

ISOLATION AND CHARACTERIZATION OF STARCH FROM MATURE SOYBEANS¹

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ABSTRACT

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Starch was identified and isolated from mature Amsoy 71 soybeans. Starch also was identified in ten different soybean cultivars. The starch from Amsoy 71 soybeans contained 15–20% amylose, was estimated to be $0.80 \pm 0.03\%$ of the dry weight, had a density of 1.51 g/ml, and had a

gelatinization temperature in the range of 73–81°C. The starch granules were smooth ovoids averaging 3 μm in length, existed as compound granules in the amyloplast, and were abundant near the center of the cotyledon but less prevalent near the periphery.

Currently, starch is thought to be present during development of the soybean but disappears during the last few days before maturity (1,2). Hence, mature soybeans would contain little or no starch. One of the few existing references describing the presence of starch in soybeans is an article by Meissl and Böcker (3). They described soybean starch granules as being smaller than the smallest rice starch granules, having a lenticular shape and existing in cells in groups or bundles of granules. The total content was estimated as less than 5%, but the maturity of soybeans studied was not indicated.

Blondel (4) reported that "it is impossible to discover, in preparation treated with iodine, the least trace of violet, blue, or black coloration." MacMasters et al (5) had never observed starch microscopically in soybeans. Bailey et al (6) reported an average of 5.6% "starch-like substance by diastase" in hundreds of analyses of soybeans, but did not describe the analytic technique. Street and Bailey (7) found the equivalent of 0.5% starch in soybeans. Aspinall et al (8) studied the polysaccharide component of soybean hulls and cotyledons and did not report any amlose or amylopectin; however, they were looking for pectic substances and the procedures used did not solubilize the starch so that it could be detected.

Boonvisut and Whitaker (9) recently estimated the amount of starch in soybeans to be 0.52% based on digestion with α -amylase.

We became interested in the starch content of mature soybeans when we consistently observed microscopically small, dense granules in wet mounts of soybean dispersions (soy milks) that had not been heated but did not observe the granules in heated soy milks. After becoming convinced that the granules were starch, we isolated and characterized the starch and estimated the quantity present. This article presents the results of these investigations.

MATERIALS AND METHODS

Soybeans

Soybeans were the Amsoy 71 cultivar produced as certified seed from the 1974 crop. Other cultivars tested were from the Iowa Agriculture and Home

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Economics Experiment Station. The commercial sample of soy flour was Archer Daniels Midland's Nutrisoy 7B.

Microscopy

Both freehand and microtome sections of soaked soybeans were examined with light and electron microscopy. Some sections were stained with iodine-potassium iodide solution (2.0 g of potassium iodide plus 1.0 g of iodine crystals dissolved in 100 ml of water), while others were observed under polarized light.

Soybean cotyledons also were fixed in 3.0% glutaraldehyde, 1.5% paraformaldehyde, and 1.0% osmium tetroxide in 0.1M phosphate buffer, pH 7.2. Tissues were dehydrated through a graded series of ethanol solutions. The tissues were embedded in a mixture of Epon and Araldite epoxy resins that were polymerized at 60°C until hardened. Sections were cut with an LKB III ultramicrotome, stained in uranyl acetate and lead citrate, and viewed with an Hitachi HS-8 electron microscope.

Starch was sprinkled onto silver-coated specimen stubs and either coated directly with carbon and then gold or treated with osmium tetroxide vapors for 1.5 hr before the coating step previously mentioned. The specimens were viewed and photographed using a JOEL, JSM-35 Scanning Electron Microscope.

Total Starch

We analyzed for total starch in the soybeans using Hassid and Neufeld's (10) method. This method involves removing sugars with 80% ethanol, extracting starch by perchloric acid, precipitating starch with iodine, and decomposing the starch-iodine complex with alkali. The soluble starch is determined colorimetrically with anthrone reagent.

Proximate Analysis

Total nitrogen was determined by a modification of the AOAC (11) micro-Kjeldahl procedure. Cupric selenite (0.2 g) was used as a catalyst in place of mercuric oxide. A Lab Con Co microdistillation unit was used to recover ammonia. Crude lipid was determined by extracting the starch in hexane in a Goldfish apparatus (12). Ash was determined by AOAC method 15.016 (11). Duplicate samples were analyzed in all cases.

