Wheat Hardness. I. A Method to Measure Endosperm Tensile Strength Using Tablets Made from Wheat Flour[1]

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ABSTRACT

To obtain a direct measure of tensile strength of reconstituted endosperm from common wheat (*Triticum aestivum* L.), tablets were made by compressing flour that contained 28% moisture. Those tablets were then dried under controlled relative humidities. Dried tablets were fractured in diametral compression to measure tensile strength. As expected, tablets made from hard wheat flour had greater tensile strength than those made from soft wheat flour. In tablets made from hard or soft wheat flours, contact between starch granules and the surrounding protein phase was visually similar to that of typical hard or soft wheat endosperms. A hard and a soft wheat flour were fractionated into starch, gluten, and water solubles. These fractions were reconstituted into doughs, which were dried and ground. Tablets made from these reconstituted flours had tensile strength similiar to that of tablets made from the parent flours. Factors influencing tablet tensile strength remained active through the processes of storage, milling, fractionation, reconstitution, and tablet making.

In common wheat (*Triticum aestivum* L.) endosperm texture (hardness) is usually identified as hard or soft. Endosperm texture can be characterized by causing mechanical failure of endosperm under the four basic kinds of stress: tension, compression, shear, and bending. The process of milling wheat probably involves all of these stresses. During milling, hard wheat behaves differently from soft wheat: Hard wheat requires more force to fracture kernels, maintains larger particles through the milling process, passes through sieves more easily, and typically yields flour that contains more damaged starch.

The mechanism by which endosperm texture is expressed is unknown. Literature reviews on possible mechanisms for phenotypic expression of endosperm texture have been written by Simmonds (1974), MacRitchie (1980), and Hoseney et al (1988). Endosperm texture in common wheat is mainly controlled by one major gene (Symes 1965, 1969), with other genes modifying its influence (Symes 1965, Yamazaki and Donelson 1983, Law and Krattiger 1987). Environment during growth has only a minor influence on endosperm texture (Miller et al 1984, Pomeranz et al 1985).

Starch granules isolated from hard and soft wheats were equal in their resistance when pierced by a micropenetrometer (Barlow et al 1973). Endosperm protein fragments from hard and soft wheats were also equal when tested by that technique. The matrix protein was about 20% harder than the starch granules in both hard and soft wheats. Therefore, differences in endosperm texture are not caused by starch granules alone or by the protein matrix alone. The remaining logical explanation is that, in hard wheat, matrix protein adheres more strongly to starch granules (Barlow et al 1973).

To study factors causing endosperm hardness in corn, sorghum, and millet, Abdelrahman and Hoseney (1984) used tablets made from flour. Those tablets were laid flat and compressed until they crumbled.

Adhesion between matrix protein and starch granules is assumed to be measured by endosperm tensile strength. Procedures involving the use of tablets to measure tensile strength in pharmaceutical research are well established (Rudnick et al 1963, Shotton et al 1976; Ejiofor et al 1986). A round tablet is placed on its edge, then compressed between parallel, flat surfaces until fracture occurs. The maximum load sustained is a measure of tensile strength.

The objectives of this study were to first develop methods to make reproducible tablets from reconstituted wheat endosperm and, second, to determine whether the tensile strength of the tablets reflected the hardness of the wheat from which the flour was milled.

MATERIALS AND METHODS

Flour

Three flours were used in this study. Logan soft red wheat (SRW) was milled in the Kansas State University (KSU) pilot mill to straight-grade flour. A composite of hard red wheat (HRW) was milled to straight-grade flour, and Newton HRW was milled to a patent flour on Ross experimental mills in the KSU milling laboratory (Table I).

Making Tablets

The flour was hydrated to 12% moisture by placing it in an aluminum petri dish and holding it for one hour at 100% relative humidity (rh). The top edge of the dish had been squared and smoothed. The inside surface of the dish had been smoothed.

The flour was showered with water mist generated by a common utility spray bottle with the nozzle set to produce the finest mist. A mist shield was used to keep water from the petri dish, minimizing adhesion of flour to the dish. The flour was misted and then mixed by hand. This cycle of misting and mixing was repeated to achieve 27.9% moisture.

