# Amylase Activity and Sprout Damage in Sorghum (*Sorghum bicolor* L. Moench)[1]

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Cereal Chem. 61(5): 415-418

ABSTRACT

Sorghum grain samples from several genotypes affected by wet and humid weather during maturation were studied. Amylase activity in the rain-affected grains ranged from 6.1 to 18.0 units per gram, whereas values below 6.7 were observed for dry-season harvests. Enzyme activity in the range of 8.2–12.6 units per gram was associated with incipient damage, while values above 12.6 were associated with visible symptoms of sprouting. Laboratory-sprouted samples (30 hr) of 15 cultivars showed an average enzyme activity of 22.8 units per gram and poor rolling, gel, and roti quality characters. Increased amylase activity was associated with decreased food quality characteristics. Studies on composites of normal and sprouted grain samples showed that 20% sprouted grains in the composite resulted in significant changes in the food-quality properties.

In many regions of the world, the maturity of the sorghum crop coincides with prolonged wet and humid weather. This may result in grain deterioration caused by molds, discoloration, and preharvest sprouting (Murty et al 1980, Castor and Frederiksen 1980). The effects of discoloration and molds on the composition and quality of the grain have been reported (Glueck and Rooney 1980, Murty et al 1982a). However, the precise effects of preharvest sprouting, as distinct from those caused by molds, have not received much attention in the past. Some immature seeds of sorghum are vulnerable to sprouting in wet and humid weather, as sorghum grain has no strong dormancy mechanisms (Kersting et al 1961, Gritton and Atkins 1963, Clark et al 1967, Maiti et al 1984 in press). Sprout damage results in the modification of storage carbohydrates of the grains and affects the quality of the food product. In wheat, the level of $\alpha$-amylase activity in flour has been used to assess sprout damage and to predict bread quality (Pratt 1977, Campbell 1980, Buchanan and Nicholas 1980). Recently, Mathewson et al (1982) reported the level of $\alpha$-amylase activity in market samples of sorghum and have proposed a colorimetric assay to assess sprout damage.

The development of such objective techniques for evaluating grain and food quality are useful in crop improvement, marketing, and food processing. The objective of the present study was to survey the level of amylase activity in grain from rainy-season harvests and from laboratory-sprouted samples of improved sorghum cultivars and to explore the possibility of utilizing an enzyme assay as a technique to predict the food quality of wet-season harvested grain.

## MATERIALS AND METHODS

Two groups of sorghum grain samples were studied: harvests from the rainy season crop, and laboratory-sprouted samples.

### Harvests from Rainy-Season Grain Samples

Bulk grain harvests from 25 diverse genotypes belonging to early- and medium-maturity groups planted in advanced yield trials at the ICRISAT Center were studied. The yield trials were planted on June 19, 1982, and were harvested in October, a week after physiological maturity. Grains were physiologically mature when black-layer formation in the hilar region at the bottom portion of the panicle was complete. The crop was entirely rain-fed. Extremely wet and humid weather prevailed for about 10 days before harvest time. All grain samples were moldy, and samples of some cultivars showed symptoms of sprouting. The harvested samples were sun-dried to 11% moisture content and were stored at room temperature for about a month before being assayed for amylase activity.

Grain samples of cultivars SPV 351, SPV 386, and SPV 475 were collected from an experiment planted in late July of 1982 for comparative studies. These grains matured during relatively dry weather and did not show any visual symptoms of weathering. Panicles from one of them (SPV 351) were collected at physiological maturity and were tested after being soaked in distilled water, then germinated at 27° C. Grain samples were taken from the panicles at 12-hr intervals and assayed for amylase activity.

### Laboratory-Sprouted Grain Samples

Bulk grain samples of 15 cultivars harvested in the postrainy season of 1981 and stored for about eight months at room temperature were chosen for sprouting studies. Grain samples were soaked in distilled water for 4 hr and then germinated at 27° C for 26 hr. After germination, the grain was dried at 35° C to a moisture content of 10%. Control and laboratory-sprouted grain samples were subjected to enzyme assay and flour-quality studies.

