

# Isolation of Small Starch Granules and Determination of Their Fat Mimic Characteristics

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## ABSTRACT

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Small starch granules from wheat and amaranth were isolated and examined by light microscopy (wheat), scanning electron microscopy (wheat and amaranth), and rapid viscosity (RVA) analysis. The isolated wheat and amaranth starches were incorporated into reduced-fat frozen dessert mixes (2%, w/w) and the frozen desserts were characterized by

sensory analysis for smoothness, creaminess, and preference. The results of the sensory analysis suggest that up to 50% of the fat in a frozen dessert may be replaced when substituted by these starches at the 2% level (w/w).

Fats are important in human growth and development. They provide essential fatty acids and serve as carriers for fat-soluble vitamins required in the diet to promote growth, reproduction, and general health. In addition, fat contributes a variety of oral sensations, imparting taste, texture, mouthfeel, and appearance to many foods. Accordingly, many high-fat foods are popular with consumers (Setser and Racette 1992) who are reluctant to give up fat in the diet, making it a major determinant of food selection (Bruhn et al 1992). However, a high intake of total dietary fat can increase the risk of coronary heart disease, obesity, and some cancers. As a result, nearly 150 million people (Anonymous 1992) in the United States are using low-calorie and low-fat foods. Reduced-fat products account for 10% of retail sales in applicable categories (Anonymous 1992). However, decreasing fat consumption is not simple because taste and texture are principal determinants in food selection (Bruhn et al 1992), and fats are partially responsible for the flavor of foods as well as such textural attributes as creaminess, viscosity, thickness, and smoothness. Low-fat diet regimens often fail because of a bland, unsatisfying taste.

Surprisingly little is known about fattiness perception and how it is related to sensory preference. Some sensory evaluations have been made of the basic psychophysics of food lipids. Kokini et al (1977) reported that thickness, smoothness, and slipperiness adequately describe liquid texture in the mouth. Work by Mela (1988) supported the idea that the perception of "fat content" may be predominantly derived from oral textural sensations. Also, it has been shown that "creaminess" appears to be associated with smoothness and thickness in milk and ice cream products (Kokini and Cussler 1983).

Starch-based fat mimetics have been postulated to achieve fat mimetic properties by stabilizing substantial quantities of water into weak gel structures. This results in lubrication and flow properties similar to those of fats in simple food systems (Yackel and Cox 1992).

The isolation of small granule starches from wheat and amaranth is described here. Recent interest in these starches has led to an examination of amaranth pasting (Jae et al 1999). Isolation of amaranth starch from amaranth flour has also been described by Zhao and Whistler (1994). Also, small wheat and amaranth starch granules have been used as partial fat replacements in frozen desserts and some of those results are reported here.

## MATERIALS AND METHODS

### Wheat Starch Isolation, Purification, and Separation

Starch was extracted from whole flour (The Pillsbury Co., Minneapolis, MN) by the dough-kneading procedure (Schoch 1957; Wolf

1964). A stiff dough was prepared by mixing 150 g of flour thoroughly with 115 mL of water. The dough was aged at room temperature for  $\approx 1$  hr, then placed in a cheesecloth bag and the doughy mass was hand-kneaded under water (2,000 mL in four equal portions) for  $\approx 30$  min. The material remaining in the cheesecloth (mostly gluten and bran) was discarded. The starch suspension was passed through a 60-mesh screen and then a 140-mesh screen. Material remaining on screens (mostly bran) was discarded. Filtrate was centrifuged at  $1,000 \times g$  for 15 min. Purification of the centrifugant was based on the method described by Wu and Seib (1990). Centrifugant was suspended in 900 mL of 0.2% sodium metabisulfite and steeped in this solution for 24 hr. Then, this suspension was centrifuged at  $1,000 \times g$  for 20 min. The top slimy grey layer of centrifugant, which consisted of protein and a part of B-type starch, was scraped off. Remaining centrifugant was washed with 200 mL of 0.15% NaOH in centrifuge tubes and centrifuged at  $1,000 \times g$  for 20 min. Supernatant was discarded and the top grey-yellow layer of centrifugant was scraped off. These two steps were repeated twice more. Remaining centrifugant was washed with 200 mL of water in centrifuge tubes and centrifuged at  $1,000 \times g$  for 20 min. Supernatant was discarded and the top grey-yellow layer of centrifugant was scraped off. These two steps were repeated two times. Remaining centrifugant was suspended in 200 mL of water. Then, the slurry was neutralized with dilute hydrochloric acid and centrifuged at  $1,000 \times g$  for 15 min.

