Rapid Determination of Germ Damage in Cereal Grains[1]

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ABSTRACT

The Christensen-Qasem bleaching method, used to reveal germ damage in wheat and corn, was studied for possible modification. Increasing the concentration of NaOCl to 6% made it easier to detect discolored germ. A bleaching-time study on wheat showed the pericarp sufficiently altered after 3 min. in hot bleach solution to show germ color readily. Modifications in the bleaching procedure were developed for rye, grain sorghum, oats, and barley. Grading-time studies with federal grain inspectors showed that the sum of processing and grading times for the modified bleaching method was significantly less than the grading time required with the present method. Germ-damage percentages shown by the modified and the current methods correlated significantly ($r = 0.88$, $n = 87$). Germination and glutamic acid decarboxylase activity for wheat and sorghum each correlated more highly with germ-damage percentage determined with bleached than with unbleached grain.

Accurately assessing germ damage in cereal grains is a major problem in determining their grades. Visual inspection of germ color is possible after tedious and time-consuming scraping of the germ covering. Studies with 50 licensed grain inspectors showed that germ damage is the most commonly overlooked grain damage (1). Accurate evaluation of grain for germ damage is required for good storage and final product quality. Numerous methods have been suggested as possible replacements for the present visual-inspection technique of germ damage. Fat acidity (2,3) and fluorescence (4) have shown significant correlations with percentages of graded germ damage. Grain viability, which relates closely to graded

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germ-damage percentages, correlates significantly with the viability test with tetrazolium dyes (5) and to a lesser extent with inorganic phosphorus levels (6). Linko (7) showed that glutamic acid decarboxylase activity (GADA) and graded germ-damage percentage correlated highly (r = 0.878). Christensen and Qasem (8) treated wheat and corn with boiling solutions of 2% sodium hypochlorite (NaOCl) to reveal discolored germ. Their grading studies resulted in a good relationship between the current grading procedure and the bleaching method. The percentage of germ-damage was determined by counting the wet kernels. Feller and Buchholz (9) confirmed Christensen and Qasem's results and applied the method to rye with no major alteration.

This paper describes the development of a modified bleaching procedure and its comparison with the present techniques of visual germ-damage evaluation and kernel viability.

MATERIALS AND METHODS

Grain Samples

Commercial grain samples with 0 to 80% germ damage were obtained from the Grain Division of the Consumer and Marketing Service in Kansas City, Mo. Additional samples of oats and sorghum were obtained from Kansas inspection stations at Atchison and Salina. Two rye samples with high germ damage were obtained from the Department of Agronomy at Kansas State University. Sprouted kernels were prepared in the laboratory by placing kernels in moist paper towels for 2 to 4 days.

Bleach Concentration and Time Series

Six 30-g. portions of 20% germ-damage grain were divided from a commercial wheat sample and placed in 1,000-ml. beakers. Bleach solutions were prepared by diluting a 12% NaOCl commercial bleach to concentrations of 2, 4, 6, 10, and 11%. Seventy-five milliliters of the diluted and full-strength bleach solutions was added to the beakers and heated to boiling. The hot bleach was discarded and the grain was washed three times with cold tap water. The air-dried grain was inspected visually to determine which bleach concentrations most effectively revealed germ color.

Samples for the evaluation of bleaching time were prepared as above. Wheat samples were treated with 6% NaOCl commercial bleach for 1 to 20 min. Timing started after the bleach was added and the beaker was placed on a hot plate. Dried grain was evaluated visually to determine which samples had been adequately bleached.

One additional 10-g. sample, treated with 75 ml. of 6% NaOCl commercial bleach for 24 hr. at room temperature, was washed, dried, and inspected visually.

Different Bleaches

The bleaching actions of reagent grade 6% NaOCl and commercial bleach containing 6% NaOCl were compared, using the 3-min. heat treatment on wheat. A comparison of the action of NaOCl commercial bleach after aging was made. Additional testing was made to check the action of three other bleaches. Sorghum and wheat samples were treated with chlorine gas for 24 hr. Three-minute heat treatments, with 30% H_2O_2 and commercial powdered bleach containing 70% calcium hypochlorite [$Ca(OCl)_2$] mixed in water, were evaluated on wheat samples.

