylase and β-amylase, depending on the position of their nonreducing ends with respect to a crystalline micelle.

Cooked tapioca pearls differ from the cooked starch noodles in that the pearls contain ~40% ungelatinized starch. Tapioca starch has only one-half the level of amylose (17%, Young 1984) in mung bean starch (Biliaderis et al 1981a). On the other hand,

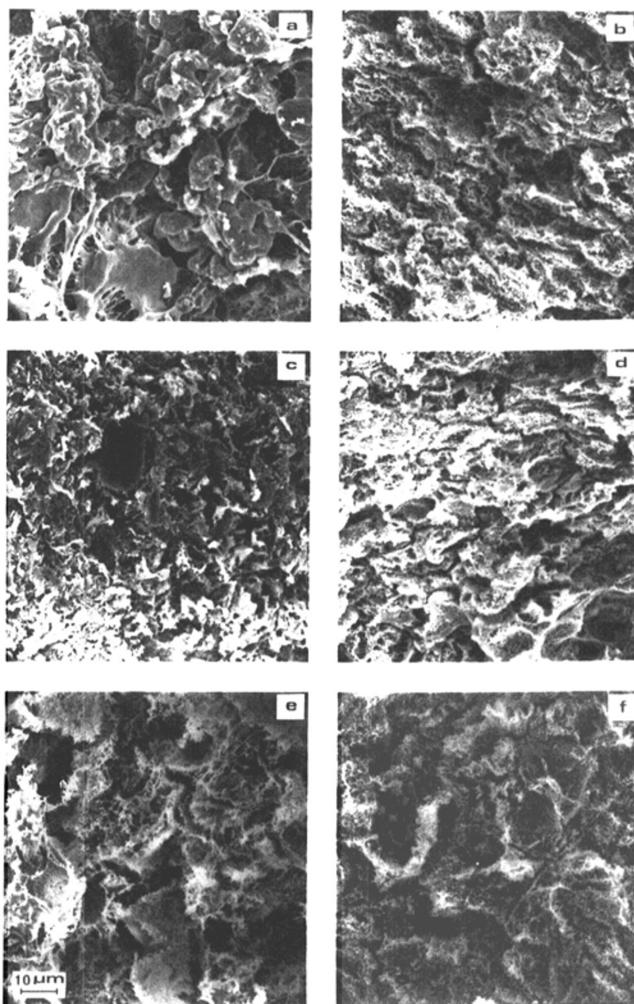

Fig. 11. Scanning electron micrographs (1,000×) of the surface of a cooked tapioca pearl without further treatment (**a**), after α-amylase digestion (**c**), and after isoamylase digestion (**e**) and of the cross-section of a cooked tapioca pearl without further treatment (**b**), after α-amylase digestion (**d**), and after isoamylase digestion (**f**). Digestion conditions as given for Fig. 9.

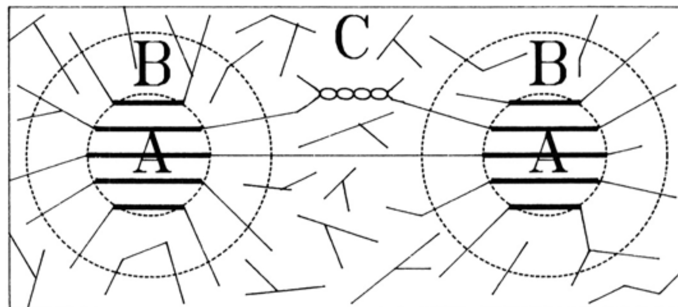

Fig. 12. Conceptual model of the structural zones in tapioca pearls and mung bean-starch noodles. **A**, micelle or junction zone. **B**, paracrystalline zone. **C**, filler (amorphous) zone.

amylose molecules from tapioca are large (DP_n 2,600) (Takeda et al 1987, Hizukuri 1988); those from mung bean starch have DP_n 1,900 (Biliaderis et al 1981b). The net result is fewer junction zones and micelles within, and between, swollen starch granules in tapioca pearls, which is evidenced by the generally reduced yields of acid- and enzyme-resistance material from cooked pearls versus cooked noodles and by the thin filaments observed in the enzyme-resistant fractions for cooked pearls (Figs. 10d and 11e) as opposed to the thick ones in cooked and digested noodles (Fig. 9c).

