Navy Bean Flour Fractions in Composite Doughs: Effect of Bean Grade on Rheology Parameters and Microstructure of Wheat Dough[1]

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ABSTRACT

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To increase the economic feasibility of producing dry-roasted, air-classified legume flour fractions for use in composite flours, the effect of using prime and cull navy beans to produce high protein flours (fines) and cotyledon flours was investigated. These flours were used to produce wheat flour-bean composite doughs (at ratios of 95:5 and 90:10) for farinograph and low-temperature scanning electron microscopic micro-structural evaluation. Composite flours with increased protein exhibited the expected increase in absorption, delay in arrival and peak time, and reduction in stability. An exception to this was the composite flour containing 5% prime navy bean fines. The stabilities of composites containing high protein fines were greater than those containing cotyledon flour. Bean grade produced few significant differences in farinograph parameters. For composites with 10% cull fines, absorption was higher, whereas arrival and peak times were significantly shorter. Microstructures of wheat-bean flour blend evaluations differed from those of the control (100% wheat flour) for all variables. No differences in farinograph parameters due to bean grade were found for any composites containing cotyledon flour. The microstructure of composites with cotyledon flour from cull beans showed slightly greater gluten disruption than was evident in the doughs from the wheat-prime cotyledon composites.

Legume flours can be an excellent choice for improving the nutritional value of bread. The high-lysine, low-methionine content complements that of wheat flour proteins, which are poor in lysine and relatively higher in the sulfur-containing amino acids. Nevertheless, legume flours are not ideal for breadmaking, as they do not posses the important gluten-forming proteins of wheat.

Many factors affect the quality of the final product in bread baking from composite flours (Bloksma and Bushuk 1988). Quality and quantity of flour protein, level of replacement, process used to produce the legume flour, flour fraction used, water absorption, and dough-mixing characteristics are among the most important factors. Knorr and Betschart (1978) suggested that the weakening effect of foreign proteins on wheat flour doughs was the result of a dilution of the gluten structure by the added bean protein. This results in a lower loaf volume and subsequently has a negative effect on other quality attributes, such as crumb grain and tenderness.

Dough-mixing studies showed that inclusion of pinto or navy protein delayed farinograph arrival time and decreased dough stability when substituted for wheat flow in a bread system (Deshpande et al 1983). These researchers found that as the level of bean flour in composite doughs increased, farinograph absorption and mixing tolerance index increased, but mixing time and dough stability decreased. D'Appolonia (1978) reported that water

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absorption increased and doughs were weakened with the addition of navy bean flour to wheat flour doughs. Sathe et al (1981) reported that water absorption and mixing tolerance increased and dough stability was reduce when wheat flour-bean flour composites were compared to control wheat flour doughs. In baking experiments, loaf volume of breads prepared with bean protein substituted for wheat flour decreased, crust color darkened, crumb color became more gray, and crumb grain showed evidence of thickened cells. Breads containing 15% or more bean flour were characterized as having a beany and bitter flavor.

In an effort to overcome flavor and color problems of flours produced by conventional wet processing followed by drum or spray drying, Aguilera et al (1982a,b) used dry-milling techniques followed by air classification to produce four flours for use as food ingredients: a whole flour, a hull flour, a high-protein flour, and a high starch flour. Dry-roasting the beans prior to milling decreased trypsin inhibitor activity and inactivated hemoglutinins.

Silaula et al (1989) observed the effects of air-classified pinto and navy bean protein flours on the rheological and physical characteristics of white and whole wheat dough and resulting bread quality. As in previous studies, farinograph evaluation showed a progressive increase in water absorption, arrival time, and development time, while dough stability decreased with increased protein content. Although the loaf volumes of breads prepared with bean protein flours were lower than those of the control loaves, inclusion with potassium bromate and sodium stearoyl-2-lactylate in the formula partially restored loaf volume.

Even though the dry-milling process is a cost-effective method of preparing bean flours (Aguilera et al 1982a,b), substantial year variation in the price of prime grade beans makes it desirable to use cull beans (those with discolored and cracked hulls, which can also be split). These cull beans can be substituted for the prime beans only if the flours produced from them are comparable. The purpose of this study was to compare the use of cotyledon (i.e., dehulled and degermed beans) and high-protein flours produced by air-classification of cotyledon flour navy beans on the rheological properties of wheat-bean flour doughs. Low-temperature scanning electron microscopy (LTSEM) was used to relate the microstructures to the rheological data.