Finalized Bleaching Procedure

A grain sample of 20 to 30 g. was placed in a 1,000-ml. beaker and covered with 75 ml. of 6% NaOCl commercial bleach. The beaker was transferred to a magnetic-stirring hot plate that had warmed enough to bring 250 ml. of water at 25°C. to a rolling boil in 10 min. After being heated and stirred moderately for 3 min., the beaker was removed and the bleach decanted. The grain was washed three times with cold tap water and dried in an air oven at 150°C. for 5 min. A preliminary step was required to bleach oats and barley. Treating these grains with 100 ml. of boiling 15% HCl for 2 min. was required to loosen the hulls. After the acid was discarded and the grain washed, the above bleaching procedure was used. Proper ventilation or a chemical hood is required with this procedure.

Comparison of Bleaching and Present Grading Methods

Preliminary testing of the bleaching method employing three sorghum samples and one rye sample was designed, using six Kansas grading stations. Each station received a bleached and an unbleached sample, numbered differently to conceal matching pairs. Visits were made to the stations to conduct the grading tests for germ damage and observe grading practices. Characteristics of germ damage in bleached grain were described to the inspectors before grading the samples. Grading times and damage percentages were recorded for each sample. Similar time studies were done on corn, wheat, and rye by the Grain Division of the Consumer and Marketing Service in Kansas City, Mo.

To increase the number of observations and, thus, the reliability of the regression analysis, additional bleached and unbleached samples of corn, sorghum, wheat, and rye were graded by both state and federal laboratories. Six bleached and unbleached sample pairs were sent to each of the six Kansas stations; 35 sample pairs were sent to the Kansas City federal laboratory. This provided a total of 87 observations when the time-study data were included. A second sorghum study of 24 observations was made at a later date at the Kansas City federal laboratory.

Viability Tests and Graded Germ Damage

Ten different wheat samples were each divided into four representative portions to compare bleaching and current grading methods with grain-viability tests. Sorghum samples were prepared identically for the two viability tests, germination, and GADA.

Germination. Samples of 100 kernels were surface-sterilized with 0.1% $HgCl_2$ solution for 2 min., washed five times with tap water, wrapped in wet paper towels, placed in 600-ml. beakers, covered, and stored at room temperature. Germinated seeds were removed every 2 days for a week before termination of the test.

Glutamic Acid Decarboxylase (GADA). Grain samples were ground in a Wiley mill with a 20-mesh screen; 30-g. samples of ground grain were mixed with 15 ml. of 0.1M glutamic acid in 0.067M phosphate buffer in pressure-meter cups. Sandstedt-Blish pressure meters, with violet-colored ethyl lactate in the manometer, were assembled and placed in a 30°C. water bath. After 5 min. equilibration, the manometers were adjusted to zero, and pressure readings were recorded after 30 min. (7).

Statistical Analyses

Simple analysis of variance and tests for interaction, assuming normal population and homogeneous variance, were determined (10).

A multiple regression program supplied by the Department of Statistics at Kansas State University provided an F-test of the coefficient of determination (r^2) and a t-test of the regular regression line coefficient (B). Significance for the r values was determined from the appropriate table (11).

RESULTS AND DISCUSSION

Bleach Concentration and Time Series

Christensen and Qasem (8) used NaOCl in 2% solution to bleach grain. Figure 1, however, indicates that stronger NaOCl solution would bleach better. With less than 5% NaOCl, some bleached kernels do not show the characteristics of bleached grain and resemble frostbitten wheat rather than the polished kernels resulting from stronger bleaching solutions. Increased concentrations of NaOCl produce fewer dull nonbleached kernels. The bleach concentration series indicates 6% NaOCl is adequate. Concentrations of 6 to 11% show acceptable results and basically the same coloration.

Results of the time studies are shown in the first two rows of Fig. 1. Kernels bleached 1 min. display none of the characteristics of bleached grain. After 2 min., bleach characteristics are visible, but color is still darker than in samples treated 3 min. or more. The kernels treated in hot bleach for 20 min. show some germ deterioration and irregular coloration. The effect of prolonged bleach is shown by the sample soaked in 6% bleach for 24 hr. at room temperature. Long periods and high concentrations cause kernel deterioration.