The round mist shield was made of anodized aluminum 0.92 mm thick, with an outside diameter of 137 mm and a round, 59 mm-diameter hole in the middle. This outside diameter was small enough to fit inside the weighing chamber of the balance that was used (Mettler Instrument Corp., model AE 100, Hightstown, NJ). The 59 mm-diameter hole was small enough to shield the sides of the petri dish from falling water mist and large enough to expose an adequate area of flour. On the bottom of the mist shield, four small pieces of aluminum were attached with epoxy glue in order to position the petri dish in the middle of the shield. The hole in the shield was covered with a round,

TABLE I
Flours Used to Make Tablets[a]

Characteristic	Logan SRW[b]	Composite HRW[c]	Newton HRW[c]
Extraction	...	69	34
Protein (N × 5.7)	10.5	≈14	11.5
Ash	0.62	0.44	0.56
Fat	1.2	...	1.3
Crude fiber	0.23	...	0.23

[a] Analytical values are reported on a dry basis.
[b] Soft red wheat.
[c] Hard red wheat.

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77 mm-diameter piece of glass 2.2 mm thick, which had a handle formed by applying ≈0.5 cm^3 of silicone rubber adhesive in the center on one side. The mist shield was attached to the top of the petri dish using adhesive filament tape. The tape was kept away from the top of the mist shield, so that water droplets were easily wiped off the top surface.

The mist shield was replaced with a dispensing lid that minimized moisture loss during transfer of the damp agglomerated flour. The round dispensing lid was made of Plexiglass 2.95 mm thick and had a dispensing hole 12.7 mm in diameter centered 19.9 mm from the outside edge. A rubber stopper plugged the hole. When the dispensing lid was positioned on the petri dish, the part of the rubber stopper that protruded on the underside of the lid was positioned to touch the inside edge of the petri dish at one point. On the top exterior side of the lid, the dispensing hole was surrounded by a silicone rubber lip molded to match the top edges of the 10-ml beakers that held the damp flour.

For each tablet, 0.560 g of the damp flour was weighed into a 10-ml beaker. Moisture loss was minimized by sealing the beaker with Parafilm M. These subsamples were left undisturbed for 2 hr.

The tablet-forming die of hardened steel had an inside diameter of 12.7 mm, a length of 28.5 mm, and a Teflon coating on the inside. The Teflon coating was DuPont's 958-203 Teflon S primer with DuPont 856-200 FEP top coat (8-403, Precision Coating, Dedham, MA). The Teflon surface was smoothed with a series of abrasives, then polished with jeweler's rouge. The smooth finish on the inside of the die allowed tablet-forming pressure to be maintained without a major portion of the plastic tablet material being forced out from the tablet area to the space between the die and the dowels. Two hardened steel dowels (55 mm long) were exactly sized to slide freely through the die (≈0.0254 mm clearance). Each dowel was held at 90° to a flat, abrasive surface when the face was formed. The faces of the dowels were coated with 0.011 mm-thick Poly-Ond—nickel and phosphorus embedded with Teflon (Poly Coatings of the South, Sarasota, FL)—then polished with 3-μm silicon carbide abrasive disks.

The dowels were used to apply the pressure necessary to form the tablets. Before damp flour was put in the die, the lower dowel was inserted into a loose-fitting cylinder 42 mm high, and the die was placed on top of this cylinder with the dowel positioned 13 mm into the die. The damp flour was quickly transferred into the die through a smooth aluminum funnel.

The upper dowel was immediately started into the die, and the die was positioned in the press. The tablet was formed with a hydraulic laboratory press (Carver). For ≈20 sec, the load on the die was gradually increased from 0 to 272 kg, which equaled ≈21,100 kPa. This load was maintained for 7 min, then gradually released during ≈20 sec. The compressed, damp flour had a plastic consistency, indicating that equal pressure was exerted at all points in the tablet during compression.

The tablet was removed from the die. Care was taken at all times to avoid unnecessary stress on the flexible tablet. The upper dowel was released from the tablet with only twisting force, never with pulling force, and then the lower dowel was slowly pushed through the die, pushing out the tablet. The tablet was cut from the lower dowel with a razor blade.