Grain samples of two cultivars, CS 3541 and SPV 352, were given the same treatment as that mentioned above; sampling was at 4-hr intervals from four to 24 hr. The samples were similarly dried and then subjected to the enzyme assay and flour-quality tests.

Grain samples of SPV 475 were soaked in distilled water for 4 hr and then germinated at 27° C. They were removed from the germinator after 18 hr and dried at 35° C to a moisture content of 10%. The sprouted grain was used to study the flour quality of mixtures from control and sprouted grains.

### Isolation and Assay of Amylase from Sorghum Grains

Grain samples were ground in a Udy mill to pass through a 0.4-mm screen. Extraction and assay of amylase in the flour were performed in duplicate, as reported by Chrispeels and Varner (1967), with minor modifications. Flour samples (0.5 g) were extracted with 10 ml of 0.2*M* sodium chloride for 30 min. The slurry was centrifuged at 3,000 × *g* for 15 min at 4° C. The resulting supernatant, referred to as enzyme extract, was used for the assay.

A fresh starch solution was made daily using 150 mg of nonsolubilized potato starch (Sigma Chemical Co.), 600 mg of $KH_2PO_4$, and 2 mg of $CaCl_2$ in a final volume of 100 ml. The starch solution (pH 4.65) was boiled for 2 min, cooled, and centrifuged at 3,000 × *g* for 15 min. The clear supernatant was used as the substrate.

The amylase assay was done at room temperature, using an aliquot of 1 ml of enzyme extract. The reaction was started by the addition of 1 ml of starch solution and lasted for 5 min before being stopped by the addition of 1 ml of iodine reagent (0.1% iodine in potassium iodide). Distilled water (5 ml) was added to each tube and the absorbance read at 620 nm. The initial absorbance of the starch solution was usually about 0.82. A correction for absorbance of soluble starch in the enzyme extract was made by subtracting the absorbance of the enzyme extract (boiled in water for 10 min) from the absorbance of the starch solution. The decrease in absorbance

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at 620 nm by the action of the enzyme is proportional to the quantity of amylase present in the sample. Amylase enzyme obtained from porcine pancreas (Sigma Chemical Co., No. 6880) was used for calibration of enzyme activity. Assay with 10 μg of enzyme equivalent to 1.6 units resulted in a 0.07 increase in absorbance at 620 nm. This gave a conversion factor of 0.229 units per 0.1 change in absorbance at 620 nm for a 5-min assay period at pH 4.65.

TABLE I
Amylase Activity in Sorghum Grain Samples Harvested in Rainy Season

| Genotype | Days to 50% Flower | Amylase Activity (units/g) |
|---|---|---|
| Varieties | | |
| M60272 | 64 | 14.1 |
| M60351 | 64 | 9.5 |
| M60936 | 64 | 10.8 |
| M60394 | 63 | 7.9 |
| M60336 | 62 | 18.0 |
| M60370 | 62 | 8.7 |
| M60350 | 62 | 12.6 |
| CSV 4 | 61 | 13.0 |
| M60044B | 61 | 9.6 |
| M60013B | 59 | 6.1 |
| M60005B | 58 | 7.4 |
| M60027B | 58 | 7.1 |
| M60031B | 58 | 8.2 |
| M60050B | 58 | 6.7 |
| 623B | 57 | 7.3 |
| Hybrids | | |
| 2219A × MR 802 | 59 | 7.9 |
| 296A × MR 836 | 59 | 7.2 |
| 296A × MR 839 | 59 | 8.0 |
| 296A × MR 844 | 59 | 11.2 |
| 296A × MR 858 | 59 | 8.9 |
| 2077A × CS 3541 (CSH 5) | 59 | 6.7 |
| 296A × CS 3541 (CSH 9) | 59 | 10.5 |
| 2077A × MR 824 | 58 | 7.6 |
| 296A × MR 841 | 58 | 11.1 |
| 296A × MR 846 | 58 | 9.2 |
| SE | | 0.55 |