Different Bleaches

The action of NaOCl on grain was unqiue among the bleaches tested. Exploratory tests with sorghum and wheat showed that chlorine gas produced from a chemical generator severely blackens grain. Boiling solutions of various concentrations of $Ca(OCl)_2$ gave grain a chalky appearance. Wheat treated with boiling 30% H_2O_2 for 3 min. was whitened, but failed to reveal germ damage (Fig. 1, row 4).

The identical bleached appearance of kernels bleached with 6% NaOCl and those bleached with a 6% commercial bleach (sections 2 and 3, last row of Fig. 1) show that bleaching is not significantly affected by the additional agents used in the commercial bleach. Using commercial bleaches instead of a reagent-grade NaOCl will reduce costs considerably. Section 4 of the last row of Fig. 1 shows the results of using an old 12% commercial bleach on wheat. Six months earlier, the same 12% bleach produced the bleached wheat shown in row 3, column 4.

Characteristics of Bleached Grains

Sorghum presents a very difficult germ-grading problem because of its small kernel size, shape, and well-concealed germ. Certain pigmented sorghums, such as brown sorghum, are difficult to bleach and may require more than one treatment. Bleaching greatly aids germ-damage evaluation in sorghum, as shown in Fig. 2. Bleaching also reveals the brown pigment of sorghum to assist in determining sorghum class.

All classes of wheat and rye were bleached successfully with 6% bleach. The response of rye to bleaching is similar to that of wheat (Fig. 2). The color changes during bleaching make heat damage easier to detect.

Grains such as oats and barley, having heavy hulls, are difficult to grade. Electric

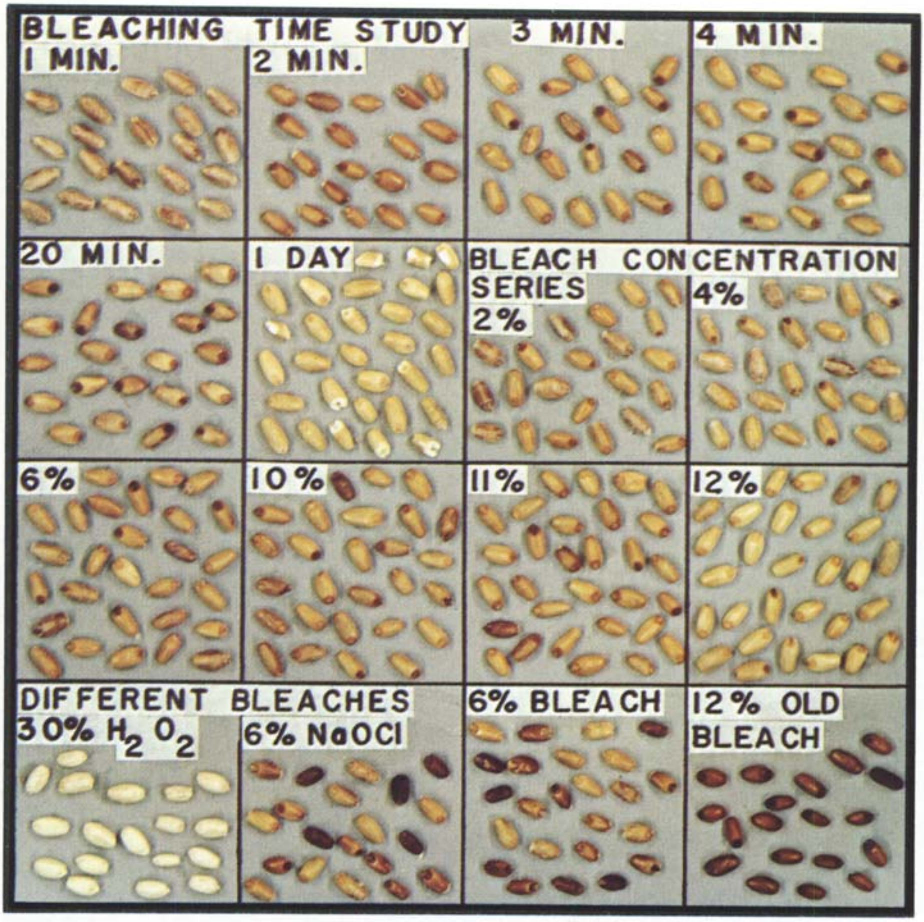

Fig. 1. Bleached wheat—using various concentrations, times, and types of bleach.