Translucence was observed while the tablet was standing on its edge. A penlight was used to shine a very bright light through the tablet. The tablet was flattened by laying it bottom-side-down on a flat surface that had been covered with Teflon. The face of the upper dowel was placed onto the tablet; an additional 617 g of weight was applied and left for 10 sec. No attempt was made to remove any slightly protruding edges of the tablet that typically resulted from a slight oozing of material between the die and the dowels. The tablet was weighed in a weighing bottle and placed in a 100% rh environment until the drying process began.

Typically, eight tablets were dried together by placing them bottom-side-down on a flexible nylon screen of 16 × 18 strands per 2.54 cm (commonly used in window screens). The nylon screen had a diameter of 7 cm with a 2-cm hole in the middle. Under the nylon screen was a stiff screen made of high-density polyethylene with 3 × 3 strands per 2.54 cm (Cole-Parmer Instrument Co., catalog J-9403-30, Chicago, IL) and a diameter of 7 cm. One stiff screen always stayed in each drying container, while another traveled with each set of eight tablets as they were moved to different drying containers. Tablets were moved by attaching a locking forceps to the traveling stiff screen through the hole in the flexible screen. The tablets were weighed with their pair of screens.

The screens were placed on a plastic cylinder ≈6.1 cm in diameter, ≈8.7 cm high, cut from a 250-ml, linear polyethylene bottle, with a space of ≈2.2 cm between the top of the cylinder and the top of the drying container. Holes of ≈13 mm diameter were drilled in the cylinder to allow free circulation of the drying solution.

Tablets, screens, and the supporting cylinder were placed in a wide-mouth pint jar containing a saturated solution of KNO_3 (≈3 cm below the screens) to maintain ≈93% rh. The jar lid was closed tightly, the solution was stirred with a magnetic stirring bar at 120 rpm for 15 min, and then the jar was left undisturbed for 24 hr.

Every 24 hr, tablets were weighed together, turned over, and turned one third of a revolution clockwise on their axes. Every 24 hr, this one-third revolution was alternately clockwise or counterclockwise. After the first 24 hr of drying, tablets were put in a second, identical drying container, except this one contained saturated KCl to maintain 84% rh. Every time the tablets were put in a drying container, the jar lid was closed tightly, the solution was stirred at 120 rpm for 15 min, and then the jar was left undisturbed for 24 hr.

After the second 24 hr of drying, tablets were put in a third drying container with a saturated solution of NaCl to maintain 75% rh, which was used until the tablets had been dried a total of 120 hr. Moisture loss was typically slow in 93% rh, rapid in 84% rh, then moderate in 75% rh, approaching equilibrium after three days in 75% rh (Fig. 1).

Relative translucence was observed from both sides of the tablets, using a light box. Each tablet had equal lighting and viewing conditions.

Each tablet was weighed and then viewed through a dissecting microscope (StereoZoom 7, Bausch & Lomb, Rochester, NY) to look for internal cracks. Shining a bright light from various angles and diffracting the beam with a pencil or similar object revealed internal cracks in the tablets under the microscope. The locations of any cracks were marked with a pencil. The diameter and thickness of each tablet were then measured.

Tablets were broken by diametral compression, using a universal testing machine (UTM) (Instron, Canton, MA) set to a

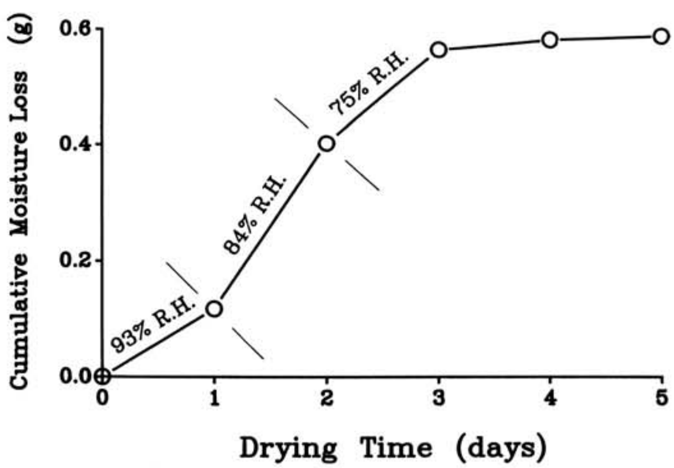

Fig. 1. Drying eight tablets. Before drying, this set of tablets had a total mass of 4.3836 g and ≈27.9% moisture, giving a calculated moisture of ≈16.6% in the dry tablets.