MATERIALS AND METHODS

Sources of wheat flour, starch, and beans are described by Lorimer et al (1991). In addition, cull beans used in this study were processed in the same manner used for prime grade beans.

Moisture contents of the wheat and bean flours were determined in duplicate using AACC Method 44-40 (AACC 1983). Nitrogen analyses were determined in duplicate using micro-Kjeldahl procedures (AOAC Methods 2.057, 14.026, and 4.068) (AOAC 1980).

Farinograph Studies

The effect of bean flour fractions on farinographs was investigated. Fines, dull fines, cotyledon, and cull cotyledon fractions adjusted to 14% mb were substituted for 5 and 10% of the weight of the hard wheat bread flour. Procedures for the farinograph studies were outlined by Lorimer et al (1991).

LTSEM

Preparation of fresh samples which have not been chemically fixed or dehydrated can be achieved by rapid freezing by submerging the sample in a cryogenic liquid, slight surface dehydration, gold coating, and examination in a scanning electron microscope. If it is desirable to examine the interior of the sample (fractured surface), the surface can be cracked using a supercooled knife. Use of the SP-2000 Sputter-Cryo system and the scanning electron microscope allows for continuous maintenance of the sample at very low temperatures and high vacuum. This helps maintain small water crystal size, which aids in decreasing potential artifacts. Preparative and examination techniques of this type are referred to as LTSEM or cyro-SEM.

Doughs used in this study were developed in the farinograph to peak maximum stage. These doughs were removed from the farinograph bowl and viewed in an SP-2000 Sputter-Cryo system in conjunction with a JEOL JSM-35C scanning electron microscope as described by Lorimer et al (1991).

Statistical Analysis

The effects of bean flour fractions on farinograph parameters were analyzed using the statistical package for IBM PC (SPSS Inc., Chicago). Duncan's multiple range test was used to determine significant differences among means.

RESULTS AND DISCUSSION

Proximate analysis of the bean flour fractions established that grade did not significantly affect flour protein content. Air classification, which was used to produce high protein flours (i.e., fines), resulted in a flour with double the protein content of the original cotyledon fraction (46 vs. 23% protein, dry weight basis).

Farinograph Studies

Farinographs of wheat and bean flour fractions composite flours showed distinct curves for each bean flour fraction. Few differences were visually evident between grades. Representative curves are shown in Figure 1.

Means and standard deviations of farinograph parameters for composite flours containing 5 or 10% levels of substitution are presented in Tables I and II, respectively. Absorption increased with increased protein. At the 5% substitution level, fines delayed arrival time significantly longer ($P < 0.05$) than the cotyledon blends, which had significantly longer ($P < 0.05$) arrival times than the control dough. No differences were found between flour systems replacing wheat flour with prime or cull fines or between those replacing wheat flour with prime or cull cotyledon fractions. The blends containing the fines had significantly ($P < 0.05$) delayed arrival times.

Peak time, often termed development time, is the time from the first addition of water to the time the dough reaches the point of greatest torque. During this phase of mixing, the water hydrates the flour components and the dough is developed. Farinographs showed that only when the prime cotyledons replaced flour in the dough system did the peak time differ significantly ($P < 0.05$) from that of the control (Table II). When substituted for flour at the 10% level, prime fines dramatically lengthened peak time. No differences in peak time were found between the wheat flour-cotyledon flour blends milled from prime or cull material at either level of substitution.