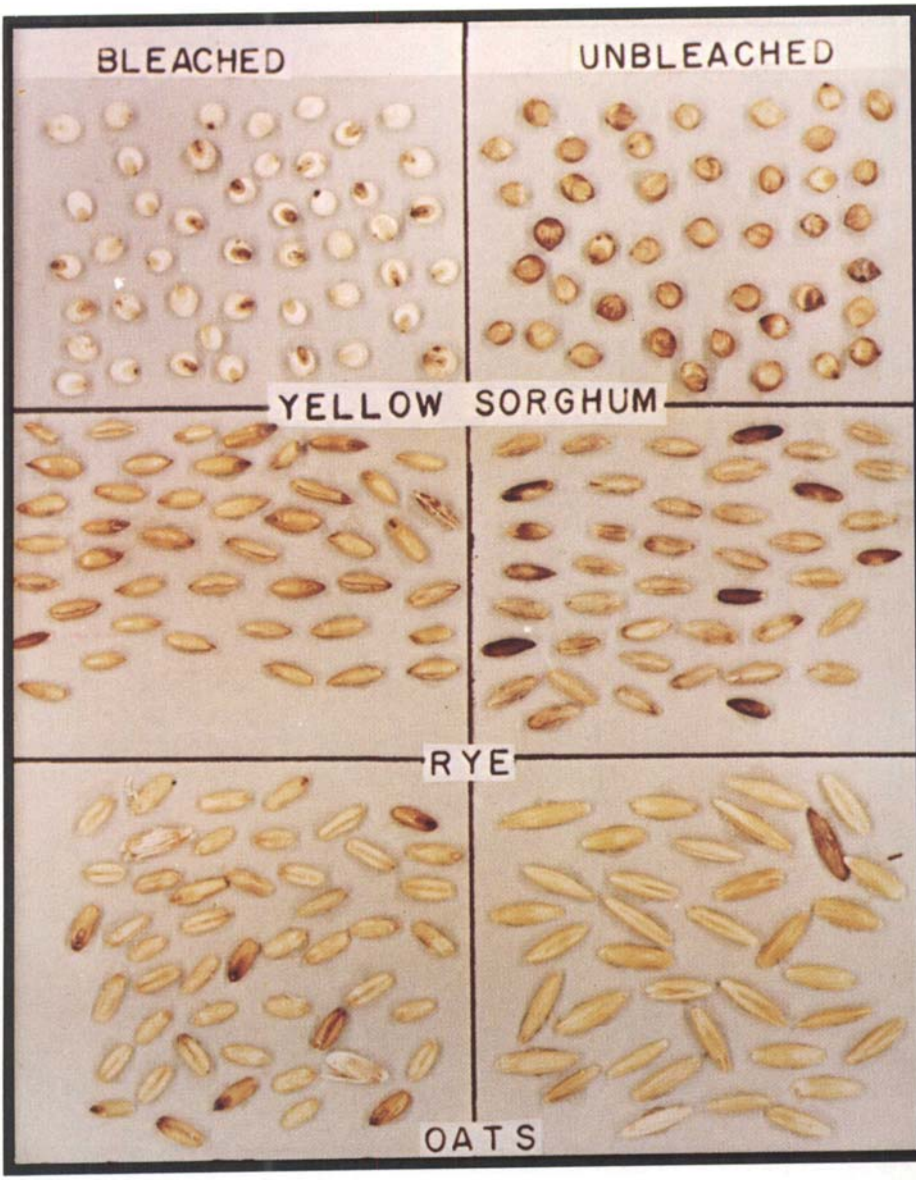

Fig. 2. Comparison of bleached and unbleached sorghum, rye, and oats.

