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A Screening Test for Grain Hardness in Sorghum Employing Density Grading in Sodium Nitrate Solution

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An indirect, non-destructive, inexpensive and simple method for testing hardness of sorghum grain was developed using density grading in sodium nitrate solution with a density of 1·315 g/ml at 25 °C. The percentage of floating kernels was highly correlated with percentage vitreousness, the grain hardness determined as work required for grinding, flour particle size, and with rheological properties of a sorghum flour dough.

Introduction

The relative proportion of vitreous endosperm to the whole endosperm within a cereal grain is often referred to as endosperm texture. Numerous studies[1–8] have shown the importance of sorghum endosperm texture to the milling performance of the grain and to physical and chemical characteristics of widely different food preparations. Rooney and Murty[5] found that grains with 60–100% vitreous endosperm were preferred for the preparation of stiff porridges like *tô* (Upper Volta, Mali), *ugali* (Tanzania), *bogobe* (Botswana), and *sankati* (India). Floury endosperm types (0–40% vitreous) produced the best quality *kisra* and *injera* (fermented breads of Sudan and Ethiopia) while grains with intermediate kernel texture (40–60% vitreous) were the most suitable for unleavened breads like *roti* (India).

Measurements of endosperm texture are often performed by cutting the grain longitudinally[1,2] or transversally[6] and then visually rating the proportion of vitreous endosperm on a scale from 1 to 5[1,5], or by quantitative measurement of the image from a microscope[2,6]. Due to variations in texture within a given sample, large numbers of grains must be analysed which makes this method very laborious. A number of pearling and milling methods have also been used for determining the hardness of bulk samples of sorghum grain[1–3]. However, measurements by these methods are affected by the nature of pericarp, size and shape of the grain and the milling or pearling equipment.

The difference in density between the floury and vitreous part of the endosperm has been the basis for a number of indirect methods for estimating endosperm texture. Kirleis

Abbreviations: PF = 'percentage floaters', i.e. the percentage of total grain that floated or remained suspended.

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and Crosby[2] graded grain samples on the basis of their density in an organic solvent. They found a highly negative correlation between endosperm texture and the percentage of floating kernels in a mixture of tetrachloroethylene and kerosene. Sodium nitrate has been used previously in a study for grading wheat for baking quality[9], and for separating the germs from sorghum grits[1].

In this paper a convenient method for assessing the hardness and vitreousness of sorghum grain using a sodium nitrate solution is described and related to kernel characteristics and food quality parameters.

Experimental

Sorghum genotypes

Grain samples of 15 sorghum genotypes (Table I) differing in endosperm texture were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, India). These were grown at the ICRISAT Center (Hyderabad) during the post-rainy season under irrigated conditions in 1981. Grain of all 15 genotypes had a white pericarp without a pigmented testa. The samples were equilibrated in paper bags at ambient temperature in an air-conditioned laboratory for 3 weeks to a moisture content of $10{\cdot}1 \pm 0{\cdot}4\%$. Grains of S-29 (photosensitive) genotype were harvested prematurely.

Density grading in flotation tests

For the inorganic medium, sodium nitrate (approximately 335 g, Merck, analytical grade) was dissolved in distilled water (500 ml). The organic medium was prepared by mixing trichloroethylene (800 ml, technical grade) and petroleum ether (176 ml, 40–60 °C, Merck, analytical grade). Both the inorganic and the organic solutions had a density of 1·315 g/ml at 25 °C. The specific gravity of the solution was measured with a hydrometer, and adjusted if necessary before each test. The tests were performed on samples of 100 kernels and three independent observations were made for each genotype. A grain sample was placed in a 500 ml glass beaker and the solution added. After stirring with a glass spatula the grains were allowed to equilibrate to their resting positions. The inorganic solution was stirred for 60 s. Due to the lower viscosity of the organic solution, a satisfactory density test took less time and the grains were stirred for 10 s. The floating and suspended kernels were removed by a small tea sieve and counted as a percentage of the total number of kernels graded; these are termed 'percent floaters' (PF). The remaining kernels resting at the bottom were removed by sieving the whole liquid.