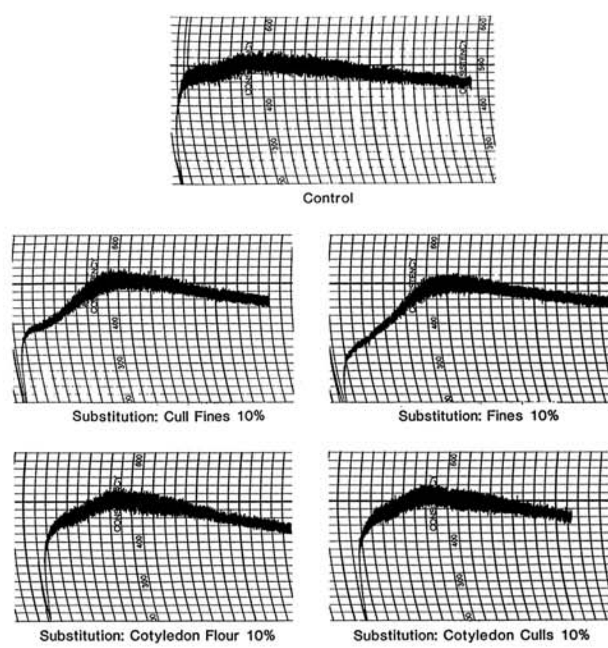

Fig. 1. Representative farinograph curves.

Departure time is a measure of when the dough has broken down sufficiently so that the curve falls below the 500 Brabender-unit line. All composite doughs except the 95-5 blend with prime fines had significantly shorter ($P < 0.05$) departure times than those of the wheat flour dough (Table I). Composites with cotyledon flour had shorter departure times than those with fines. Grade did not influence departure time except for the 95-5 composite flour with prime fines flour type. At the level of 5% substitution, no differences in stability were determined between the bread flour control and the system substituted with prime fines (Table I). Stability of wheat blends with cull fines, cotyledons, or cull cotyledons was significantly less ($P < 0.05$) than that of the control and the prime fines system. However, stability was not significantly different ($P < 0.05$) among the blends. At the 10% level of substitution (Table II), stability decreased for all blends, with the prime fines having the greatest detrimental influence on stability. No differences were found between prime or cull fine blends, nor were differences detected between blends made from prime or cull cotyledon bean flours. Differences were noted between bean flour fractions. The wheat flour-cotyledon

TABLE I
Farinograph Measurements of Doughs Substituted (at 5% level) with Navy Bean Flour (Mean ± SD)[a-d]

Flour Fraction	Absorption[e] (%)	Arrival Time (min)	Peak Time (min)	Departure Time (min)	Stability Time (min)
Control	62.3 ± 0.0 c	2.0 ± 0.0 c	5.0 ± 0.0 a,b	10.0 ± 0.0 b	8.0 ± 0.0 a
Prime fines	64.2 ± 0.1 a	3.5 ± 0.5 a	5.3 ± 0.3 a	11.8 ± 1.4 a	8.3 ± 1.9 a
Cull fines	64.4 ± 0.1 a	3.5 ± 0.0 a	5.2 ± 0.3 a	9.5 ± 0.0 b,c	6.0 ± 0.0 b
Prime cotyledon	63.6 ± 0.1 b	2.8 ± 0.3 b	4.5 ± 0.0 c	7.8 ± 0.3 d	5.0 ± 0.5 b
Cull cotyledon	63.5 ± 0.3 b	2.7 ± 0.3 b	4.7 ± 0.3 b,c	8.3 ± 0.3 c,d	5.6 ± 0.6 b

[a] Values with the same letters in a column are not significantly different ($P < 0.05$) from each other as judged by Duncan's multiple range test.
[b] $n = 3$.
[c] Farinograph values were measured to 0.1.
[d] When the standard deviations are zero, the measures from the three replicates were the same.
[e] 14% mb.

TABLE II
Farinograph Measurements of Doughs Substituted (at 10% level) with Navy Bean Flour (Mean ± SD)[a-d]

Flour Fraction	Absorption[e] (%)	Arrival Time (min)	Peak Time (min)	Departure Time (min)	Stability Time (min)
Control	62.3 ± 0.0 d	2.0 ± 0.0 d	5.0 ± 0.0 c	10.0 ± 0.0 a	8.0 ± 0.0 a
Prime fines	66.7 ± 0.1 b	4.5 ± 0.1 a	6.5 ± 0.0 a	9.2 ± 1.3 b	4.7 ± 1.3 b
Cull fines	67.2 ± 0.3 a	4.0 ± 0.0 b	5.7 ± 0.3 b	9.0 ± 0.0 b	5.0 ± 0.0 b
Prime cotyledon	65.8 ± 0.1 c	3.0 ± 0.6 c	4.5 ± 0.0 d	7.2 ± 0.3 c	4.2 ± 0.3 c
Cull cotyledon	65.6 ± 0.3 c	3.0 ± 0.6 c	4.5 ± 0.0 d	7.2 ± 0.3 c	4.2 ± 0.3 c

[a] Values with the same letters in a column are not significantly different ($P < 0.05$) from each other as judged by Duncan's test.
[b] $n = 3$.
[c] Farinograph values were measured to 0.1.
[d] When the standard deviations are zero, the measures from the three replicates were the same.
[e] 14% mb.