### Food-Quality Measurements

Whole-grain samples were ground with a Milcent Domestic Size 2 laboratory Carborundum stone mill (Balaji Mill Traders, Secunderabad, India). Flour samples (25 g) were sifted by hand, using sieves with openings of 75 and 125 μm. Flour was sieved continuously until no more flour passed through the sieve. Flour mixtures from sprouted and control samples were obtained by grinding a mixture of grains in the required proportions. The gel spread and roti-quality tests were performed according to the procedures of Murty et al (1982b) and Murty and Subramanian (1982), respectively.

## RESULTS AND DISCUSSION

### Amylase Activity of Rainy-Season Samples

Grain samples harvested from some genotypes of the rain-affected crop showed chits and acrospires of 2–4-mm length were occasionally observed. Visual observations showed that among these genotypes (M60272, M60351, 296A × MR 836, M60336, and 296A × MR 844), the number of grains suspected to be sprout-damaged was about 3–4%. No visual symptoms of germination were observed in the other genotypes. The levels of amylase enzyme activity among the 25 cultivars varied from 6.1 to 18.0 units per gram (Table I). Some late-maturing genotypes showed a high level of enzyme activity, while some early-maturing genotypes showed relatively low enzyme activity. Mathewson et al (1982) noted that α-amylase content varied widely among field-sprouted sorghum lines and that some genotypes that exhibited bad sprouting contained relatively small amounts of α-amylase. We found it very difficult to rate samples for sprouting, especially when only a percentage of the grains showed visual symptoms like bulging and chitting at the embryo region and of varied severity. Visual methods for measuring sprouting do not distinguish samples that vary in the degree of sprouting and cannot detect incipient damage in which α-amylase has been produced in the grain but in which the acrospire is not visible (Mathewson et al 1982). Acrospires are often broken off during threshing and grain processing.

Several grain samples collected from the three cultivars SPV 351, SPV 475, and SPV 386, which were grown in the late plantings

TABLE II
Flour and Roti Properties of Normal (C) and Sprouted (S) Sorghum Grain Samples