The separation into A- and B-type starch was done according to Soulaka and Morrison (1985). The centrifugant was transferred to 2L beaker and suspended in  $\approx 1,700$  mL of water using a mechanical stirrer. After 5 min, the stirrer was removed and the suspension was left to settle for 2 hr. The supernatant was carefully decanted and the sediment was resuspended and sedimented as before. This procedure was repeated two more times. Then, the sediment containing A-type starch was washed with 100 mL of 95% alcohol, filtered under vacuum using Whatman filter No. 1 and dried under vacuum over  $\text{CaCl}_2$  for two days at room temperature. The combined supernatants containing B-type starch (prime B-type starch) were centrifuged at  $1,000 \times g$  for 15 min. Centrifugant was washed with 100 mL of 95% alcohol in centrifuge tubes and centrifuged at  $1,000 \times g$  for 15 min. This was repeated twice. The centrifugant was suspended in 100 mL of 95% alcohol and filtered on a sintered glass funnel. A latex dam was used during filtration to prevent water absorption and subsequent hornification. Resulting starch was dried under vacuum over  $\text{CaCl}_2$  for two days at room temperature and screened through a 140-mesh screen. All scraped-off top layers containing protein and B-type starch were suspended in 100 mL of water and neutralized with dilute hydrochloric acid. The B-type starch was recovered by sedimenting as before. Protein sedimented first and was on the bottom. This B-type starch (impure starch) was not as pure as prime B-type starch because some protein was still present in it. When sodium metabisulfite and sodium hydroxide treatment was repeated,  $\approx 70\%$  of the material was recovered as pure B-type starch in 46% yield. The relative weights of isolated starches were 76% A-type starch, 16% prime B-type starch,

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and 8% impure B-type starch. According to Evers et al (1974), B-type starch represents 30% of the total wheat starch isolated from grain.

### Amaranth Starch Isolation and Purification

Amaranth starch was isolated from amaranth seeds (Arrowhead Mills, Hereford, TX) by a modification of the method of Wu and Seib (1990). Seeds (210 g) were steeped in 1,200 mL of a 0.2% solution of sodium metabisulfite for 24 hr and then blended in a Waring blender for 1 min. After the slurry stood for 1 hr, the top foamy layer was removed and discarded and the suspension was passed through a 60-mesh screen and then a 140-mesh screen. Material left on screens was mostly bran. This was washed with 100–200 mL of water which was then added to the filtrate and discarded. Combined filtrate was centrifuged at low speed ( $25 \times g$ ) for 5 min and the supernatant was decanted. Centrifugant was stirred with 400 mL of water and centrifuged at low speed ( $25 \times g$ ) for 5 min. Supernatant from this centrifugation was combined with the previous supernatant and the centrifugant discarded. The combined supernatants were centrifuged at  $1,000 \times g$  for 20 min, and the supernatant and top slimy grey layer were discarded. Remaining centrifugant was washed with 200 mL of 0.15% sodium hydroxide solution and centrifuged at  $1,000 \times g$  for 20 min and the top grey-yellow layer of centrifugant was scraped off using a small amount of water. These steps were repeated twice. Remaining centrifugant was stirred with 200 mL of water in centrifuge tubes and centrifuged at  $1,000 \times g$  for 20 min. Supernatant was discarded and any top layer of protein removed using a small amount of water. These last two steps were repeated twice. Resulting centrifugant was suspended in 200 mL of water, neutralized with dilute hydrochloric acid, and centrifuged at  $1,000 \times g$  for 20 min. Centrifugant was mixed with 200 mL of 95% alcohol in centrifuge tubes and centrifuged

