

# Study of Proteins Extracted from the Surface of Wheat Starch Granules with Sodium Dodecyl Sulfate

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## ABSTRACT

Wheat prime starch granule surface proteins were extracted with 1% sodium dodecyl sulfate (SDS) solution containing 1% 2-mercaptoethanol at room temperature without swelling and gelatinization of the starch granules. The extracted prime wheat starch granules showed no staining with amido black 10B, indicating no stainable proteins remained on the surface of the starch granules. SDS gel electrophoresis of the extracts stained with Coomassie brilliant blue R-250 and silver stain showed the pattern of the extracted surface protein bands. These protein bands were

also subjected to periodic acid-Schiff and Sudan black staining; however, neither glycoproteins or lipoproteins were observed. A low molecular weight band was observed in the gel and identified as lysophosphatidyl choline after electro-extraction following thin-layer chromatography. After five successive extraction treatments of the same mixture, changes in the prime starch granules became apparent under observation with phase-contrast microscopy.

The surface characteristics of wheat prime starch granules exert considerable influence on the textures of various starchy foods. The starch surface seems to play a dominant role, particularly when products are only slightly gelatinized. Few papers report research in this field (Lowy et al 1981, Greenwell et al 1985, Seguchi 1986); however, further information is needed to explain the textures of bakery products (Seguchi 1987).

Seguchi (1984a-c) reported that chlorination and heat treatment of wheat prime starch granules changes the surface characteristics from hydrophilic to hydrophobic (lipophilic) and is dependent on the changes of the starch granule surface proteins. Seguchi and Matsuki (1977) reported that in the pancake baking test using chlorinated wheat flour, the lipophilic starch granules are essential to the improvement of pancake texture characteristics such as springiness or gumminess. Furthermore, Seguchi (1986) showed that protein-specific dyes (Coomassie brilliant blue, eosin Y, amido black 10B, and fluorescamine) bound to the surface of wheat prime starch granules, which indicates the presence of proteins on the surface of the wheat starch granule. The characteristics of these surface proteins are not known. Greenwell and Schofield (1986) reported that all soft wheat possesses a prominent 15 K band in the starch granule protein extracts, and this protein plays an important role in conferring endosperm softness on wheats.

In this paper we report that most surface proteins of wheat prime starch granules could be extracted with 1% sodium dodecyl sulfate (SDS) solution containing 1% 2-mercaptoethanol and studied by SDS gel electrophoresis. The surface structure of the extracted wheat prime starch granules was shown after five successive extractions with the same solution.

## MATERIALS AND METHODS

### Materials and Reagents

Wheat flour used in this study was Alps brand (Nitto Milling Co.) from western white wheat. The protein content was 7.2% and ash was 0.39% at 12.8% moisture. Wheat prime starch granules were prepared from nonchlorinated wheat flour by the acetic acid fractionation technique described by Sollars (1958). All reagents were purchased from commercial sources.

### Extraction Method

Wheat prime starch granules (10 g) and 200 ml of 1% SDS solution containing 1% 2-mercaptoethanol (2-ME) mixed in a 300-ml conical glass flask were stirred (300 rpm) for 24 hr at room

temperature and centrifuged at 600 × g for 10 min. Supernatant was filtered and subjected to protein and carbohydrate determination by the method of Lowry et al (1951) and the phenol sulfate method, respectively. The presence of starch granule surface proteins was studied by amido black 10B staining (Seguchi 1986) of the extracted and water-washed wheat prime starch granules.

Wheat proteins (salt and water-soluble, alcohol-soluble, and acetic acid-soluble proteins) were extracted sequentially following the procedure described by Chen and Bushuk (1970).

### SDS Gel Electrophoresis

A 40-ml aliquot of the starch granule supernatant extract was concentrated to 3-4 ml by vacuum dialysis and was subjected to 15% SDS disc gel electrophoresis (Weber and Osborn 1969) and 10% slab gel electrophoresis (Laemmli 1970). SDS disc gels and slab gels were stained with Coomassie brilliant blue R-250 and silver stain (Sammons et al 1981), respectively. Molecular weights of the proteins were determined from marker proteins. Periodic acid-Schiff (PAS) staining after electrophoresis was performed by the method of Glossmann and Neville (1971). Sudan black staining was used for checking the lipoprotein bands in SDS disc gel electrophoresis (Blix et al 1940).

