Note on Mill for Pulverizing Single Kernels of Cereals for Isoelectric Focusing

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In genetic studies, because of limited availability of grain and variations among kernels, some investigators have extracted meals from single kernels to compare gel electrophoretic patterns of storage proteins in wheat (Bietz et al 1975, Kasarda et al 1976, Shepard 1968), rye (Shepard 1968), corn (Soave et al 1978), and barley (Shrewry et al 1978). Commercial mills such as the Wiley mill and the Udy laboratory cyclone mill can grind small samples (5–10 g) of grain, but they give low recoveries of a fine meal when used for grinding single kernels. A mortar and pestle has been used in research on wheat, barley, and rye to grind single and half kernels before analysis. Grinding is usually tedious and time-consuming. Corn and sorghum are difficult to grind this way, especially if the kernels are hard and vitreous. We designed a simple, inexpensive, and efficient mill to pulverize a single kernel of any of the common cereal grains into a fine meal size. It is simple to construct and requires little effort to pulverize a single or half kernel of grain in a short time. Because breeders and geneticists need to use only the germ portion of the seed for growth of the new plant on agar media, the endosperm tissue is available to be analyzed for prolamine proteins. Zeins in extracts of the ground endosperm of a single kernel of corn can be effectively resolved by isoelectric focusing (IEF) in polyacrylamide gels.

MATERIALS AND METHODS

Mill

The pulverizing mill was made of SAE 10.20 steel. Cylindrical parts were turned on a metal lathe to the specified diameters (Fig. 1B). The mill consists of three parts: a base with a short round pedestal, a retaining ring that fits closely around the pedestal, and a pulverizing rod of diameter to fit snugly in the ring. The pedestal is joined to the base plate by a metal screw.

Samples

Sorghum, triticale, wheat, barley, corn, and oat grains in initial milling studies were common commercial varieties. Corn endosperm sections used in the demonstration of IEF of zein proteins were dissected by hand from single kernels of different races after being soaked in water for 15 min at room temperature.

Pulverization

A single grain was placed on the pedestal, around which was placed the retaining ring. The pulverizing rod was inserted in the retaining ring. The grain was ground by hitting the rod with a hammer. The rod was removed, and the meal was scraped into a pile to the side of the retaining ring. The rod was reinserted and hit again with the hammer. This operation was repeated until a fine meal was obtained. Vitreous kernels require more blows with the hammer than do floury ones. Removal of the retaining ring permits ready access to all of the ground meal, which is then brushed onto weighing paper.

Isoelectric Focusing

About 200 mg of pulverized corn endosperm meals containing about 8% protein and prepared from single kernels of several South American corn races was extracted in 1.5 × 15 cm test tubes with 5 ml of 70% (v/v) aqueous ethanol for 10 min on a vortex mixer. The extracts were next centrifuged in a bench-top centrifuge at 2,000 × *g* for 5 min. Two milliliters of supernatant was removed and evaporated to dryness under vacuum. The residue was reduced and alkylated in 8*M* urea (total volume 70 μl) according to a previously described procedure (Paulis and Wall 1977). Wrigley's procedure (1968) was adapted to the use of a slab gel. Samples of about 5 μl of each extract were absorbed on separate paper wicks and inserted into a 0.3 × 13 × 15.5 cm polyacrylamide gel slab containing 4.75% acrylamide and 0.25% *N,N'*-methylene bisacrylamide, 2% carrier ampholytes prepared with equal volume of pH 4–6, pH 5–7, pH 6–8, and pH 7–9 range Ampholines (LKB Products) and 8*M* urea. IEF was conducted for 17 hr at about 4 W with 0.01 *M* H_3PO_4 in the cathode well and 0.02*M* NaOH in the anode well. The gel slab was cooled by water circulating through base and cover plates. For

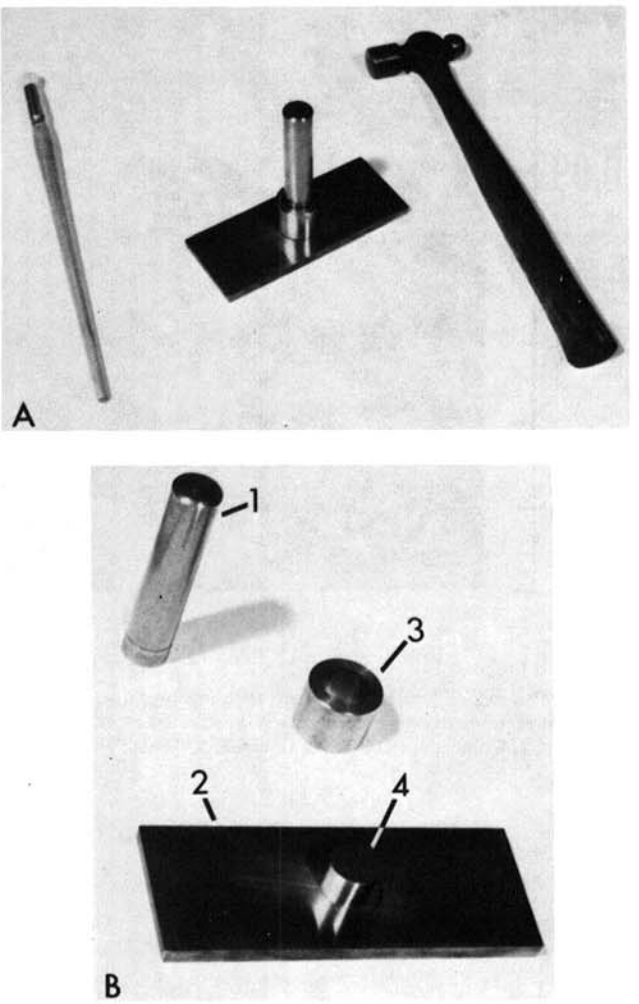

Fig. 1. A, Microhammer mill. Hammer strikes the pulverizing rod, and meals are recovered by dusting with a brush. **B,** Dimensions of mill parts: **1,** Pulverizing rod, 0.740 in. OD × 3.5 in.; **2,** base plate, 0.313 × 2.5 × 5.5 in.; **3,** retaining ring, 0.751 ID × 1.10 OD × 1 in.; **4,** pedestal, 0.75 OD × 0.625 in. on base plate center.