| | | Flour[a] | | | | | | | | Roti[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Amylase Activity (units/g) | | % Flour Through 75μm | | Gel Spread (mm) | | Rolling Quality (cm) | | Taste Score | | Keeping-Quality Score | |
| Genotype | Percent Germination | C | S | C | S | C | S | C | S | C | S | C | S |
| M 35-1 | 82 | 4.7 | 20.7 | 66.1 | 67.8 | 57 | 57 | 24.2 | 21.6 | 2.0 | 2.5 | 2.0 | 2.5 |
| IS 10504 | 88 | 7.4 | 21.6 | 73.1 | 83.3 | 73 | 94 | 16.0 | 17.1 | 3.0 | 4.0 | 3.0 | 4.0 |
| SPV 351 | 91 | 10.4 | 23.1 | 48.4 | 62.9 | 58 | 62 | 22.2 | 19.7 | 2.5 | 3.0 | 2.5 | 2.7 |
| SPV 475 | 96 | 6.5 | 24.5 | 43.9 | 53.1 | 55 | 57 | 23.7 | 21.5 | 2.7 | 3.0 | 2.5 | 2.7 |
| CSH 1 | 43 | 4.4 | 20.7 | 53.5 | 61.5 | 57 | 57 | 23.7 | 21.5 | 2.7 | 3.0 | 2.5 | 2.5 |
| CSH 6 | 44 | 5.0 | 21.3 | 52.2 | 50.2 | 58 | 62 | 22.5 | 21.2 | 2.5 | 3.5 | 2.7 | 3.2 |
| 2077B | 82 | 4.7 | 23.7 | 64.9 | 72.8 | 58 | 58 | 23.5 | 17.4 | 2.7 | 4.0 | 2.7 | 3.7 |
| CSV 4 | 94 | 6.5 | 23.0 | 39.4 | 52.9 | 56 | 57 | 22.4 | 17.7 | 2.7 | 3.5 | 2.7 | 3.2 |
| Mothi | 98 | 5.1 | 22.4 | 41.3 | 54.5 | 59 | 64 | 23.5 | 20.2 | 2.5 | 3.7 | 2.7 | 3.7 |
| Safra | 98 | 5.9 | 22.0 | 53.1 | 70.9 | 61 | 70 | 23.5 | 17.2 | 3.0 | 3.7 | 3.0 | 4.0 |
| SPV 352 | 98 | 5.5 | 22.9 | 47.9 | 54.8 | 59 | 63 | 23.7 | 19.1 | 3.0 | 3.5 | 3.0 | 3.0 |
| CSH 9 | 98 | 5.2 | 24.7 | 44.4 | 62.8 | 61 | 64 | 23.2 | 19.1 | 2.5 | 3.0 | 2.7 | 3.0 |
| CSH 5 | 81 | 4.7 | 24.8 | 59.2 | 69.1 | 60 | 64 | 22.0 | 19.2 | 2.2 | 3.0 | 2.2 | 2.5 |
| CSH 8 | 63 | 4.2 | 22.9 | 63.8 | 69.4 | 56 | 55 | 24.7 | 20.7 | 2.8 | 3.5 | 2.5 | 2.7 |
| E 6954 | 66 | 3.9 | 23.4 | 42.5 | 57.2 | 56 | 58 | 25.5 | 20.5 | 3.0 | 4.0 | 2.5 | 2.7 |
| SE ± | | 0.42 | 0.35 | 2.70 | 2.40 | 1.1 | 2.5 | 0.55 | 0.42 | 0.07 | 0.12 | 0.07 | 0.13 |
| C vs S mean difference | | | **[c] | | **[c] | | *[d] | | **[c] | | **[c] | | **[c] |

[a] Values are averages of two observations.
[b] Roti taste and keeping quality were rated on a scale of 1–5, where 1 = good and 5 = very poor.
[c] ** = significant at $P = 0.01$ by paired $t$-test.
[d] * = significant at $P = 0.05$ by paired $t$-test.

(July 1982) and thus were not exposed to rain during maturation, showed enzymatic activity in the range of 5.0–6.7 units per gram. These samples were free of molds. Physiologically mature grains of SPV 351 showed enzyme activity values around 6.4 units per gram. Hourly sampling showed that such grains sprouted on the panicle only after 48 hr of continuous wet and humid conditions in the germinator. However, in nature, alternate wetting and drying of panicles is more common. Sampling at 12-hr intervals showed that enzyme activity in the grain was 6.4, 6.7, 8.9, and 17.5 units per gram at 12, 24, 36, and 48 hr, respectively. These studies show that enzyme activity levels up to 6.7 units per gram can be encountered in sound seeds harvested from the wet season. Incipient damage should be suspected in seeds showing enzyme activity of 8.9–12.6 units per gram. Seeds with some visible sprout damage had amylase-activity values greater than 12.6 units per gram. Germination tests of all the grain samples confirmed these findings. These results agree with those of Mathewson et al (1982), reported from samples of sorghum grown in the United States.

### Amylase Activity and Flour Quality of Laboratory-Sprouted Samples

Amylase activity of laboratory-sprouted grains was very high (Table II). The control samples showed a very low value—5.6 units per gram.

Average particle size of flour from the sprouted samples was significantly less than that of the control. The particle size of the flour from sprouted grain was reduced by 20–30%, as reflected by the increased amount of flour passing through the 75-$\mu$m sieve. A moderate particle size (65% of flour $<75$ $\mu$m) is suitable for making a good dough and roti (Murty et al 1982a).