Gelatinization

Gelatinization of soybean starch was observed by two different methods: loss of birefringence and optical transmittancy.

A Kofler hot stage mounted on a Balplan research microscope equipped with polarizing lens was used to observe loss of birefringence according to the method of Schoch and Maywald (13). Temperatures were recorded that corresponded to the initial loss of birefringence and to when the majority of the starch granules had lost their birefringence.

The increase in optical transmittance of a 0.1% soybean starch suspension was measured at 625 nm using a Bausch and Lomb Spectronic 20.

Amylase Treatment

The susceptibility of the starch granules to amylase attack was investigated by subjecting an aqueous suspension of powdered starch grains to α -amylase

(Sigma type 111A) and β -amylase (Sigma type 11B) for 1 hr at room temperature. The hydrolysis products were detected by thin-layer chromatography (TLC) with the procedure of DeStefanis and Ponte (14).

Amylose Content

The amylose content of soybean starch was determined by a modification of the colorimetric procedures that McCready and Hassid (15) and Wolf et al (16) described. The procedure was modified to include 90% dimethyl sulfoxide to obtain increased solubilization. Two milligrams of starch per 100-ml sample (final dilution) was used instead of the 1 mg of starch/100-ml sample to remove the interference from excess iodine. Absorbance values were measured at 625 nm. Potato and rice starch were used to verify the above procedure.

RESULTS

After being dispersed in water to make a soy milk, soaked soybeans were microscopically observed to contain small, dense granules. The granules were not observed to be present when soybeans were heated for several minutes in boiling water before dispersal. Light microscopy showed that the granules, when viewed with polarized light, gave the typical birefringence pattern (centric polarization crosses) of starch grains and that the granules readily stained dark blue when treated with iodine-potassium iodide mixture (Fig. 1 and 2). These observations convinced us that the granules were starch, and our next step was to isolate the granules for further characterization of the starch.

An isolation procedure that gave a purified starch preparation is shown in Fig. 3. After the first centrifugation, the starch layer is gray, but no obvious contamination appears when the starch grains are viewed under light microscopy. Analyses for protein and lipid, however, showed the preparation to be contaminated with approximately 20% protein and 20% lipid. Under scanning electron microscopy (SEM), the starch granules were found to be embedded in this protein-lipid matrix. Washing the starch preparation several times in dilute alkali effectively removed the protein and lipid contaminants. The starch preparation was white after alkali treatment and practically free from contaminating material when observed under SEM (Fig. 4). After the starch was dried at room temperature, analysis of the starch yielded 26.7% moisture, no protein, no lipid, and 2.4% ash. The high ash content was probably due to contaminating sodium from the alkali treatment.

For this starch granule preparation we hydrolyzed isolated starch granules by using α - and β -amylase and analyzed the hydrolysis products by TLC. As would be expected, hydrolysis with α -amylase gave glucose, maltose, and oligosaccharides, and hydrolysis with β -amylase gave glucose and maltose.

Because previous reports had noted the absence of starch in soybeans, we examined several other varieties by using iodine staining of freehand sections and light microscopy. We had no difficulty in identifying starch in all the cultivars examined—Beeson, Corsoy, Harcor, Hark, Hodgson, Hawkeye, Marion, Wells, and Woodworth. Also, starch granules were observed in commercial soy flours that had protein dispersibility index values of 87.5 to 20.7.

The amount of starch present in Amsoy 71, other tested cultivars, and flour was determined and is listed in Table I. We estimate that the mature Amsoy 71