CONCLUSIONS

Apparently, few starches possess the combination of two properties thought to be important in making a starch "pearl": ease of swelling and solubilization under moist heat with high molecular weight amylose. About 50% of the tapioca starch gelatinizes during the preparation of a pearl, and the gelatinized granules exude a portion of their amylose. The high molecular weight amylose creates a network of micelles between and within granules, and those zones resist disintegration of a pearl during cooking. Thus, the cooked pearl maintains a high concentration of starch molecules and, upon cooling, sets to a rubbery, gelled particle.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Official Methods of the AACC, 8th ed. Method 30-20, approved April 1961, revised October 1975, reviewed October 1982; Method 44-15A, approved October 1975, revised October 1981; Method 46-16, approved October 1988. The Association: St. Paul, MN.

BILIADERIS, C. G., GRANT, D. R., and VOSE, J. R. 1981a. Structural characterization of legume starches. II. Studies on acid-treated starches. Cereal Chem. 58:502.

BILIADERIS, C. G., GRANT, D. R., and VOSE, J. R. 1981b. Structural characterization of legume starches. I. Studies on amylose, amylopectin, and beta-limit dextrins. Cereal Chem. 58:497.

BILIADERIS, C. G., PAGE, C. M., SLADE, L., and SIRETT, R. R. 1985. Thermal behavior of amylose-lipid complexes. Carbohydr. Polym. 5:367.

CHIANG, B.-Y., and JOHNSON, J. A. 1977. Measurement of total and gelatinized starch by glucoamylase and o-toluidine reagent. Cereal Chem. 54:429.

DONOVAN, J. W., and MAPES, C. J. 1980. Multiple phase transitions of starches and Nägeli amylodextrins. Starch/Staerke 32(6):190.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

FRENCH, D. 1984. Organization of starch granules. Chapter VII in: Starch Chemistry and Technology. 2nd ed. R. L. Whistler, J. N. BeMiller, E. F. Paschall, eds. Academic Press: Orlando, FL.

GRACE, M. R. 1977. Cassava Processing. Food and Agriculture Organization; pp. 55-61: Rome.

HINRICHS, W., BÜTTNER, G., STEIFA, M., BETZEL, C. H., ZABEL, V., PFANNEMÜLLER, B., and SAENGER, W. 1987. An amylose antiparallel double helix at atomic resolution. Science 238:205.

HIRAO, K., NISHIOKA, I., and TAKAHASHI, S. 1989. Studies on the cooking of pearl starch. I. Cooking conditions of tapioca pearls. J. Home Econ. Jpn. 40(5):363.

HIZUKURI, S. 1988. Recent advances in molecular structures of starch. J. Jpn. Soc. Starch Sci. 35(5):185.

HIZUKURI, S., TAKEDA, Y., YASUDA, M., and SUZUKI, A. 1981. Multi-branched nature of amylose and the action of debranching enzymes. Carbohydr. Res. 94:205.

HOLM, J., BJÖRCK, I., ASP, N.-G., SJÖBERG, L.-B., and LUNDQUIST, I. 1985. Starch availability in vitro and in vivo after flaking, steam-cooking and popping of wheat. J. Cereal Sci. 3:193.

JANE, J-L., and ROBYT, J. F. 1984. Structure studies of amylose-V complexes and retrograded amylose by action of α-amylase, and a new method for preparing amylodextrins. Carbohydr. Res. 132:105.