crosshead speed of 2.5 mm/min. Each tablet was placed on its edge and centered between a smooth, flat base and a smooth, flat face on a 12.7 mm-diameter aluminum rod. A tablet was positioned using two squared pieces of 9 mm-thick Plexiglass. Any internal cracks were positioned away from expected fractures as much as possible. Fractures were typically vertical, running between the contact points, with one wedge nearly parallel to the main fracture and extending almost to the middle of the tablet (Figs. 2 and 3). Any visibly flattened edge of a tablet was not used as a contact point. After a load of ≈2 kg had been applied, the crosshead was stopped, the Plexiglass supports were removed, and the top and bottom of the tablet were labeled with pencil, according to the tablet's position in the UTM. Crosshead motion was then resumed until fracture occurred. When the tablet had broken, the fracture pattern was sketched, and all tablet fragments were collected and stored. UTM load vs. distance compressed was a smooth, slightly s-shaped curve before the fracture (Fig. 4).

Tensile strength (which is independent of tablet diameter and thickness) was calculated from the breaking load according to the following formula (Rudnick et al 1963):

$$\text{maximum tensile stress} = \frac{2 \times \text{load}}{\pi \times \text{diameter} \times \text{thickness}}$$

Fractionation of Flour

Flour was fractionated using modifications of the procedure of Rogers and Hoseney (1989). Flour (250 g) was added to distilled water (750 ml) in a gallon jar, then mixed constantly for 10 min by hand. The mixing process was shaking and inverting the jar, with intermediate vigorous shaking. The slurry was centrifuged at 1,000 × *g* for 20 min, and the supernatant was shell-frozen and lyophilized at ≈100 mtorr. The centrifugate was mixed at 34 rpm for 4 min in a pin mixer (TMCO National Mfg. Co., 450-g model, Lincoln, NE). Starch was washed out of the mixed centrifugate with small amounts of water. The centrifugate was lightly squeezed with the back of a rubber-gloved hand until the wash water was slightly cloudy. Cloudy wash water was poured into a container cooled in an ice-water bath. After a time, the remaining gluten required moderate massaging before the wash water became cloudy. Washing continued until the wash water did not become cloudy with vigorous massaging of the gluten. Gluten washing required ≈2 hr. The gluten was separated into several pieces ≈2 cm in diameter, frozen in a glass jar at −22°C, and lyophilized at ≈100 mtorr.

The starch fraction was centrifuged at 1,000 × *g* for 20 min, then washed twice by being slurried with water and centrifuged at 1,000 × *g* for 20 min. After being slurried with a minimum of water, the starch was shell-frozen and lyophilized at ≈100 mtorr. The proportions obtained for all fractions and flours are shown in Table II.

Reconstitution of Flour

The gluten was ground in a porcelain mortar and pestle by hand until particles passed through a 150-μm (100-mesh) sieve. Frequent sieving ensured that particles were not subjected to grinding after they were small enough to pass through the sieve. This was done with the intent to minimize starch damage. The starch fraction and gluten were brought to 10.8% moisture by exposure to air with 100% rh. The water solubles were free-flowing powders and were assumed to have 10% moisture. Fractions were combined in the proportions obtained from the parent flours (Logan SRW and the composite HRW, Table II). The powders were dry-blended, then mixed in a 10-g mixograph, using 8.2

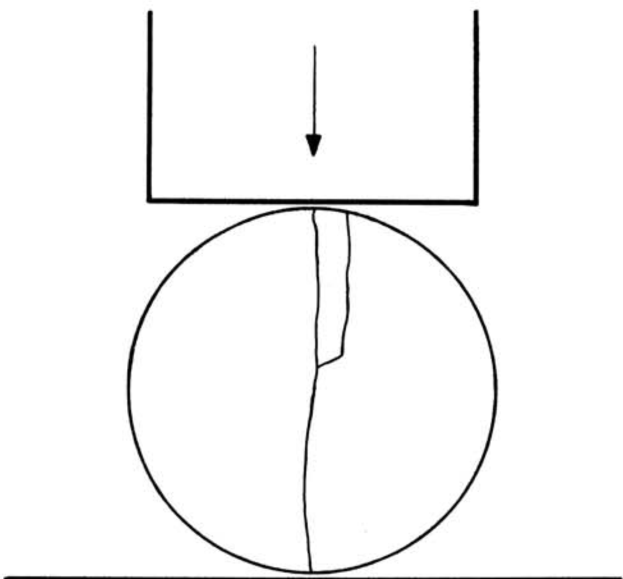

Fig. 2. Diametral compression to break a tablet using a universal testing machine.