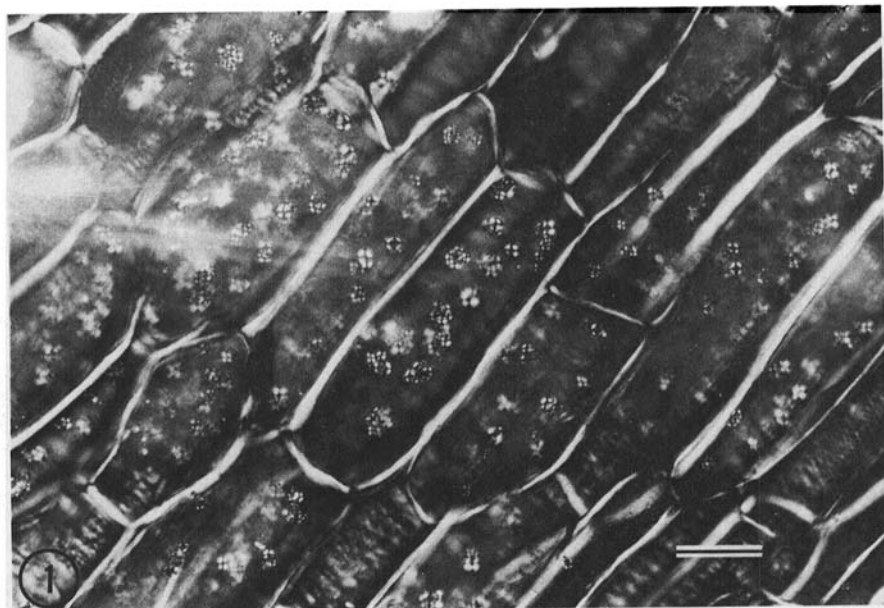


Fig. 1. Unstained bright field photomicrograph of longitudinal section of cotyledonous tissue from mature soybean viewed through crossed polarizers. Starch granules show typical birefringence pattern (centric polarization crosses). Bar = 20 μm .

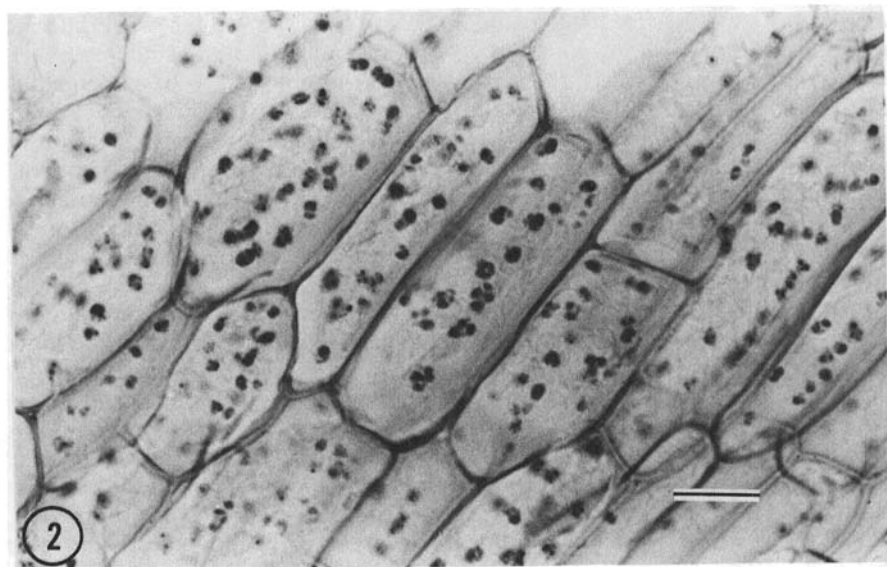


Fig. 2. Same view as in Fig. 1 after staining with iodine. Dense bodies correspond with starch granules under polarized light. Bar = 20 μm .

soybeans contained $0.80 \pm 0.03\%$ starch. Hawkeye was found to have the lowest value at 0.19% and Marion the highest at 0.91%. The soy flour was found to have values from 0.56 to 0.58%.