Average rolling quality of sprouted samples was much lower than that of control samples. However, rolling quality of flour from sprouted grains of CSH 6 was not significantly lower than that of control samples (Table II), probably because the amount of germination in this lot was only 44%. Taste and keeping quality of the rotis made from sprouted samples was poorer than those from control samples.

Gel-spread values for the sprouted samples were significantly higher than that of the control samples (Table II). A thick gel is an important attribute of sorghum porridges (Rooney and Murty 1982).

Hourly sampling of sprouting grain from the two cultivars showed that, as sprouting progressed, amylase activity increased (Table III) and roti quality became poorer. A significant increase in the amylase activity occurred after 20 hr. Some changes in flour particle size, gel spread, and roti quality were observed even after 8 hr.

The studies with flour from mixtures of control and sprouted grain showed that rolling quality was significantly reduced only when 40% of the grains in the composite had sprouted (Table IV). Significant reduction in the flour particle size occurred when only 10% of sprouted grain was added. Taste and keeping quality of roti decreased significantly with a mixture of 30% or more sprouted grains. An increase in the amount of sprouted grains in the mixture was accompanied by an increase in amylase activity and a decrease in roti quality. Gel spread significantly increased even when 20% of the grains in the sample had sprouted.

TABLE III
Changes in the Grain, Flour, and Roti Qualities of Sorghum Grains During Sprouting

| | Flour[a] | | | | Roti[b] | |
|---|---|---|---|---|---|---|
| Treatment | Amylase (units/g) | % Flour Through 75 $\mu$m | Gel Spreading (mm) | Rolling Quality (cm) | Taste | Keeping Quality |
| Control | 5.9 | 44.1 | 57.5 | 23.2 | 2.3 | 2.4 |
| Sprouted 8 hr | 6.3 | 54.0 | 59.0 | 22.9 | 2.5 | 3.0 |
| Sprouted 12 hr | 5.4 | 57.0 | 59.5 | 22.8 | 2.6 | 3.0 |
| Sprouted 16 hr | 9.8 | 59.1 | 59.5 | 23.1 | 3.0 | 2.8 |
| Sprouted 20 hr | 13.4 | 56.3 | 61.0 | 23.0 | 3.5 | 2.8 |
| Sprouted 24 hr | 23.6 | 59.0 | 61.0 | 19.5 | 4.0 | 3.7 |
| SE M± | 2.86 | 1.69 | 0.53 | 0.42 | 0.25 | 0.14 |

[a] Averages of two observations over two cultivars, CS 3541 and SPV 352.
[b] Roti taste, texture, and keeping quality were scored on a scale of 1–5, where 1 = good and 5 = poor.

TABLE IV
Flour and Roti Properties of Mixtures of Sprouted and Normal Sorghum (SPV 475) Grains

| | Flour[a] | | | | Roti[b] | | |
|---|---|---|---|---|---|---|---|
| Treatment | Amylase Activity (units/g) | % Flour Through 75 $\mu$m | Gel Spreading (mm) | Rolling Quality (cm) | Taste | Texture | Keeping Quality |
| Control (C) | 5.0 | 44.5 | 53 | 24.7 | 2.7 | 2.7 | 3.0 |
| 90%C + 10% sprouted | 7.3 | 50.1 | 54 | 23.7 | 2.7 | 2.7 | 3.2 |
| 80%C + 20% sprouted | 10.9 | 51.7 | 55 | 24.2 | 3.0 | 2.7 | 3.2 |
| 70%C + 30% sprouted | 13.6 | 51.7 | 54 | 24.4 | 3.0 | 3.0 | 3.2 |
| 60% + 40% sprouted | 16.1 | 51.3 | 55 | 22.6 | 3.2 | 3.2 | 3.5 |
| 50%C + 50% sprouted | 17.7 | 50.4 | 55 | 22.5 | 3.5 | 4.0 | 3.2 |
| 100% sprouted | 23.4 | 54.9 | 56 | 21.5 | 4.5 | 4.5 | 3.7 |
| SE M± | 2.65 | 0.85 | 0.24 | 0.19 | 0.22 | 0.25 | 0.08 |

[a] Average of two observations.
[b] Roti taste, texture, and keeping quality were scored on a scale of 1–5, where 1 = good and 5 = poor.