[1]Mention of firm names or trade products does not imply their endorsement or recommendation by the USDA over other firms or similar products not mentioned.

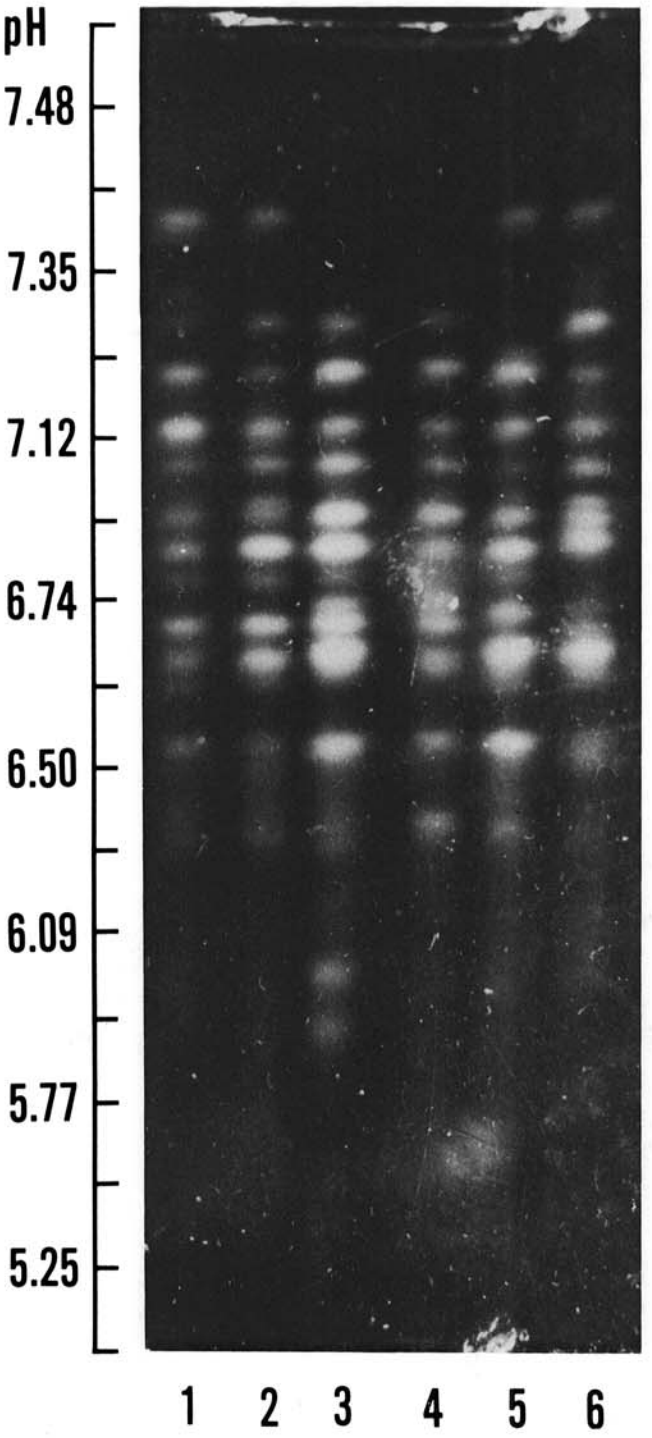

Fig. 2. Polyacrylamide slab isoelectric focusing of zein proteins from endosperm sections of single corn kernels of selected races: **1,** Palomero Toluqueño; **2,** Conico; **3,** Pepitilla; **4,** Calqueñ; **5,** Conico Norteño; and **6,** Coroico.

visualization, the proteins were precipitated with a sulfosalicylic acid solution described by Vesterberg et al (1977). The gel was put on a glass plate placed over a black background with perpendicular illumination from a translucent light box and photographed using Polaroid P/N 55 film. The pH was determined at 1-cm intervals on the gel with a combination pH electrode.

RESULTS AND DISCUSSION

The mill described here (Fig. 1) can be used to pulverize single whole or half kernels and endosperm sections of single kernels to a flour 80–85% of which passes through a U.S. Standard Sieve No. 60 (250-μ opening). The apparatus can be constructed in most machine or instrument shops. A rod or pipe near the specified diameter can be purchased. The mill works very easily with vitreous kernels, such as dent corns, which require time-consuming and tedious hand grinding with a mortar and pestle. Recoveries for all grains are from 92–95%.

Figure 2 shows IEF patterns on a polyacrylamide slab of reduced and alkylated zein extracts from ground endosperm meals of single kernels from selected races of corn. The results demonstrate that only about 10–15% of the extracts of the zein proteins from pulverized meals of single kernels are needed for IEF studies on polyacrylamide gels to give distinct patterns. This technique indicates that reduced and alkylated zein polypeptides obtained from single kernels of various races of corn exhibit sufficient differences in IEF pattern to be useful in evolutionary studies.

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