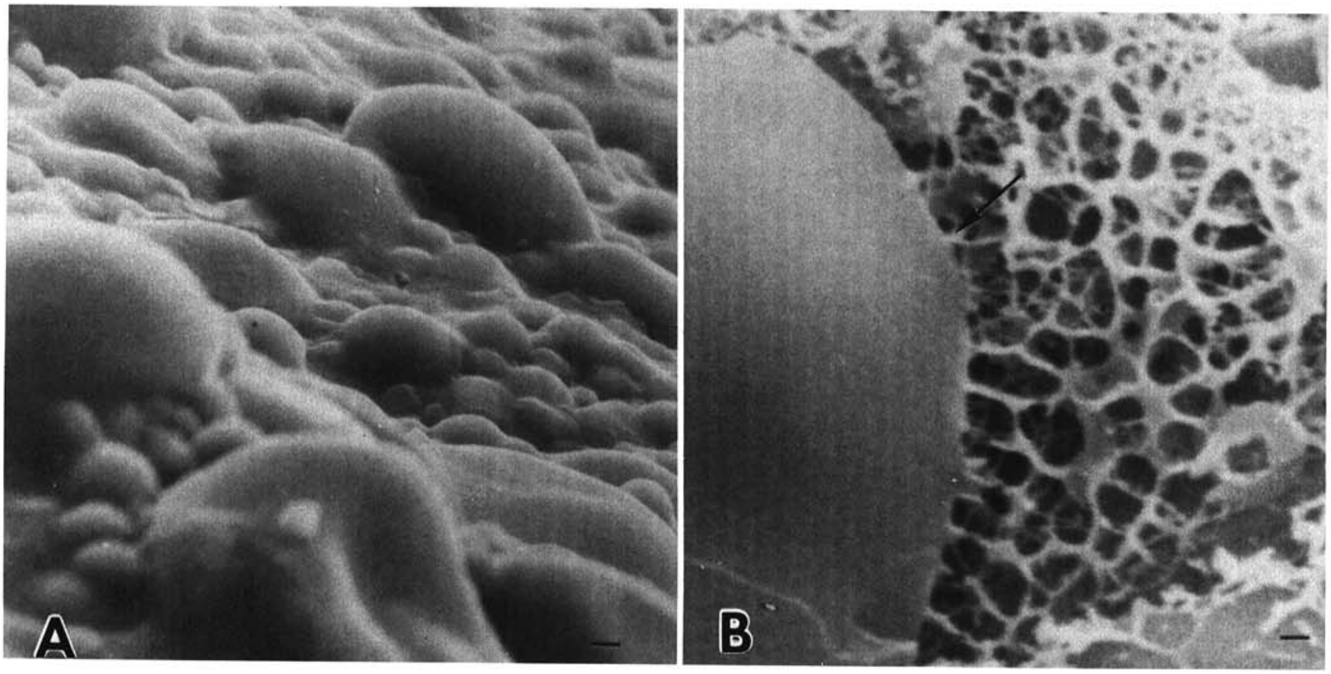

Fig. 2. Microstructure of a wheat flour dough. **A**, surface view; **B**, fractured view (arrow indicates protein network attached to starch grains). Bar = 1 μm.

blends were significantly less stable ($P < 0.05$) than their wheat flour-fine counterparts.

LTSEM Studies

LTSEM (cryo-SEM) is a relatively new technique, and few reports have been published describing its application to food products using commercial LTSEM systems such as the SP-2000 used in this study (Sargent 1988a, Beckett and Read 1989). Sargent (1988b) examined the surface morphology of intact cereal endosperms and gelatinized starch granules using LTSEM. He found that rapid freezing in a cryogenic liquid decreased the artifactural surface pitting in wheat starch granules encountered when freeze-drying was used as a fixation technique. Sargent (1988b) also examined pizza dough development using the low-temperature technique. He concluded that cryopreservation was an excellent preparative means to preserve the ultrastructure of dough samples in order to follow structural changes resulting from dough preparation and baking. Chemical fixation of dough specimens is known to alter dough structure, thus creating artifacts (Aranyi and Hawrylewicz 1969, El-Minyawi 1980). Therefore, LTSEM was a reasonable choice for observation and analysis of the microstructure of doughs prepared using navy bean flour fractions. All micrographs presented in this article are representative of the ultrastructure observed on the whole sample surface and three replicates.