The Amsoy 71 granules were suspended in various mixtures of carbon

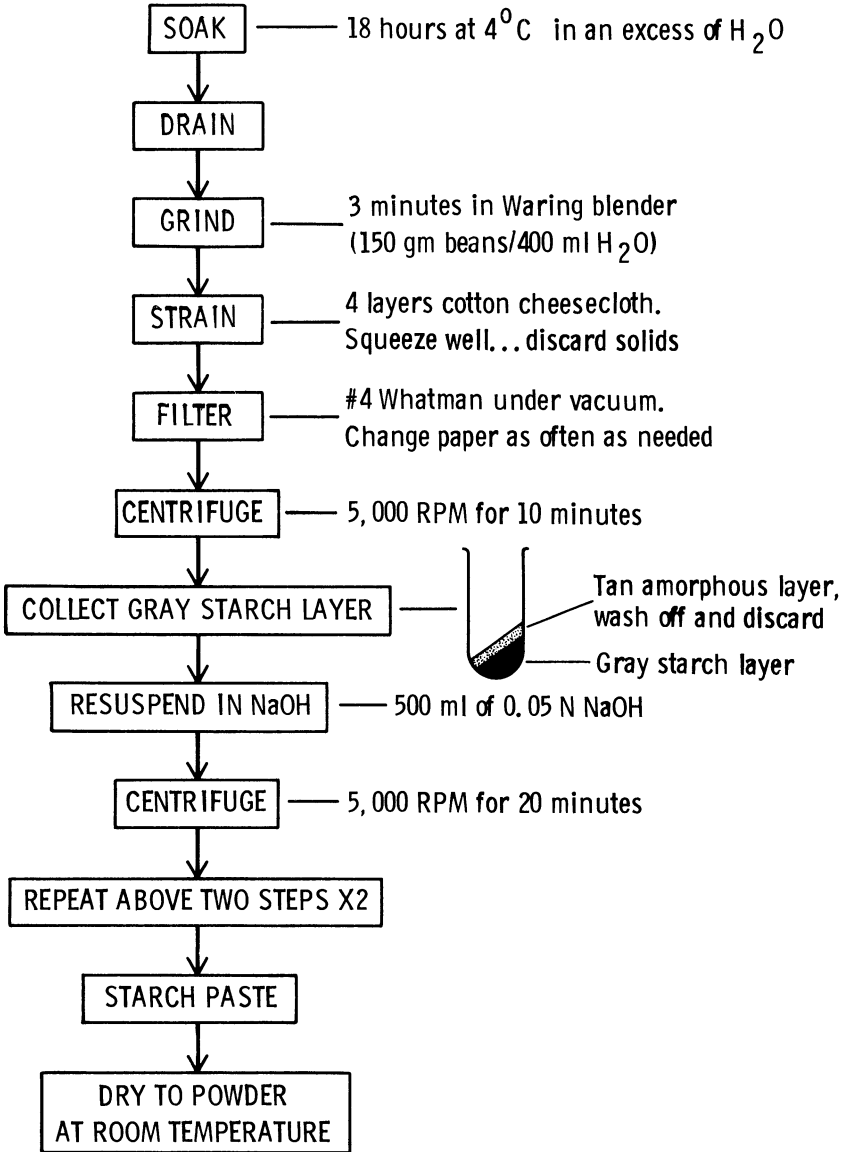


Fig. 3. Procedure used to isolate starch from mature soybeans. Washing with dilute NaOH was necessary to remove adhering protein and lipid.

tetrachloride and chloroform to determine density. The density was estimated to be 1.51 g/cc based on failure to sediment or float in the appropriate mixture after centrifugation. This is slightly higher than the density of wheat (1.473–1.476) found by Medcalf and Gilles (17).

TABLE I
Starch Content of Various Soybeans and Soybean Flours

Variety	Starch Content ^a (%)
Amsoy 71	0.80 ± 0.03
Beeson, 1976	0.36 ± 0.00
Corsoy, 1976	0.42 ± 0.03
Harcor, 1976	0.23 ± 0.09
Hark, 1976	0.28 ± 0.09
Hodgson, 1976	0.48 ± 0.01
Hawkeye	0.19 ± 0.01
Marion, 1976	0.91 ± 0.08
Wells, 1976	0.76 ± 0.00
Woodworth, 1976	0.23 ± 0.04
ADM, Nutrisoy 7B Flour, 063-130	0.57 ± 0.03
ADM, Toasted Nutrisoy Flour-40, 063-158	0.58 ± 0.02
ADM, Toasted Nutrisoy Flour, 063-160	0.56 ± 0.04

^aAverage of two samples ± difference from mean.