For the preparation of sorghum samples with different initial moisture levels, the grains were dried in a vacuum oven for 48 h at 25 °C to a moisture content of 8·4% (w/w). Distilled water was added to give a moisture range from 8·4 to 17·8% (w/w). The samples were equilibrated in air-tight, sealed plastic containers for 36 h before the floatation tests and moisture determinations were performed.

Moisture determination

Moisture content of the grain was determined by the method of Jacobsen[10]. In this method the samples are weighed before grinding thus eliminating the risk of gain or loss of moisture from/to the surrounding atmosphere.

Endosperm texture

Fifty grains from each genotype were selected at random and embedded in plastic clay using the Carlsberg Seed Fixation System[11], equipped with a matrix designed to accommodate sorghum grain. The samples were sanded until the seeds were approximately halved. The endosperm texture of individual grains was studied in a Polyvar microscope (Reichert AG, Vienna, Austria) using incident polarised light. In this light the smooth vitreous part appears dark while the floury part appears bright. The image was transmitted to an image analyser (Quantimet 720, Cambridge Instruments, U.K.). The floury part and the whole endosperm area were deliniated on the TV-screen with a light pen. The endosperm texture was defined as the percentage of vitreous endosperm area in the total area of endosperm. Statistical analyses were performed with a HP-9835 computer.

Measurement of the work required to grind sorghum

Two different grinding principles were used to determine the work required to grind sorghum grain. Both were modified to record the energy used when pulverising a small sample.

Brabender hardness test. A Brabender hardness and structure tester (Duisburg, West Germany) was connected to a Brabender Do-corder type E in a manner similar to that described by Miller *et al.*[12] for determining the work required for grinding wheat.

The torque was recorded on a strip chart recorder (600 mm/min) and transferred to a HP-9825 computer via an analog/digital converter and a BCD-interface. The hardness and structure tester is a burr-type mill for grinding 50 g samples with a Do-corder speed of 63 rpm. We obtained reproducible results with 10·00 g samples by setting the clearance close to zero (2·5 on the scale ring) and the Do-corder speed at 30 rpm. The torque measurement range was between 0 and 25 J. The total work used for grinding was recorded automatically in the computer by integrating the torque/time plot. The following formula was used to calculate the work required to grind a sample:

Work (J) = Torque (J) × angular velocity (s^{-1}) × time (s).

Alpine universal mill hardness test. An Alpine laboratory universal hammer mill model 100 LU (Augsburg, West Germany) equipped with a perplex rotor, was operated at 19,000 rpm, with a 0·3 mm mesh triangular screen. The torque developed during grinding a 5·00 g sample was recorded and the work (in arbitrary units) required to grind the sample was calculated from the area under the curve by a digitiser (HP-9864A).

Sieve analyses

The flour from the Brabender mill was sieved on an Alpine laboratory air-jet sifter (Augsburg, West Germany) equipped with a 125 μm mesh screen. The sifting time was 60 s for a 20 g sample. The sieve analyses were based on a single determination for each genotype.

Breaking strength

The breaking strength of individual kernels was performed with a Kiya rice hardness tester[7].

Germination tests

The flotation tests were performed as described and both the floating grains and the settled grains were tested for germination efficiency. The grain was removed immediately from the liquid after flotation and washed with tap water for 3 min. After washing, the kernels were soaked in distilled water for 16 h. The grains were then germinated in Petri dishes containing fine sand (60 g) and water (15 ml). The sprouted seeds were counted after an incubation period of 48 h at 25 °C.