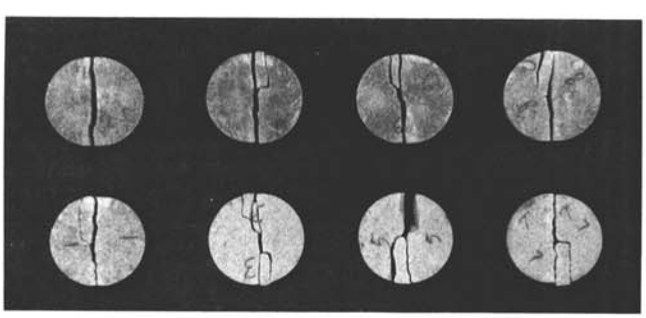

Fig. 3. Tablets made from hard wheat flour (top) or soft wheat flour (bottom). Fragments are in the same orientation as they were when the tablets were broken.

TABLE II
Flour Fractions Used to Make Tablets[a]

Fractions	Logan SRW[b]	Composite HRW[c]
Starch	88.9	85.7
Gluten	7.0	10.0
Water-solubles	4.1	4.3
Total	100.0	100.0

[a] Analytical values are reported on a dry basis.
[b] Soft red wheat.
[c] Hard red wheat.

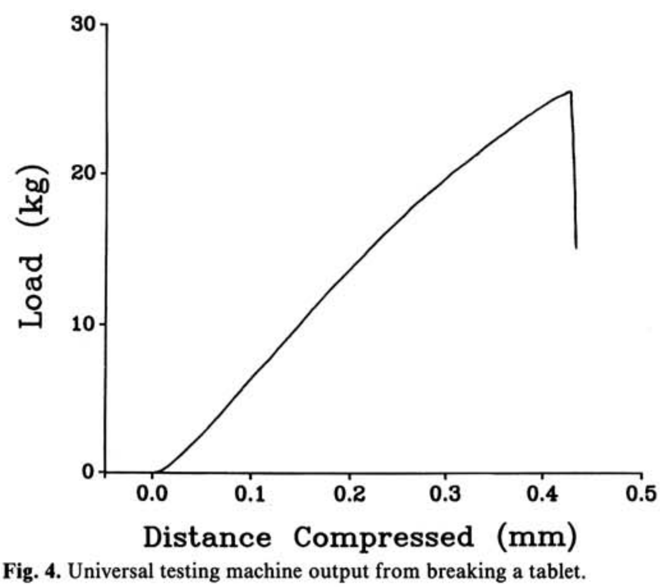

Fig. 4. Universal testing machine output from breaking a tablet.

g of powder and 14% moisture basis (mb), with ≈60% baker's absorption (percent of flour weight, 14% mb), to form dough that was similar to an optimally mixed bread dough. The dough was dried to a constant weight (by being divided into pieces of ≈0.33 g and subjected to a 37°C air current), ground in the same way as the gluten, and hydrated to 12% moisture by exposure to air with 100% rh.

In some preliminary experiments, tablets were made from flour fractions that were dry blended but not mixed into a dough. However, in these experiments gluten particles were distinctly visible when the dried tablets were viewed through a dissecting microscope and had obviously not made the desired contact with starch granules. Therefore, the formation of a dough was used in all other reconstitutions reported in this study.