at  $1,000 \times g$  for 20 min. This was repeated twice. Then the final centrifugant was suspended in 200 mL of 95% alcohol and filtered on a sintered glass funnel. A latex dam was used during filtration to prevent hornification due to drawing air through the starch. Starch was dried under vacuum over calcium chloride at  $25^\circ\text{C}$  for two days and passed through a 140-mesh screen. Yield of starch was 20%.

### Protein

Protein content of starch (0.12%) was determined by the Kjeldahl method ( $N \times 5.85$ ). This is somewhat higher than the level of 0.05% protein reported by Uriyapongson and Rayas-Duarte (1994).

### Light Microscopy

Light microscopic investigations were performed on isolated starch granules in a 50% glycerol suspension using a Leitz Leica microscope (W. Nuhsbaum, McHenry, IL) at a magnification of  $300\times$ .

### Scanning Electron Microscopy

Scanning electron microscopy (SEM) of purified starch was performed using a JEOL JSM-840 scanning electron microscope. The samples were mounted on 12-mm aluminum stubs and sputter-coated with AuPd. Magnification was  $1,200\times$  for wheat starch and  $4,500\times$  for amaranth starch; voltage was 5 kV.

### RVA Analysis

Pasting properties of wheat starch and amaranth starch were determined using a Rapid Visco Analyser (RVA) (Newport Scientific Inc., Australia). The appropriate starch (3 g) was mixed with 25 mL of water and this slurry was subjected to RVA analysis.

### Frozen Dessert Formulation and Preparation

Full-fat ice cream and reduced-fat frozen desserts were experimentally prepared using the formulation shown in Table I. The control ice cream contains 12% fat. Ingredients used included sugar (Domino pure cane granulated), distilled water, corn syrup solids (DE 24; Amaizo), heavy whipping cream (30% fat), stabilizer (Kontrol, Germantown Mfg. Co., Broomall, LA), nonfat dry milk (Carnation, extra grade), starch-based replacers (Whistler Center for Carbohydrate Research, West Lafayette, IN), and vanilla extract (Durkee-French). Whipping cream, skim milk, and fat-replacer contents were adjusted to obtain the desired fat reduction.

Dry ingredients were blended with water using an electric blender (Sunbeam). When fat replacers were used, they were suspended in

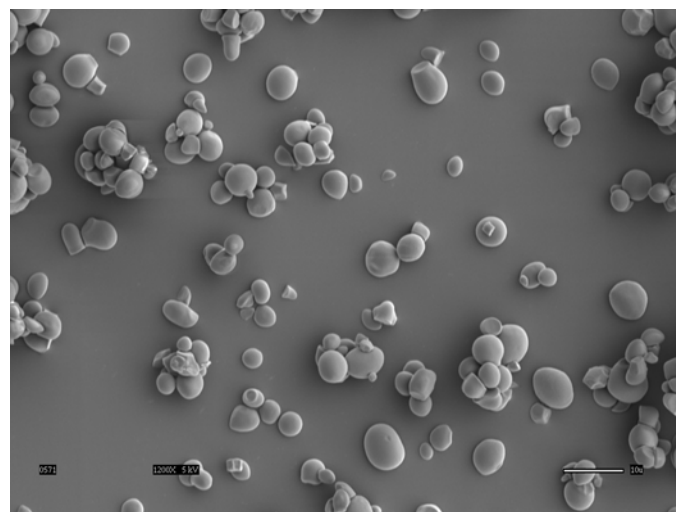


Fig. 1. SEM of small wheat starch granules (type B)