The doughs obtained from wheat flour and wheat flour substituted at the 10% level with bean flour were formed by mixing to the maximum peak stage (optimum) in the farinograph. Examination of the surface view of control dough (Fig. 2A) microstructures showed starch granules continuously united by a gluten

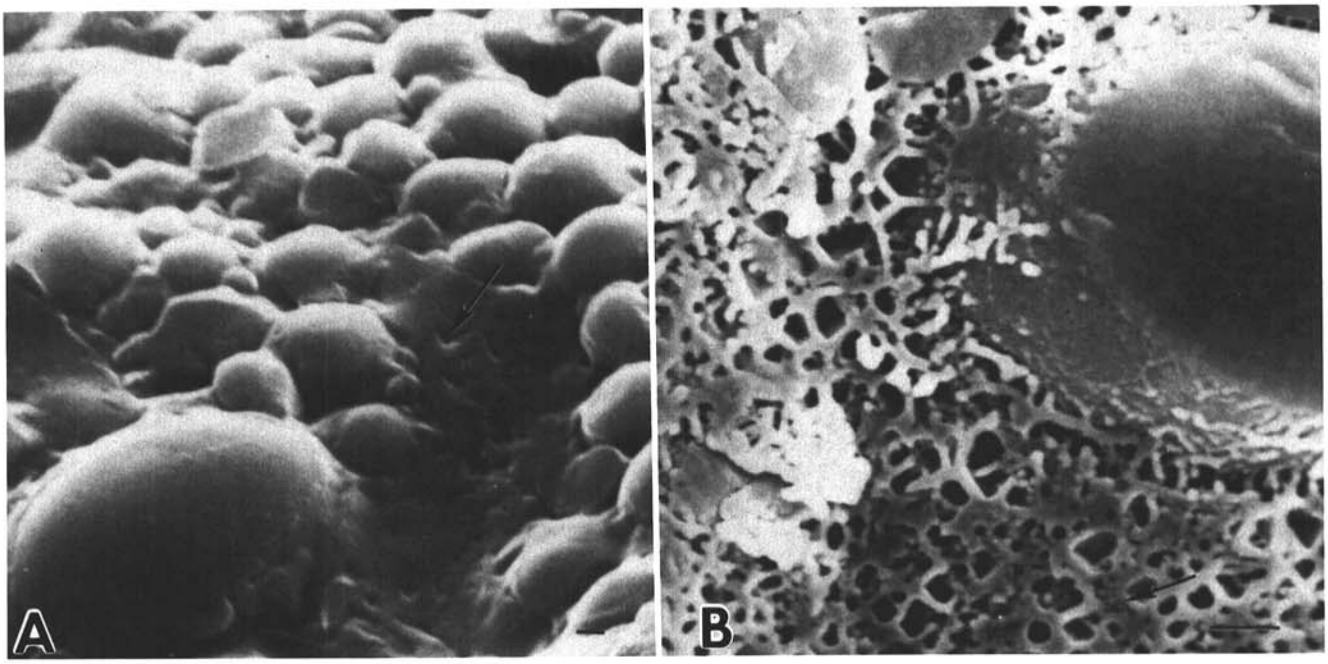

Fig. 3. Micrograph of dough from wheat flour-prime fines blend. **A**, surface view (arrow indicates pits in gluten sheet structure); **B**, fractured surface. Bar = 1 μm.