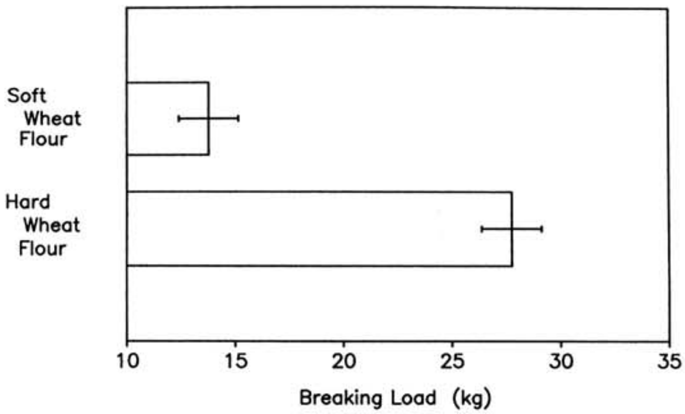

Fig. 5. Tensile strength of tablets made from hard wheat flour or soft wheat flour. Each horizontal bar shows the mean from four tablets in one experimental unit. Error bars show the 95% confidence interval for replicate experimental units.

RESULTS AND DISCUSSION

Hard Wheat Flour and Soft Wheat Flour

The first question to answer was whether flour from hard kernels could be made into tablets that had a high tensile strength. Tablets made from the composite hard wheat flour did have a greater tensile strength than tablets made from soft wheat flour (Fig. 5). Tablets from Newton flour or from the same Logan flour gave results nearly identical to Figure 5. In all cases tablets made from hard wheat flour had significantly greater tensile strength than tablets made from soft wheat flour.

The 95% confidence interval for replicate experimental units was obtained by pooling the sums of squares from later treatments that had little or no effect on tablet tensile strength. Therefore, the estimated standard deviation of the breaking load, 0.60 kg (df = 8), might be too large because small, potential treatment effects were treated as if they were experimental error. The estimated standard deviation of tensile strength was 0.86 kg/cm^2 (df = 7). Typically, tablets that retained more translucence during drying also shrank ≈30% more during drying. Translucent tablets typically shrank from 12.7 mm to ≈11.8 mm in diameter; opaque tablets typically shrank to ≈12 mm in diameter.

A tablet made from hard wheat flour had a fractured surface, showing that gluten remained in contact with the starch granules after drying (Fig. 6). This resembled the appearance of hard wheat endosperm (Hoseney and Seib 1973). Similarly, in a tablet made from soft wheat flour, gluten did not remain in contact with the starch granules after drying (Fig. 7), resembling the appearance of soft wheat endosperm. These results showed that this procedure for making tablets was suitable for use in the search for components of wheat flour that influenced endosperm texture.

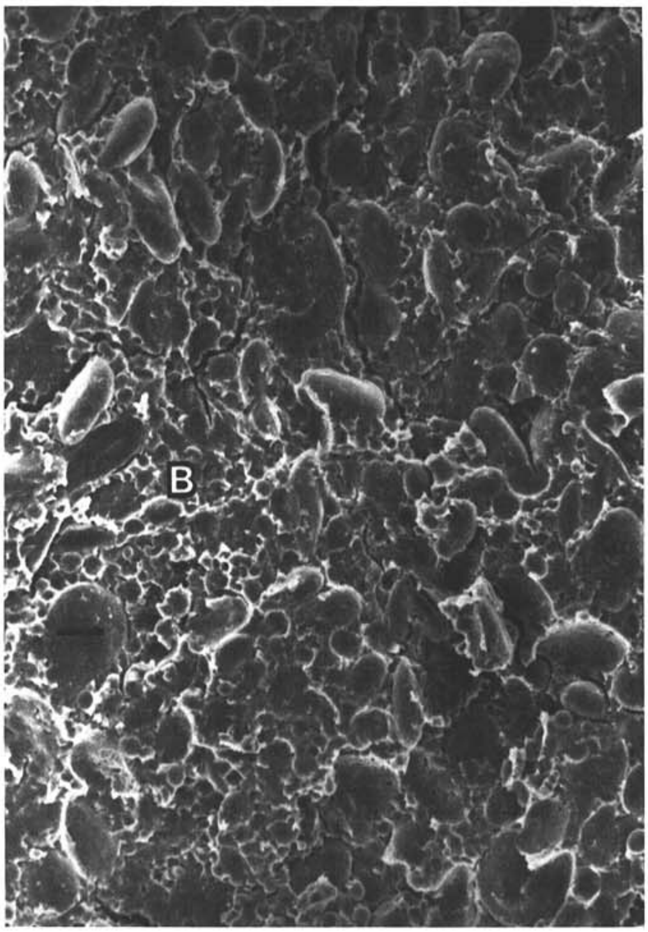

Fig. 6. The fractured surface of a tablet made from hard wheat flour. Note the broken starch granule (**B**). Bar = 10 μm.