Gel spread tests

Sorghum flour was obtained by milling the grain samples on a Domestic Milcent (size-2) grinder equipped with two carborundum stones. A suspension of flour (10 g) in tap water (70 ml) was taken

TABLE I. *Mean properties for some grain, flour, and dough quality attributes of 15 sorghum genotypes*

Genotypes	Endosperm* texture (%)	PF† in $NaNO_3$ (%)	Grinding work‡ Brabender 10 g grain (J)	Grinding work‡ Alpine 5 g grain (arbitrary units)	Kernel breaking strength§ (kg)	Flour particle size‖ (% < 125 μm)	Dough rolling quality§ (cm)	Gel spread§ (mm)
2219B	78	5	70·0	27·5	6·5	14·0	22·4	55
E35-1	74	17	75·6	24·1	11·0	19·5	22·3	56
CSH-6	66	2	64·4	21·4	6·1	16·5	23·1	57
IS-5604	66	23	67·0	24·5	5·0	18·0	23·1	59
S-29	65	87	68·0	21·7	7·2	20·0	22·0	60
SPV-351	58	32	65·4	21·4	8·1	19·5	21·0	58
296B	48	57	56·8	18·0	6·3	26·5	21·0	62
SPV-422	48	69	59·9	18·1	7·9	25·5	18·5	61
CSH-8	47	45	59·9	18·6	5·8	25·0	22·4	59
SPV-393	45	63	62·9	20·1	6·8	22·0	22·0	62
M35-1	38	67	56·8	14·9	5·7	28·5	22·5	60
2077B	36	70	50·7	15·9	4·1	29·0	23·0	58
IS-9985	31	98	51·2	13·2	6·1	37·5	19·3	67
IS-1401	24	97	40·6	12·1	4·3	33·0	18·1	67
P721ø	17	99	43·8	9·4	5·2	29·5	14·5	67
Mean	52·6	55·4	59·5	18·7	6·5	24·3	21·0	60·6
Standard deviation	5·1	4·5	0·80	0·88	0·51	—	0·59	1·33
Coefficient of variation	9·6	8·1	1·34	4·71	7·9	—	2·8	2·2

* Means of 50 kernels.
† PF = Percentage of kernels floating in $NaNO_3$ solution. Means of triplicate determinations.
‡ Means of duplicate determinations.
§ Means of nine determinations.
‖ Single determinations.

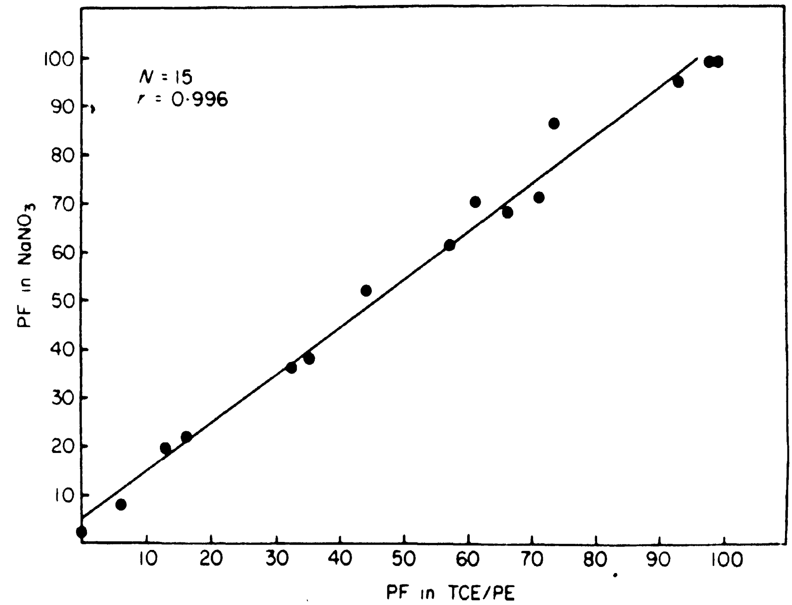

FIGURE 1. The relationship between the percentage of floating kernels (PF) in a sodium nitrate solution and in a mixture of trichloroethylene and petroleum ether (TCE/PE) for 15 sorghum genotypes. The correlation coefficient is significant at $P = 0{\cdot}001$.

and stirred into boiling water (140 ml). Cooking was continued for approximately 10 min. The resulting porridge was then poured into Petri dishes (20 x 52 mm, coated with a thin layer of oil) and cooled in a refrigerator at 10 °C for 3 h. The gel spread was determined by placing the gel on a glass surface and measuring the gel mass diameter (in mm) after 5 min at room temperature[8].