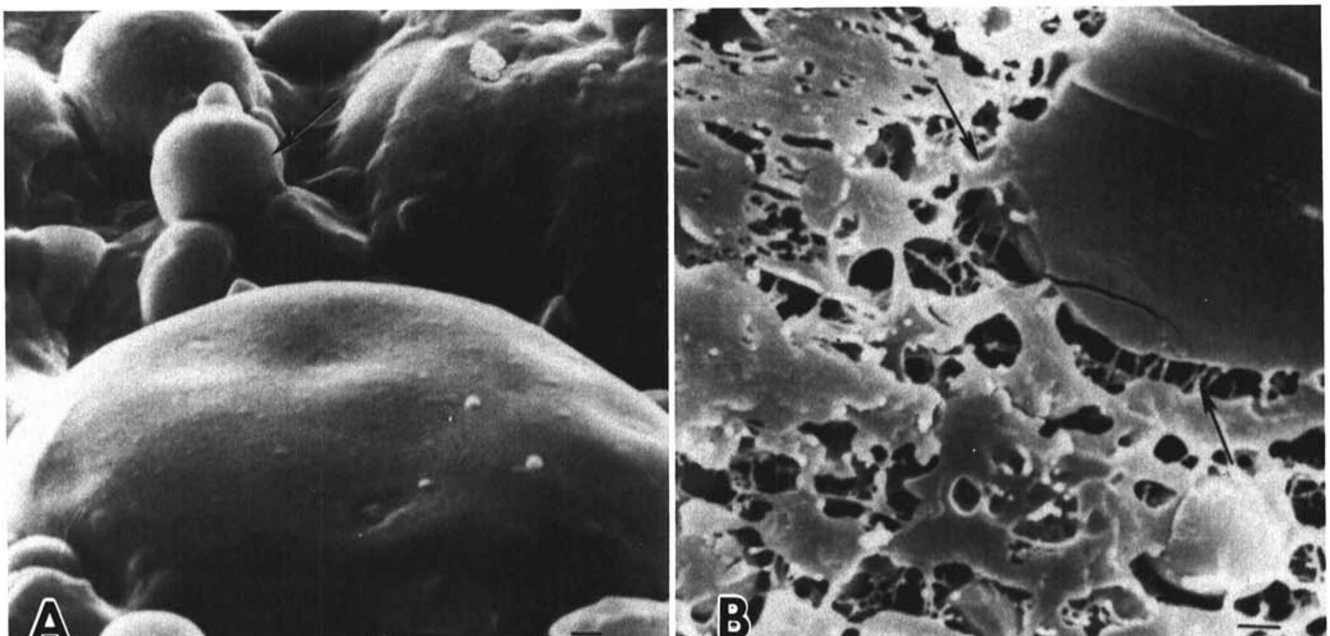

Fig. 4. Micrograph of dough from a wheat flour-cull fine blend. **A**, surface image (arrow indicates a protruding starch granule); **B**, fractured image (arrow indicates attachment of a protein filament to the starch granule). Bar = 1 μm.

coating. At a magnification of 2,000, there were no obvious pores of disruptions at the protein-starch interface. These results agreed with previous SEM microstructure descriptions of flour and water doughs (Aranyi and Hawrylewicz 1968, Evans et al 1977, Varriano-Marston 1977, El-Minyawi 1980). Because of this agreement, it appeared that the technique used to excise the dough from the farinograph bowl did not produce any more dough alteration than other reported techniques and was therefore not considered a factor when evaluating dough microstructure. El-Minyawi (1980) reported that holes and pockets found at the protein-starch interface in doughs may have been caused by the (freeze-drying sample fixation) technique. The fractured surface image (Fig. 2B) showed a fine, weblike protein network that was attached to and surrounded the starch grains. The type of microstructure observed for the control dough was considered representative of a relatively strong dough based upon the farinograph data (Table I) and was used as a reference to compare changes in microstructure and farinograph parameters in sample doughs.

The surface of the dough made from the wheat flour-prime fines blend (Fig. 3A) was not covered with as smooth a gluten sheet as that of the control (Fig. 2A). The surface was marred with pits and pock marks, which may be an artifact since this appearance was not seen in the other sample micrographs. The fractured surface (Fig. 3B) showed a network formed from thick filaments with small interspaces. The protein-starch interface was disrupted. This was obvious by a qualitative visual observation of the micrographs, which involved using the size of the 1-mm bar in each micrograph to compare relative sizes of protein strands and interspaces of the samples to the control dough. Actual quantitation of all interspaces and protein strands width for multiple samples was not determined. Prime fines significantly delayed arrival time and lengthened peak time; yet departure time occurred significantly earlier than that of the control. From these micrographs, taken at peak time, it was speculated that the areas with thin gluten coverings contribute to decreased stability.