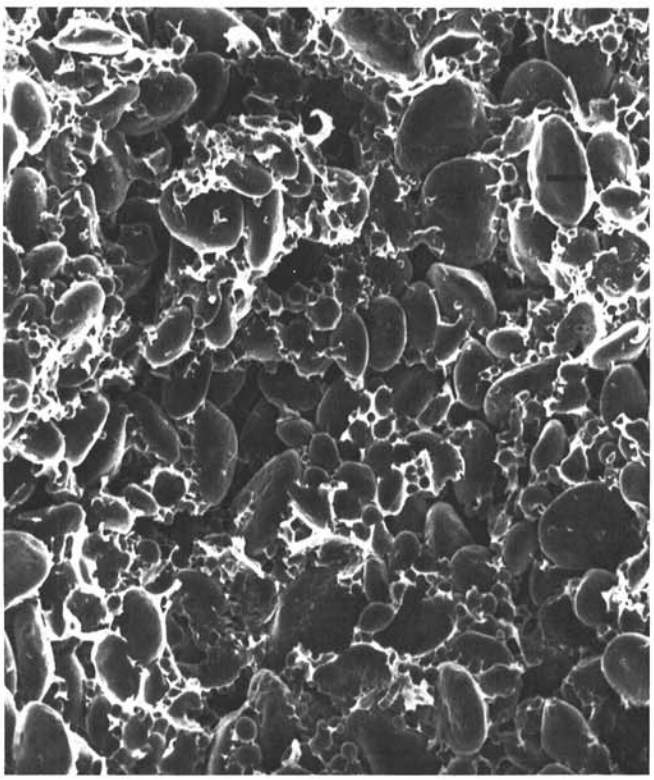

Fig. 7. The fractured surface of a tablet made from soft wheat flour. Bar is 10 μm.

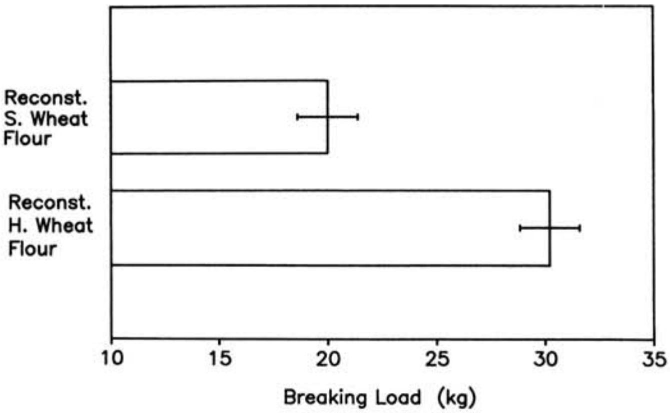

Fig. 8. Tensile strength of tablets made from reconstitutions of hard wheat flour or soft wheat flour. Each horizontal bar shows the mean from four tablets in one experimental unit. Error bars show the 95% confidence interval for replicate experimental units.

Reconstituted Flours

Tablets made from the reconstituted hard wheat flour had greater tensile strength than tablets made from the reconstituted soft wheat flour (Fig. 8). Tablets from these reconstitutions had somewhat greater tensile strength than tablets from the parent flours (compare Fig. 8 with Fig. 5), but the results were similar. Therefore, these procedures were suitable for use when combining and interchanging flour fractions (Malouf et al 1992).

CONCLUSIONS

Procedures were developed to make and fracture tablets, providing a measure of their tensile strength that was indicative of the hardness of wheat flour and reconstituted wheat flour. In tablets made from flour, contact between starch granules and the surrounding protein phase was visually similar to that of typical wheat endosperm. Fractionation-reconstitution procedures gave tablets that were similar to tablets from the parent wheat flours. Factors influencing tablet tensile strength remained active through the processes of storage, milling, fractionation, reconstitution, and tablet making.

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