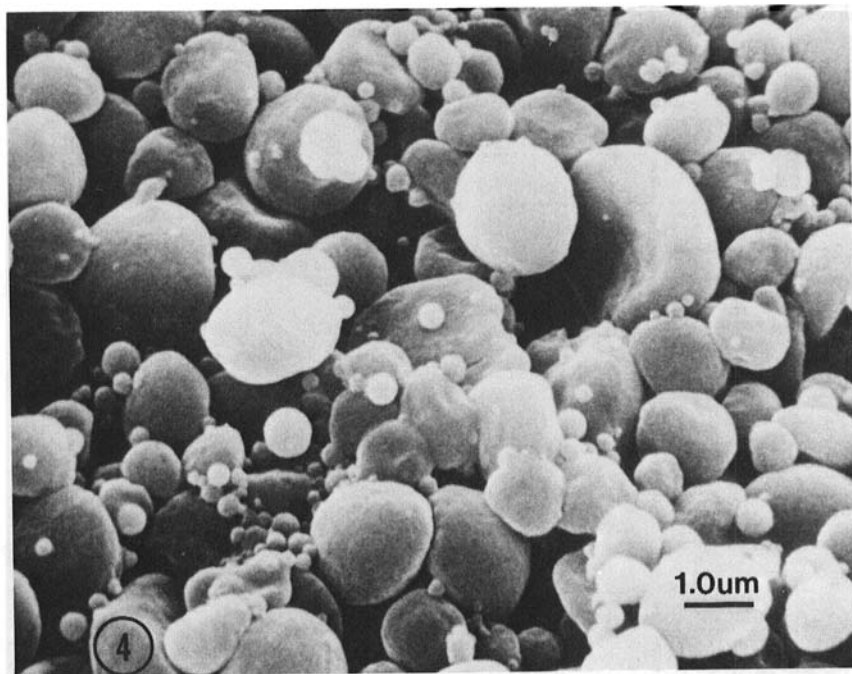


Fig. 4. Scanning electron micrograph of isolated soybean starch granules after dilute alkali treatment ($\times 7,800$).

Under the light microscope, the starch granules appeared as smooth ovoids averaging $3\ \mu\text{m}$ in length, with a range from 1 to $7\ \mu\text{m}$. SEM (Fig. 4), however, showed that some of the starch granules were $0.1\ \mu\text{m}$ in size. Although many of the granules are round to ovoid, some were observed to have one concave surface (Fig. 4). This irregularity may be due to the packing and growth of the starch in the amyloplast. Light and transmission electron microscopy showed the starch to be closely associated with one or more granules in amyloplast (Fig. 1,2,5). Under SEM, the granules were found to have relatively smooth surfaces with some slight texturing but no fissures or grooves.

The gelatinization (pasting) of isolated soybean starch is shown in Fig. 6. The light transmission was constant to 50°C and then increased as the granules began to swell. The curve shows a single-stage gelatinization from 50 to 85°C . This initial transition temperature is lower than that for rice, which also has a single transition, and wheat, which has two stages in gelatinization (18).

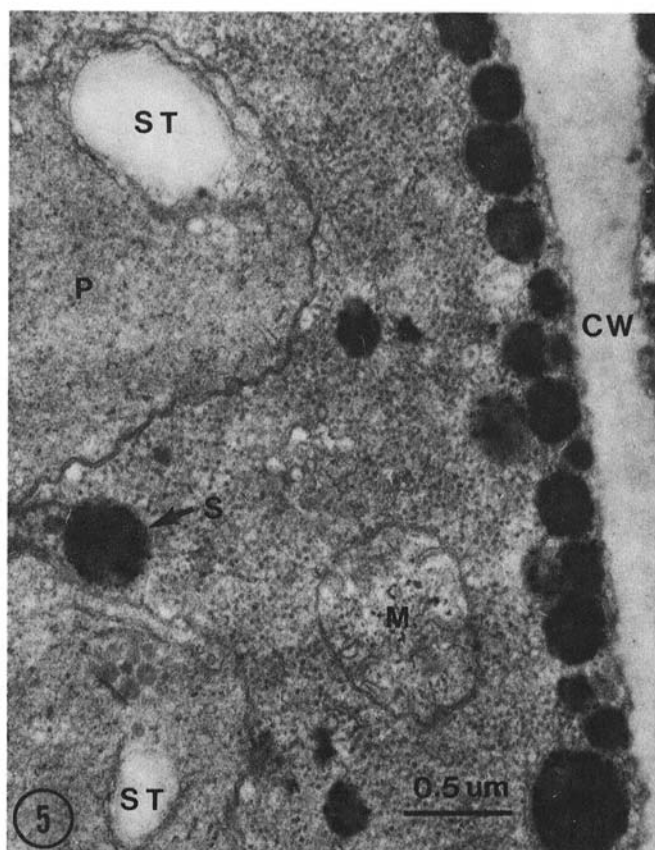


Fig. 5. Transmission electron micrograph of soybean cotyledon. Note starch (ST) in plastids (amyloplast). CW, cell wall; M, mitochondria; P, plastid; S, spherosome; ST, starch. $\times 53,460$.