## CONCLUSIONS

Amylase activity of rain-affected grain samples ranged from 6.1 to 18.0 units per gram, while grains sprouted in the laboratory for 30 hr showed a mean enzyme activity of 22.8 units per gram. Enzyme activity levels in physiologically mature panicles sampled in the dry season also confirmed that grain free from sprout damage exhibited amylase activity of 6.7 units per gram or less. Enzyme activity levels between 8.2 and 12.6 units per gram probably indicate incipient sprout damage, whereas values above 12.6 are accompanied by visible symptoms of germination. Bulk samples of rain-affected sorghum harvest showing amylase activity values less than 8.2 units per gram would be desirable. The studies on laboratory-sprouted samples were initiated so that the effects of sprouting independent of grain molds and weathering in the rainy-season field-harvested samples could be observed. Results indicated that damage to flour quality could be significant if the field harvests contain 10–20% sprouted grains. However, the combined effects of molds, in addition to sprouting, could lower flour quality to an even greater extent than the effects of sprouting alone. No attempt was made to study the direct effects of amylase on flour and food quality. However, our study shows that grains affected by wet weather could have high amylase activity in the flour and poor food quality.

## LITERATURE CITED

BUCHANAN, A. M., and NICHOLAS, E. M. 1980. Sprouting, $\alpha$-amylase and baking quality. Cereal Res. Commun. 8(1):23.

CAMPBELL, J. A. 1980. Measurement of $\alpha$-amylase in grains. Cereal Foods World 24:46.

CASTOR, L. L., and FREDERIKSEN, R. A. 1980. *Fusarium* and

*Curvularia* grain molds in Texas. Page 93 in: Proc. Intl. Workshop on Sorghum Diseases. December 11–15, 1978. ICRISAT, Hyderabad, India.
CHRISPEELS, M. J., and VARNER, J. E. 1967. Gibberallic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. Plant Physiol. 42:398.
CLARK, L. E., COOLIER, J. W., and LANGSTON, R. 1967. Dormancy in *Sorghum bicolor* (L.) Moench. I. Relationship to seed development. Crop Sci. 7:497.
GLUECK, J. A., and ROONEY, L. W. 1980. Chemistry and structure of grain in relation to mold resistance. Page 119 in: Proc. Intl. Workshop on Sorghum Diseases. December 11–15, 1978. ICRISAT, Hyderabad, India.
GRITTON, E. T., and ATKINS, R. E. 1963. Germination of sorghum seed as affected by dormancy. Agron. J. 55:169.
KERSTING, J. F., STICKLER, F. C., and PAULI, A. W. 1961. Grain sorghum caryopsis development. I. Changes in dry weight, moisture percentage and viability. Agron. J. 53:36.
MAITI, R. K., RAJU, P. S., and BIDINGER, F. R. 1985. Studies on germinability and some aspects of the harvest physiology of sorghum grain. Seeds Sci. Technol. In press.
MATHEWSON, P. R., FAHRENHOLZ, C. H., and POMERANZ, Y. 1982. Applicability of the colorimetric α-amylase assay to evaluate sprouted sorghum. Cereal Chem. 59:156.
MURTY, D. S., and SUBRAMANIAN, V. 1982. Sorghum *roti*. I. Traditional methods of consumption and standard procedures for evaluation. Page 73 in: Proc. Intl. Symp. Sorghum Grain Quality. October 28–31, 1981. ICRISAT, Hyderabad, India.
MURTY, D. S., RAO, K. N., and WILLIAMS, R. J. 1980. Breeding for grain mold resistant sorghums at ICRISAT. Page 154 in: Proc. Intl. Workshop on Sorghum Diseases. December 11–15, 1978. ICRISAT, Hyderabad, India.
MURTY, D. S., PATIL, H. D., and HOUSE, L. R. 1982a. Sorghum *roti*. II. Genotypic and environmental variation for *roti* quality parameters. Page 79 in: Proc. Intl. Symp. Sorghum Grain Quality. October 28–31, 1981. ICRISAT, Hyderabad, India.
MURTY, D. S., PATIL, H. D., and HOUSE, L. R. 1982b. Cultivar differences for gel consistency in sorghum. Page 289 in: Proc. Intl. Symp. Sorghum Grain Quality. October 28–31, 1981. ICRISAT, Hyderabad, India.
PRATT, D. B., Jr. 1977. Criteria of flour quality. Page 201 in: Wheat: Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem. St. Paul, MN.
ROONEY, L. W., and MURTY, D. S. 1982. Evaluation of sorghum food quality. Page 571 in: Sorghum in the Eighties. Proc. Intl. Symp. Sorghum Grain Quality. November 2–7, 1981. ICRISAT, Hyderabad, India.