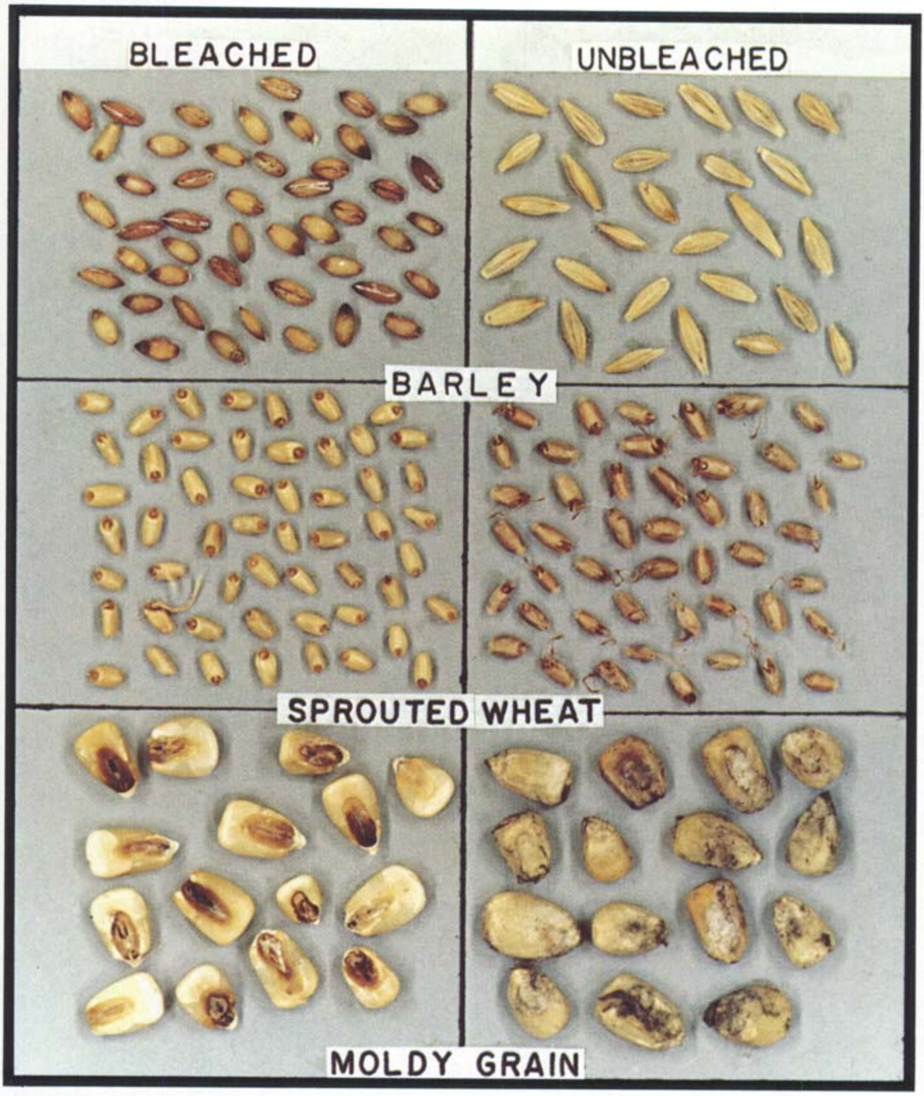

Fig. 3. Comparison of bleached and unbleached barley, sprouted wheat, and moldy corn, showing the effect of bleaching on surface damage.

barley pearlers have been used as part of the regular grading procedure. Once the barley hull is removed, heat and germ damage can be evaluated easily. However, oats must be dehulled by hand. Germ and heat damage are expressed as the percent damage after both the groat and hull are weighed. Bleaching often does not completely remove the oat hulls, as shown by two kernels with partially remaining hulls in the bottom row of Fig. 2. Bleached barley is shown in the top row of Fig. 3.

Since germ damage is considered alone only when it is the single grading factor, the effect of bleach must be considered on other types of damage. Black-tip fungus is totally removed by bleaching. Sprouts (second row of Fig. 3) are also removed in part or totally in some cases. Surface mold (bottom row of Fig. 3) is completely removed from corn. Other surface damage in bleached grain would also be difficult, if not impossible, for the grader to evaluate. Thus, germ damage must be evaluated on a separate sample and then added to other percents of damage found in an unbleached sample.

Correlation Studies at Kansas Stations

Practices in germ grading varied among the Kansas stations. Sample sizes used for evaluation of germ damage varied from 2 to 10 g. Some inspectors scraped every kernel; others scraped only those which appeared to be unsound. Most graders used pointed forceps for removing the germ covering, while others used razor blades or knives.

Table I shows the germ-damage percentages for each sample determined at each station. The correlation coefficient between germ damage in bleached and unbleached grain was 0.99**.