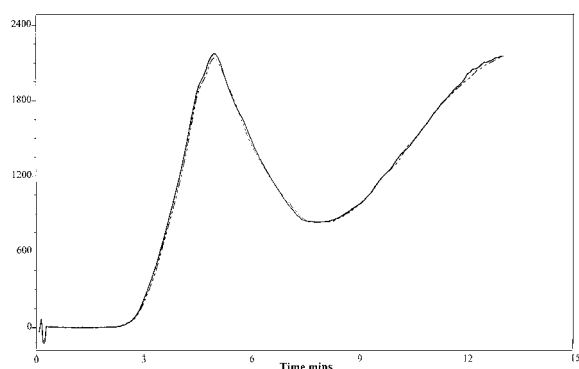


Fig. 2. RVA of small granule wheat starch (solid and dashed lines represent duplicate viscosities)

TABLE I  
Control and Fat-Reduced Frozen Dessert Composition

Ingredients	Fat Reduction (% by wt)				
	Control	15	30	50	75
Sugar	12.0	12.0	12.0	12.0	12.0
NFMS	11.0	11.0	11.0	11.0	11.0
Corn syrup solid	4.0	4.0	4.0	4.0	4.0
Stabilizers	0.3	0.3	0.3	0.3	0.3
H <sub>2</sub> O	39.7	39.7	39.7	39.7	39.7
Fat replacer	0	2.0	2.0	2.0	2.0
Heavy cream	33.0	28.0	23.0	16.5	8.5
Skim milk	0	3.0	8.0	14.5	22.5

TABLE II  
Mean Sensory Scores for Control and Small Granule Wheat Starch Substitutes, Reduced-Fat Frozen Desserts<sup>a</sup>

Attribute	Fat Reduction (% by wt)				
	Control	15	30	50	75
Smoothness	7.1a <sup>a</sup>	7.3a	7.0a	6.6a	4.9b
Creaminess	7.1ab	7.6a	7.3ab	6.6ab	4.8b
Preference	6.9a	6.9a	7.0a	6.0a	5.1b

<sup>a</sup> Values followed by the same letter in the same row are not significantly different ( $P < 0.05$ ).

water using an electric blender. These suspensions were added to the remaining mix ingredients which had been heated to 70°C for 5 min and then cooled to 40°C. Cooling was done to avoid gelatinization. Mixes (without added starch suspensions) were heated to 70°C and held for 5 min. After heating, hot mixes (65 ± 5°C) were blended at high speed for 3 min followed by medium speed blending for 2 min to simulate homogenization. Mixes were cooled to 20°C and stored overnight at 4°C. After the addition of vanilla extract, mixes were frozen using a frozen yogurt freezer (Rival) to achieve 50 ± 10% overrun. Frozen desserts were stored at -20 ± 2°C for 24 hr before evaluation.

### Sensory Analysis

Unstructured ranking sensory analysis was conducted in the sensory laboratory by eight semitrained panelists for each panel session. Sensory sessions were conducted at least in duplicate. Before sample testing, panelists were familiarized with attribute descriptors, anchor points, and instructions relating to completion of ballots. A sample of laboratory-prepared full-fat ice cream was presented to each panelist to familiarize them with test products. Seated in individual booths and lighted with standard fluorescent lighting (630 lux), each panelist received 20-g samples of frozen desserts served in 2-oz plastic cups (Solo) coded with a random three-digit number. A ballot consisting of unmarked nine-point linear scales for each attribute, room temperature water, and crackers were provided. Panelists were allowed to evaluate each sample in any order. Samples were evaluated for smoothness, creaminess, and overall preference.

### Statistical Analysis

Sensory data were decoded using a nine-point scale and analyzed by one-way analysis of variance (ANOVA). Least significant difference (LSD) tests at  $P < 0.05$  were used to separate means when significant differences were found.

## RESULTS AND DISCUSSION

The yield of starch from wheat flour and amaranth was 46 and 20%, respectively. Protein content of the amaranth starch was 0.12%.