JONES, S. F. 1983. The World Market for Starch and Starch Products with Particular Reference to Cassava (Tapioca) Starch. Tropical Development and Research Institute: London.

KAINUMA, K., MATSUNAGA, A., ITAGAWA, M., and KOBAYASHI, S. 1981. New enzyme system—β-amylase-pullulanase—to determine the degree of gelatinization and retrogradation of starch or starch products. J. Jpn. Soc. Starch Sci. 28:235.

LEVINE, S., MEADUS, F. W., and SPARKS, B. D. 1987. Theory of spherical agglomeration. II. Layering/crushing process in a rotating conical drum. Sep. Sci. Technol. 22(5):1449.

LII, C. Y., and CHANG, S. M. 1981. Characterization of red bean starch and its noodle quality. J. Food Sci. 46:78.

MATSUKURA, U., MATSUNAGA, A., and KAINUMA, K. 1983. Structural studies on retrograded normal and waxy corn starches. J. Jpn. Soc. Starch Sci. 30(1):106.

MESTRES, C., COLONNA, P., and BULEON, A. 1988. Characterization of starch networks within rice flour noodles and mung bean starch vermicelli. J. Food Sci. 53:1809.

PARK, J. T., and JOHNSON, M. J. 1949. Submicrodetermination of glucose. J. Biol. Chem. 181:149.

RING, S. G., COLONNA, P., ORFORD, P. D., and KALICHEVSKI, M. T. 1987. The gelation and retrogradation of amylopectin. Carbohydr. Res. 162:277.

RUDDLE, K., JOHNSON, D., TOWNSEND, P. K., and REES, J. D. 1978. Palm Sago—A Tropical Starch from Marginal Lands. University Press of Hawaii: Honolulu.

SCHOCH, T. J., and MAYWALD, E. C. 1968. Preparation and properties of various legume starches. Cereal Chem. 45:564.

SINGH, U., VORAPUTHAPORN, W., RAO, P. V., and JAMBUNATHAN, R. 1989. Physicochemical characteristics of pigeon pea and mung bean starches and their noodle quality. J. Food Sci. 54:1293.

SLADE, L., and LEVINE, H. 1987. Recent advances in starch retrogradation. In: Recent Developments in Industrial Polysaccharides. S. S. Stivala, V. Crescenzi, and I. C. M. Dea, eds. Gordon and Breach Science: New York.

TAKAHASHI, S., HIRAO, K., KOBAYASHI, R., KAWABATA A., and NAKAMURA, M. 1987. The degree of gelatinization and texture during the preparation of harusame noodles. J. Jpn. Soc. Starch Sci. 34(1):21.

TAKEDA, Y., HIZUKURI, S., TAKEDA, C., and SUSUKI, A. 1987. Structures of branched molecules of various origins, and molar fractions of branched and unbranched molecules. Carbohydr. Res. 165:139.

WHITTAM, M. A., NOEL, T. R., and RING, S. G. 1990. Melting behaviour of A- and B-type crystalline starch. Int. J. Biol. Macromol. 12:359.

XU, A. 1992. Structure of Tapioca Pearls. Ph.D. dissertation. Kansas State University: Manhattan, KS.

YOKOBAYASHI, K., MISAKI, A., and HARADA, T. 1970. Purification and properties of *Pseudomonas isoamylase*. Biochem. Biophys. Acta 212:458.

YOST, D. A., and HOSENEY, R. C. 1986. Annealing and glass transition of starch. Starch/Staerke 38:289.

YOUNG, A. H. 1984. Fractionation of starch. Pages 249-274 in: Starch: Chemistry and Technology, 2nd ed. R. L. Whistler, J. W. BeMiller, and E. F. Paschall, eds. Academic Press: New York.

ZELEZNAK, K. J., and HOSENEY, R. C. 1986. The role of water in the retrogradation of wheat starch gels and bread crumb. Cereal Chem. 63:407.

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