Loss of birefringence was found to start at 73°C and to be completed at 81°C. Obtaining an accurate value at 50% loss was not possible due to the small size of the starch. The gelatinization temperature was taken to be that found by loss of birefringence, due to its greater sensitivity (19). The gelatinization temperature for soybean starch is thus higher than that found for potato (56–66°C), rice (61–77.5°C), chickpea (63.5–69°C), and horsebean (61–70°C) (19,20).

We compared the amylose content of isolated soybean starch with that of potato and rice starches by using the iodine-staining properties of the starch. With this procedure, the amylose content was estimated to be 15–20%.

DISCUSSION

One of the curious aspects of research on starch in soybeans is that many people have looked for it, but not everyone has found it. In examining cross

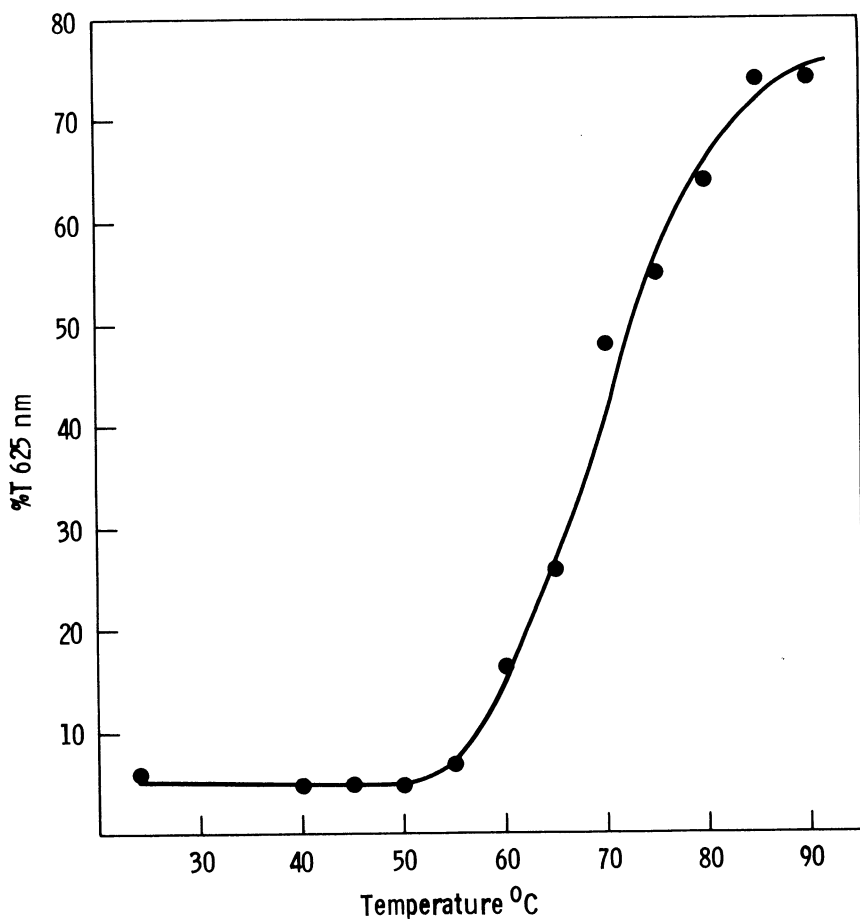


Fig. 6. Change in transluency as 0.1% isolated starch suspension was heated in water.

sections of soybean cotyledon, we learned that starch is concentrated toward the midline of the cotyledon and is practically absent near the periphery. If microscopic examination were confined to sections near the periphery of cotyledons (away from the axial midline), then the failure to find starch would be understandable.

Initially it was believed that the starch found in soybeans might contribute to the increased viscosity of heated soy milk. The small amount present, however, does not support this hypothesis.

While the presence of such a small amount of starch in soybeans has no significance to the starch industry, it may have significance nutritionally. Recently, Boonvisut and Whitaker (9) reported the presence of starch in soybeans and found increased tryptic digestion of some protein fractions after treatment with α -amylase. They speculated that starch bound to protein may be responsible for low digestibility of some soy proteins.

Acknowledgment

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