Electro-extraction of gel bands was performed using the method of Stephens (1975). The extracted sample was identified by thin-layer chromatography (chloroform/methanol/water = 65:25:4, v/v). Spots were made visible by I<sub>2</sub> vapor and Dittmer reagent (Dittmer and Lester 1964) spray.

### Microscopy

The extracted wheat prime starch granules suspended in water were observed through a phase contrast microscope (Shimadzu Kalnew SKP-1).

## RESULTS AND DISCUSSION

### Extraction of Surface Proteins from Wheat Prime Starch Granules

Although the presence of wheat starch granule surface proteins was confirmed by the dye-binding method (Seguchi 1986), surface proteins could not be completely extracted. In order to study the role of the surface proteins, they must be extracted without starch gelatinization. An extraction solution known to extract almost all the surface proteins without gelatinization is 1% SDS containing 1% 2-ME; this solution is generally used to extract hard-to-dissolve proteins. After the first extraction (24 hr), the prime starch granules were washed with a large volume of water and then dialyzed against water to completely exclude the SDS and 2-ME extraction solution. It is necessary to determine that the extracted proteins from the starch granule surfaces have no contaminants, and that proteins are absent from the surface of the extracted wheat prime starch granules. The extracted prime starch granules were subjected to a staining test of 1% amido black 10B

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(Seguchi 1986). They did not stain (Fig. 1B), which indicated that almost all wheat starch granule surface proteins were extracted by the extraction solution. Amido black 10B was found to stain the control sample of nonextracted wheat prime starch granules (Fig. 1A) (Seguchi 1986).

A 10-g sample of prime starch granules yielded 3.39 mg of protein and 9.62 mg of carbohydrate (Table I). Microscopic examination indicated that the extracted wheat prime starch granules did not change; therefore no gelatinization occurred (Fig. 2B), which suggested the extracted proteins came from only the starch granule surfaces. The extraction step was successively repeated five times with fresh solutions under the same conditions for a total of 240 hr (Table I). Repetition did not extract any more surface proteins (Table I). Carbohydrates were extracted by successive extractions, although the amounts did not exceed the first extraction and showed a fixed low level (Table I).

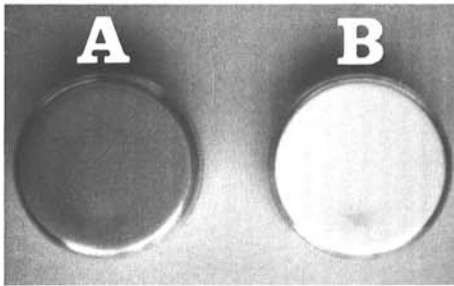


Fig. 1. Photograph of wheat starch granules before (A) and after extraction (B) that were stained with amido black 10B, washed with water, dialyzed against water, and dried in a petri dish.

### Microscopy

Changes in the appearance of wheat prime starch granules were observed throughout the extraction processes by phase contrast microscopy (Fig. 2). The uniform surface of the wheat prime starch granules (Fig. 2A and B) changed as the hilum structure appeared near the center of the starch granules after the third or fourth extraction (Fig. 2C), and further changed to a rough and patterned appearance after the fifth extraction (Fig. 2D). The presence of SDS and 2-ME could hinder gelatinization of the starch and maintain the round shape of the granules. The resulting naked starch granules may be important for future investigation of the molecular and macromolecular structures of wheat prime starch granules.

### SDS Gel Electrophoresis

The characteristics of the extracted proteins were examined by SDS gel electrophoresis of the concentrated samples. The SDS

TABLE I  
Extractions of Surface Proteins and Carbohydrates  
from 10 g of Wheat Prime Starch Granules  
with 1% Sodium Dodecyl Sulfate Solution  
Containing 1% 2-Mercaptoethanol

Washing	Total Extraction Times (hr)	Extracted Surface Proteins <sup>a</sup> (mg)	Extracted Carbohydrates <sup>a</sup> (mg)
1st	24	3.39	9.62
2nd	48	0.35	5.25
3rd	96	0.00	5.84
4th	168	0.46	8.54
5th	240	0.00	7.04

<sup>a</sup>Three replicates. Standard deviation  $\pm$  0.05.