Light microscopy of wheat starches showed that A-type starch contained mostly large granules. The amount of damaged granules was <1%. B-type starch (prime type) consisted of small granules only. Some agglomeration of starch granules was observed. Secondary B-type starch was not pure and contained dark clumps of protein. Inside these clumps, numerous small granules of starch were present.

Scanning electron microscopy (SEM) of wheat starch showed that the size of granules was 2.0–10.0 µm (Fig. 1). Most of these

granules were smooth and round to lenticular but some granules were more angular.

The results of RVA analysis of small wheat starch granules are shown in Fig. 2. The peak viscosity was 2151.5 cP (mean value). This starch dispersion showed significant breakdown (1325.5 cP, mean value) and significant setback (1319.5 cP, mean value). The pasting temperature was ≈70.15°C. The consistency of the small granule cold paste was substantially less than that of commercial wheat starches. This may suggest that small granules swell to a smaller final size or contain less amylose than larger granules.

The size and shape of amaranth starch granules determined by SEM were similar as those described by Lorenz (1981) for starch granules of *Amaranthus hypochondriacus* (Fig. 3). The size of granules was 0.5–1.2 µm. Most of these granules were angular and polygonal, but some granules were more rounded as reported by Paredes-Lopez et al (1989).

RVA viscosities of amaranth starch dispersions (Fig. 4) were lower than those of the small granule wheat starch. The peak viscosity was 1432.5 cP (mean value). Breakdown was not as extensive as in small wheat starch granules (605.0 cP, mean value). The low setback value (209.5 cP) is due to the low content of amylose (7.2%) (Becker et al 1981). The pasting temperature was 72.75°C. RVA data are comparable with Brabender data reported by Becker et al (1981), Lorenz (1981), and Yanez et al (1986).

### Sensory Evaluation

The sensory evaluation results for frozen desserts using small granule wheat starch as the fat replacer are shown in Table II. Frozen desserts containing small granule wheat starch with fat reduced 15, 30, and 50% did not significantly differ from the full-fat control.

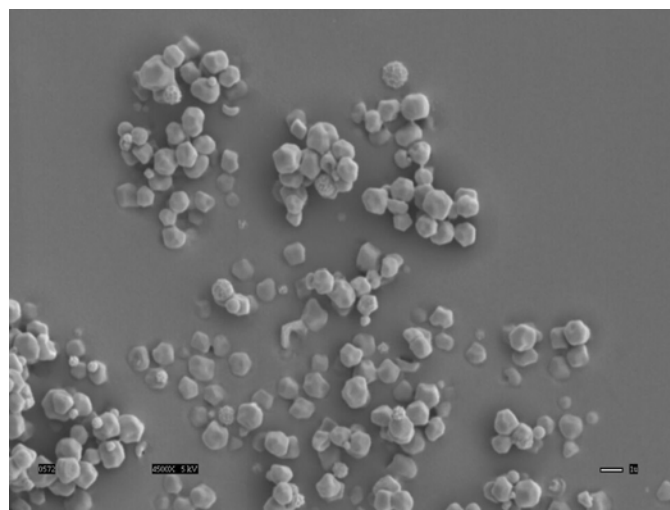


Fig. 3. SEM of amaranth starch granules.

TABLE III  
Mean Sensory Scores for Control and Amaranth Starch Substitutes, Reduced-Fat Frozen Desserts

Attribute	Fat Reduction (% by wt)			
	Control	15	30	50
Smoothness	7.6	6.8	7.5	6.7
Creaminess	7.5	7.0	7.4	6.8
Preference	7.5	6.7	7.1	6.2

TABLE IV  
Mean Sensory Scores for 15, 30, and 50% Fat-Reduced Frozen Desserts With and Without (W, W/O) Amaranth Starch as the Fat Replacer<sup>a</sup>

Attribute	15% Fat Reduction		30% Fat Reduction		50% Fat Reduction	
	W	W/O	W	W/O	W	W/O
Smoothness	6.5	6.8	7.6	5.2a	7.3	5.3a
Creaminess	7.4	7.6	7.5	7.3	7.6	6.7
Preference	7.0	6.7	7.3	6.6a	7.1	5.6a

<sup>a</sup> Within a given % fat reduction block, values followed by the same letter in the same row are not significantly different ( $P < 0.05$ ).