Rolling quality

Roti dough was prepared[7] by mixing a 30 g sorghum flour sample with the required amount of water (25–30 ml). The dough was rolled with a pin until the *roti* dough broke. The maximum diameter (in cm) was recorded as the rolling quality.

Results and Discussion

Results obtained from flotation tests on the 15 sorghum samples are presented in Table I. The percentage of total grain that floated (PF) ranged from 2 to 99% with a coefficient of variation (CV) of 8·1%. Fig. 1 shows the relationship between flotation in the organic solvent mixture and in sodium nitrate for the 15 sorghum genotypes. First we performed the flotation test in the trichloroethylene/petroleum ether mixture. The same kernels were then dried and used in the aqueous test solution. The loss of moisture after the organic flotation was insignificant. The result shows that the sodium nitrate solution can substitute for the organic solvents as a flotation medium. In the germination test using genotypes 2219B, M35-1 and 296B, neither the organic nor the inorganic test affected the germination.

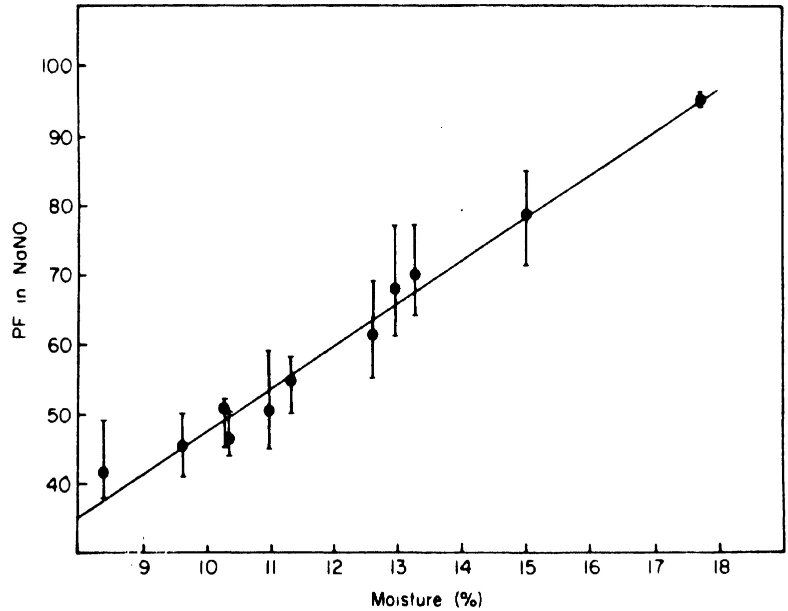

FIGURE 2. The relationship between the percentage of floating kernels (PF) in sodium nitrate solution and grain moisture content. The graph shows mean values and ranges obtained from four replicate experiments.

The influence of moisture content on the flotation test was investigated using genotype 296B (not identical with the sample in Table I) with an intermediate endosperm texture. The relationship between moisture content and PF is illustrated in Fig. 2. As grain moisture increased from 8·4 to 17·8% (w/w) the PF increased from 40 to 95%. Thus, for accurate measurements it is necessary to equilibrate the seeds at constant humidity to eliminate differences in grain moisture contents. Minor variations in grain moisture content ($\pm 0{\cdot}5\%$) presented no significant errors when using the flotation method. In

A highly significant correlation ($r = -0{\cdot}84$) was obtained between PF and endosperm texture. However, sample S-29 deviated significantly from the other samples (Fig. 3). This deviation may be due to two factors: (1) S-29 was the only sample that was harvested prematurely, and (2) the S-29 genotype contains a very thick mesocarp. Later experiments on another sample of S-29, harvested at maturity and on other thick mesocarp lines, showed only minor deviations from the regression line in Fig. 3. Thus, we conclude that the deviation of S-29 resulted from premature harvesting. Further correlation studies were carried out with only 14 genotypes, excluding S-29.