[Received September 2, 1983. Revision received April 15, 1984. Accepted May 4, 1984]

# Bread-Making Test for 10 Grams of Flour[1]

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**ABSTRACT**

Cereal Chem. 61(5): 418–423

A bread-making test for 10 g of flour was developed. Dough mixer, liquid-dispensing apparatus, shortening dispenser, dough molder, bread pans, and loaf volumeter are described. Comparisons of 10- and 100-g bread-making tests were made on fractionated and reconstituted flours, on two flour-protein series, one of station composites and the other of a single variety, on loaf-volume responses to various concentrations of glucose, and on a flour that responded to various levels of oxidation. Correlations between the 10- and 100-g bread-making tests ranged from r = 0.976 to r = 0.991.

Early fractionation and reconstitution studies conducted in our laboratory utilized a 100-g bread-making test. Preparation of gluten fractions eventually became too time-consuming for the 100-g test to be practical. Therefore, a bread-making test for 10 g of flour was developed (Shogren et al 1969). Subsequently, improvements in equipment have been made, but the basic concept of the 100-g method was retained. Details of 10-g procedures and improved equipment, together with comparisons of the 10- and 100-g methods, are reported here.

## MATERIALS AND METHODS

### Wheat and Flour Samples

Flour from the varieties Pawnee, Comanche, C.I. 12995, KS501097, and KS501099 were fractionated into gluten and starch plus water-solubles followed by fractional precipitation of the gluten from a dilute lactic acid solution (Shogren et al 1969). Reconstituted flours contained the gluten fractions and the starch plus water-solubles.

Approximately 25 hard red winter wheat varieties were harvested at each of many stations throughout the Great Plains during the years 1977–1979 and composited by station. The station composites ranged in flour-protein content from 7.9 to 17.2% (14% mb).

The hard winter wheat variety Newton was harvested at 15 stations in 1979. Flour protein content ranged from 7.3 to 14.8%.

A regional bake standard (RBS-78) consisted of many hard winter wheats harvested throughout the Great Plains in 1978. RBS-78 flour had a protein content of 12.3%. It was baked with 0–10% glucose.

The hard winter wheat variety Payne was harvested at four locations in 1979. The blend had a flour-protein content of 13.4%, a medium-short mixing time, and a good loaf-volume response to oxidation. Loaf characteristics were determined at several levels of oxidation.

### Bread-making Equipment

The fermentation pans and cabinet were those used in the 100-g test. Oven temperature was raised from 215 to 232° C, and the shelf covering of sheet asbestos doubled to 1/8 in. Sheeting rolls were spaced at 0.0984 in. (2.50 mm) for all punches. Shortening (0.3 g) was added to the flour in the mixing bowl by means of a length of

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA over other firms or similar products not mentioned.
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