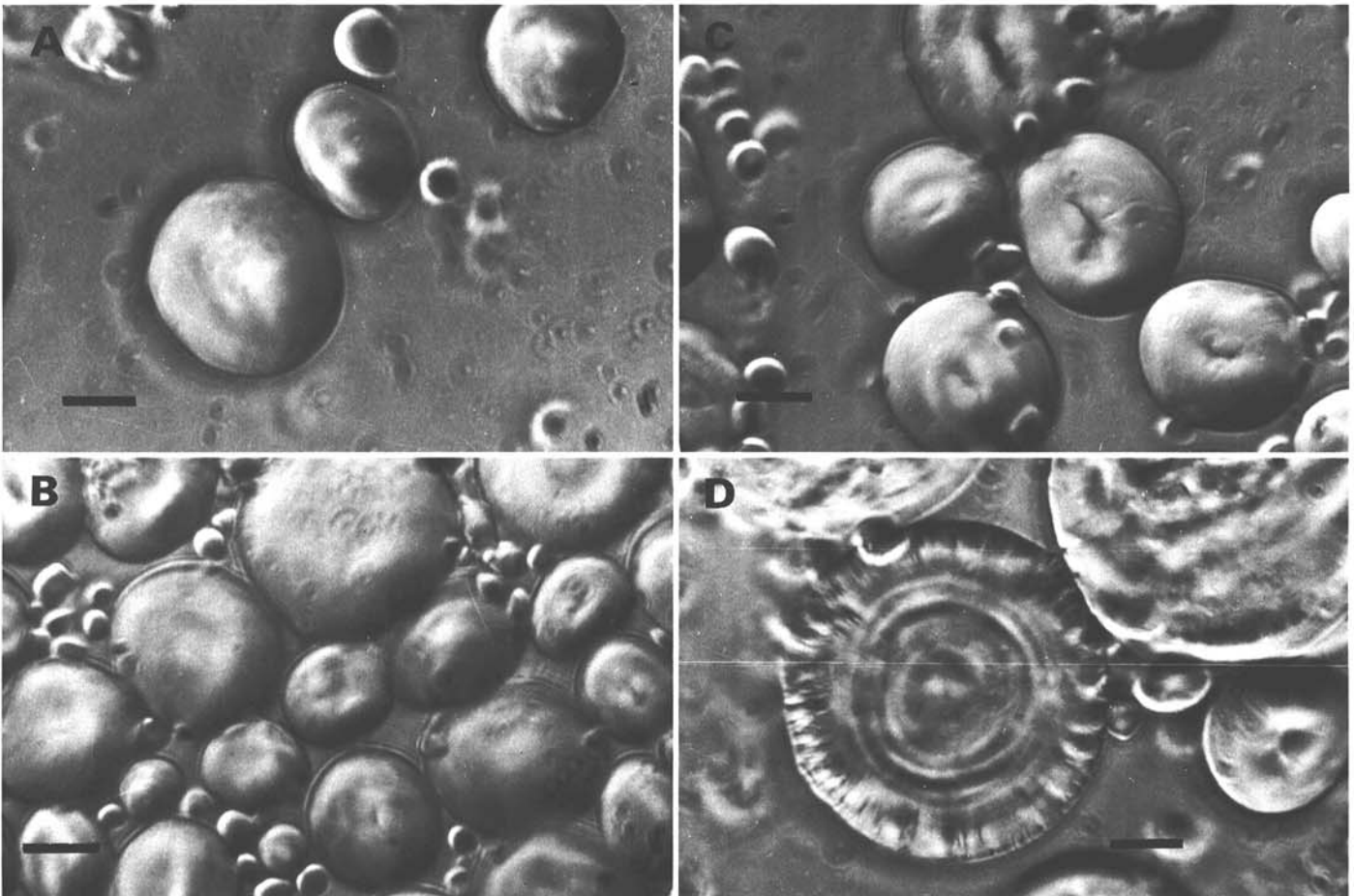


Fig. 2. Photomicrographs of wheat prime starch granules before extraction (A) and after the first (B), second (C), third and fourth (C), and fifth (D) extractions with 1% sodium dodecyl sulfate solution containing 1% 2-mercaptoethanol. The scale bar indicates 10  $\mu$ m.