The correlation between PF and percent endosperm texture ($r = -0{\cdot}96$ significant at $P = 0{\cdot}001$) indicated that determination of PF can measure indirectly the endosperm texture in a grain sample. This confirms the findings of Kirleis and Crosby[8] who found a correlation ($r = -0{\cdot}95$) between the PF in organic solvents and grain vitreousness or endosperm texture.

Kosmolak[13] and Miller *et al.*[14] used a Brabender micro-hardness tester for grinding small quantities of wheat. They found that grinding time could be used to classify wheat

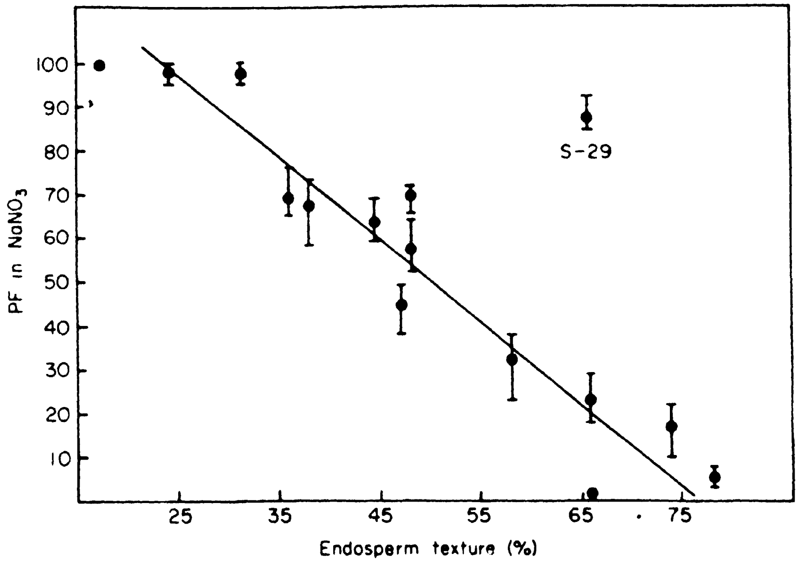

FIGURE 3. The relationship between the percentage of floating kernels (PF) in sodium nitrate and endosperm texture. Mean values and ranges are from triplicate experiments.