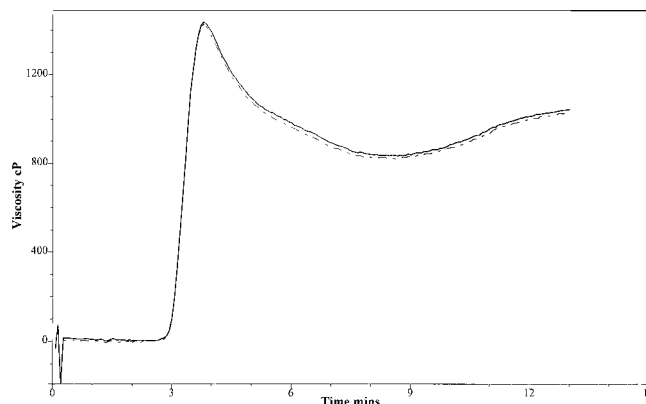


Fig. 4. RVA of amaranth starch (solid and dashed lines represent duplicate viscograms).

Sensory results indicated that the 75% fat-reduced frozen desserts failed to show acceptability.

The mean sensory scores of frozen desserts using amaranth starch as the fat replacer are shown in Table III. There is no significant difference between the four variations using amaranth starch as fat replacers in terms of the three attributes. However, the mean scores of the control frozen dessert were highest on all three attributes.

To ascertain whether the small starch granules were having any effect at all, a series of experiments were conducted at 15, 30, and 50% fat reduction. In these experiments, one frozen dessert was made using the fat replacer and an identical one was made without it. The results are shown in Table IV. This shows that at <50%, fat replacer has no effect on the smoothness, creaminess, or preference of the frozen desserts. However, at 50% fat reduction those desserts containing the small granule starch fat replacers were smoother and had a higher preference rating than the frozen desserts without the fat replacer. There was no effect on creaminess in this set of experiments even at 50% fat reduction.

The capacity of small granule starch to compensate for textural and physical properties of milk fat might be attributed to one or both of two mechanisms: small particles and colloidal properties of the carbohydrate (Specter and Setser 1994). Hydrated small starch granules may influence how the frozen mass liquefies in the mouth; slightly swollen granules lubricate ice crystals and amplify the perception of creaminess. Any starch polymers that are released through partial granule swelling may also act as hydrophilic colloids, increasing the viscosity of the continuous phase in the frozen dessert mix. Thus, the water phase can be stabilized within the starch matrix (Luallen 1994), subsequent foam formation and stability could be improved, large ice crystal formation during freezing could be restricted, the proportion of water converted into ice could be decreased, and phase separation during meltdown could be inhibited. Thus, the resulting frozen dessert could taste creamy and not watery during meltdown in the mouth.

The sensory data for both wheat and amaranth starches suggests that the 50% reduced-fat frozen desserts were acceptable by inclusion of small granule starches at a low (2%) level in the mix as fat replacers. Particle size and colloidal properties of dispersed starch may be the two factors that are important in small granule starches fat mimicking functionality. Efforts to obtain more information, such as electron microscopic study of frozen desserts, should be continued to help gain a better understanding of the functionality of small granule starches as fat replacers.

## CONCLUSIONS

Small starch granules from wheat and amaranth starches have been isolated and characterized by microscopy and viscometry. Unmodified wheat and amaranth small starch granules were utilized as fat replacers in frozen desserts and produced desserts not statistically significantly different from the control when 15, 30, and 50% of the milkfat was replaced with a 2% level of the small starch granules. At 75% fat replacement, the desserts were less acceptable than the control.

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