The wheat flour-cull fines blend produced similar images (Fig. 4). The uncut surface was disrupted, with starch granules that were not evenly coated with a fine gluten sheet. There was evidence of heavy gluten coating in some areas and protruding starch granules with a very thin or possibly no gluten coverings. The cut surface image showed both thick and fine filaments surrounding and connecting the starch. The starch grains had fewer points of association with the protein than was evident in the micrographs of the wheat flour-prime fines blend. However, since no differences were found in farinograph stability values between prime and cull fine substituted doughs, this was not thought to affect the rheological data.

The surface of the wheat-cotyledon flour dough system appeared roughly similar to that of the control dough (Fig. 5). The appearance of small irregular blisters on the gluten surface covering the larger starch granules is probably not significant. The fractured surface of this dough system is similar to the structure of the wheat flour-prime fines blend sample (Fig. 3B). Protein strands appear thicker with small interspaces compared to those of the control dough micrograph (Fig. 2B). The low farinograph stability of the wheat-cotyledon flour dough system (Table II) correlates with the coarse-textured microstructure appearance.

The blend of wheat flour and cotyledon flour obtained from cull materials produced micrographs showing greater disruption of the gluten matrix than was evident in the doughs from the wheat-prime cotyledon blend (Fig. 6). The gluten sheet was thin and torn in some areas. In a few instances, starch granules protruded from the gluten sheet. The fractured surface of this composite dough also showed a thick, compact gluten network similar to that of the wheat flour-prime fines blend sample (Fig. 3B).

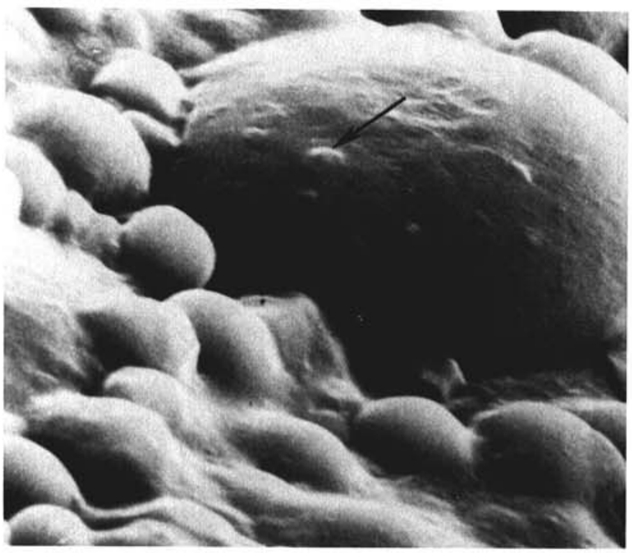

Fig. 5. Microstructure of dough from a wheat-cotyledon blend (surface image; arrow indicates blisters). Bar = 1 μm.

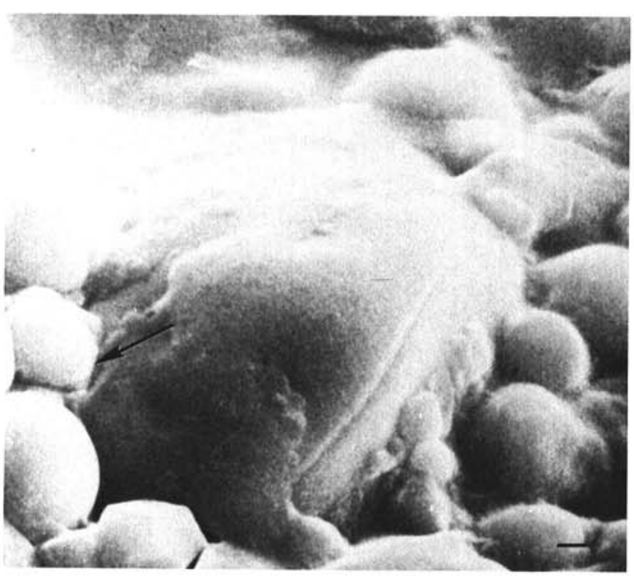

Fig. 6. Microstructure of a dough from a wheat-cull cotyledon blend (surface image; arrow indicates a protruding starch granule from the gluten sheet). Bar = 1 μm.

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