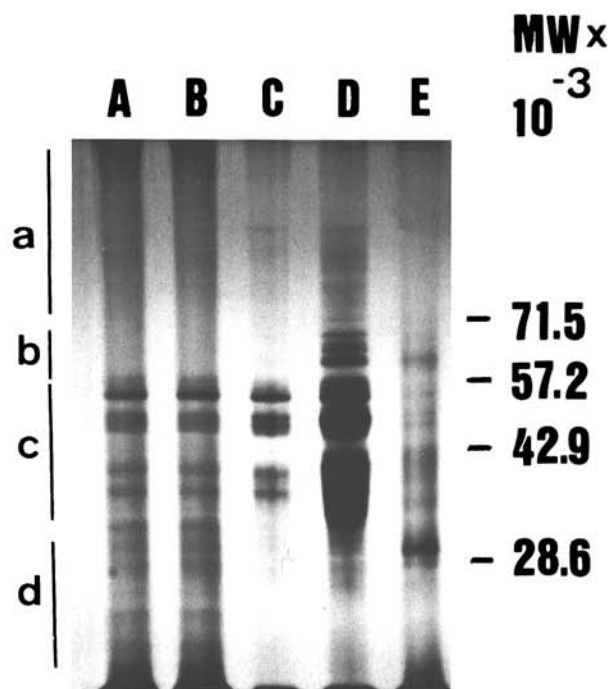
**Fig. 3.** Thin-layer chromatogram of lysophosphatidyl ethanolamine (1), electroextracted phospholipids in wheat prime starch granules (2), and lysophosphatidyl choline (3). Developed on thin-layer chromatography plate cellulose F (Merck) with chloroform/methanol/water (65:25:4, v/v) and detected by Dittmer reagent. Arrow indicates bromphenol blue.

disc gel electrophoresis (Weber and Osborn 1969) pattern was stained with Coomassie brilliant blue R-250. A broad and slight blue band appeared in the lower molecular weight side, and many other faint bands appeared in the higher molecular weight side of the gel. The broad band gradually became whitish and opaque after a few days in the destaining solution of 7% acetic acid, which might have been caused by SDS diffusion from the gel. The band was cut out of the gels with a sharp razor and electroeluted by the method of Stephens (1975). It was further analyzed qualitatively by thin-layer chromatography (chloroform/methanol/water = 65:25:4, v/v). The two resulting spots were made visible by  $I_2$  vapor and Dittmer reagent and were identified as lysophosphatidyl choline (the main spot) and lysophosphatidyl ethanolamine (the faint spot) (Fig. 3). Both phospholipids are known as complexed amylose lipids.

The Laemmli slab gel electrophoresis method (1970) with silver stain was used (Fig. 4) to aid in visualizing the samples. Many protein band patterns were made significantly more visible (via silver staining), and were then compared with proteins soluble in salt and water, alcohol, and acetic acid. As indicated in Figure 4, higher molecular weight bands (from  $M_r$  57,200 to near 30,000) of the wheat starch granule surface proteins (lanes A and B) were rather similar to those in lanes C (acetic acid-soluble proteins, mainly glutenins) and D (alcohol-soluble proteins, mainly gliadins), but lower molecular weight bands differed from lane C, D, and E (salt and water-soluble proteins) proteins and indicated unique patterns.

These protein bands were also tested with PAS and Sudan black staining; however, no clearly stained bands were observed, which suggested that these bands did not contain glycoproteins or lipoproteins.

It has been reported that chlorination and heat treatment of wheat prime starch granules change the nature of surface proteins from hydrophilic to lipophilic (Seguchi 1984a-c). If the surface proteins are extractable from chlorinated and heated starch



**Fig. 4.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of wheat starch granule surface proteins (A and B), acetic acid-soluble proteins (C), alcohol-soluble proteins (D), and salt and water-soluble proteins (E); high molecular weight glutenin subunits (a);  $\omega$ -gliadins (b);  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins; and low molecular weight glutenin subunits (c); albumins and globulins (d).

granules, studies on the characteristics of these surface proteins may be possible. The previous research on chlorinated and heat treatment of wheat prime starch granules (Seguchi 1984a-c) can be expected to progress to direct experiments.

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