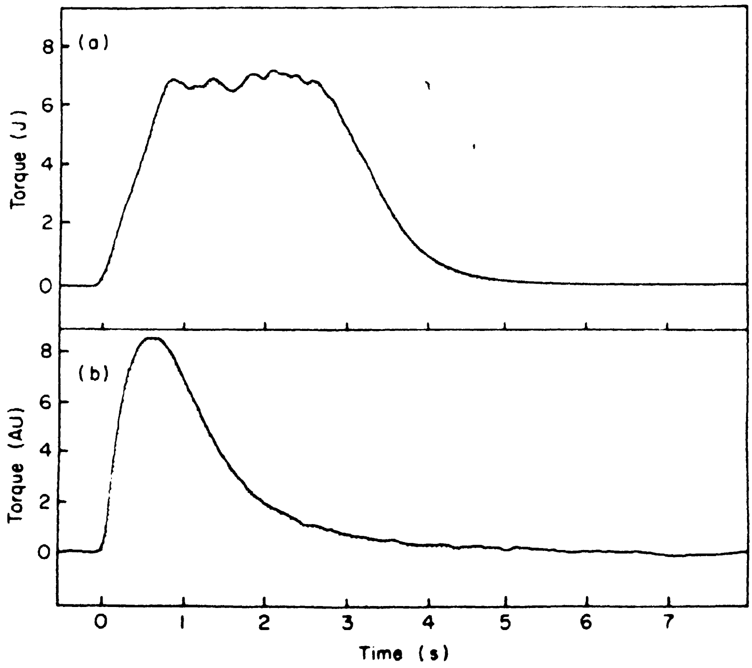

FIGURE 4. Torque-time plot obtained from grinding experiments on sorghum genotype 296B. The shaded area represents grinding work. (a) Brabender hardness and structure tester. (b) Alpine universal mill hardness tester, torque measured in arbitrary units (AU).

TABLE II. *Correlation matrix for PF, endosperm texture, grain hardness, flour, and dough quality parameters on 14 sorghum genotypes.*

	PF†	Endosperm texture	Brabender grinding work	Alpine grinding work	Kernel breaking strength	Flour particle size	Dough rolling quality	Gel spread
PF	1							
Endosperm texture	−0·96***	1						
Brabender grinding work	−0·88***	0·94***	1					
Alpine grinding work	−0·92***	0·97***	0·93***	1				
Kernel-breaking strength	−0·42	0·56*	0·70**	0·48	1			
Flour particle size	0·93***	−0·89***	−0·84***	−0·90***	−0·40	1		
Dough rolling quality	−0·71**	0·67**	0·66**	0·70**	0·13	−0·55*	1	
Gel spread	0·90***	−0·86***	−0·83***	−0·85***	−0·51	0·82***	−0·78***	1

* Significant at $P = 0{\cdot}05$; ** Significant at $P = 0{\cdot}01$; *** significant at $P = 0{\cdot}001$.

† PF = Percentage of kernels floating in $NaNO_3$ solution.

according to hardness. We believe that the work required for grinding in a Brabender hardness and structure tester as well as in an Alpine hammer mill is a good estimate of grain hardness in sorghum. The grinding work for the 15 sorghum samples in the Brabender mill and the Alpine mill is presented in Table I. The torque-grinding time curves for cultivar 296B are shown in Fig. 4. The endosperm texture was correlated highly with the work required to grind sorghum grain in both mills (Table II). A high (negative) correlation was observed between PF and the work required to grind the grain. A correlation coefficient of $r = 0{\cdot}93$ was observed between PF and the percentage of stock through a 125 μm mesh screen after grinding in the Brabender mill. Kirleis and Crosby[2] obtained a positive correlation between PF, particle size, and grain hardness (determined as pearling resistance) and our results agree with their finding.

On the contrary, for wheat, Svensson[15] found no relationship between PF in sodium nitrate and grain hardness (determined as pearling resistance). Trapped air bubbles present in the crease of the wheat kernel might cause problems in flotation in sodium nitrate, whereas this problem does not arise with the more regular, round kernels of sorghum.

In our studies, endosperm texture and Brabender grinding work were positively correlated with breaking strength. This is to be expected as breaking strength is an alternative indication of grain hardness related to grinding.

Dough rolling quality was correlated negatively with PF ($r = -0{\cdot}71$), particle size and gel spread and was correlated positively with kernel characteristics such as endosperm texture and the work required for grinding in the Brabender and the Alpine mill. Thus, parameters characteristic of soft grain such as a high PF and a fine particle size facilitate gel spreading. Endosperm texture and milling work (resistance to grinding) are negatively correlated with gel spread.

The sodium nitrate flotation method described here is simple and can be used effectively to classify a large number of grain samples on the basis of endosperm texture. Breeders can evaluate test samples rapidly and precisely against a selected check sample. Compared with organic solvents, the sodium nitrate test solution is non-toxic and non-flammable. Further investigations into the effects of factors such as poor grain filling, storage, and insect and fungal damage on sorghum grain flotation tests are in progress.

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