Percentages of germ damage varied widely. For example, unbleached sorghum 8 varied from 1.4 to 13.0%. Variation in the same bleached sample (bleached sorghum 7) was 6.0 to 12.3%. However, the bleaching method was new to the inspectors and some variation would be expected until they became proficient with the new procedure. A simple one-way analysis of variance showed that variations within were greater than among stations; values among stations were not significantly different.

Sorghum samples 1, 3, 4, and 5 were from the same sample, permitting an additional test for interaction of station and method; but no interaction was found.

Average grading rates for sorghum, determined at the six stations, in g. graded

TABLE I. PERCENTAGES OF GERM DAMAGE REPORTED AT SIX KANSAS GRADING STATIONS

Sample Graded	Germ-Damage Range	Sample Average
Sorghum 1, bleached	0.0– 1.0	0.3
Sorghum 4, unbleached	0.0– 1.1	0.4
Sorghum 3, bleached	0.0– 3.0	1.3
Sorghum 5, unbleached	0.0– 1.3	0.6
Sorghum 7, bleached	6.0–12.3	12.9
Sorghum 8, unbleached	1.4–21.0	11.0
Rye 2, bleached	0.0– 0.5	0.1
Rye 6, unbleached	0.0– 0.6	0.1

per min. were 1.58 for bleached and 0.59 for unbleached grain. Rates for bleached and unbleached rye were 3.13 and 2.35 g. per min., respectively. The time saved by grading a 30-g. sample of bleached grain rather than unbleached is 30.86 min. for sorghum and 3.19 for rye.

Time Studies

Inspectors at the Grain Division of the Consumer and Marketing Service in Kansas City, Mo., evaluated four samples of bleached and unbleached corn, rye, and wheat for germ damage. Grading speeds were measured and found to be faster for bleached grain by 1.1 g. per min. for corn; 0.2 g. per min. for rye; and 1.0 g. per min. for wheat. The r-values for comparing the two methods were 0.99***, 0.99***, and 0.99***, respectively. These were slightly higher than r-values obtained at the Kansas grading stations.

A proper comparison of grading time for bleached and unbleached samples must include the time required for the bleaching process. Processing time for bleaching requires 8 to 10 min., depending on the speed of the worker and the moisture content desired. Drying at 150°C. for 5 min. results in about 13% moisture. With grading speeds and a 9-min. bleach-processing time, a 30-g. sample of corn would require 7.67 min. more than the present procedure. However, 30-g. samples of sorghum, wheat, and rye would be graded 23.86 min., 9.00 min., and 59.66 min. faster with the bleaching method. Bleaching would enable inspectors to grade a larger sample than is presently being graded and thus increase the statistical soundness of the determination. The increased productivity of the grader might also decrease the tediousness of the work so that grading errors from fatigue might be fewer.

Summary of Regression Analysis for Grain Composites

The results for all observations for correlation of the two methods for wheat, rye, sorghum, and corn are shown in Table II. All data sets of the F-test of r^2 and the t-test of B are significant. The higher correlation determined from the sorghum

TABLE II. REGRESSION ANALYSIS FOR GRAIN COMPOSITE PERCENTAGES OF GERM DAMAGE FOUND IN BLEACHED AND UNBLEACHED GRAINS

Sample	r	r^2	Regression Coefficient B
Wheat N=34	0.98***	0.96***	0.90***
Sorghum I N=25	0.74***	0.54***	0.40***
Corn N=14	0.84***	0.70***	0.95***
Rye N=14	0.74***	0.55***	0.74***
Summary of above grains N=87	0.88***	0.77***	0.83***
Sorghum II N=24	0.98***	0.97***	0.98***

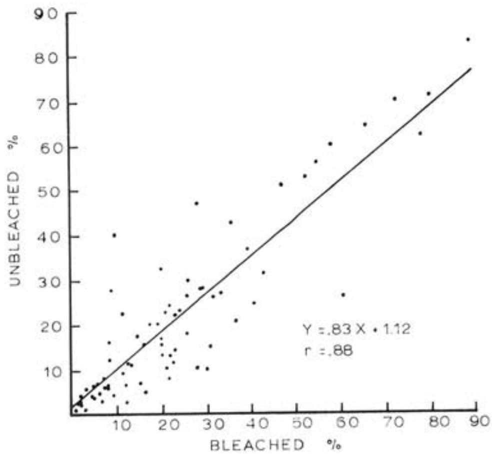

Fig. 4. Percentages of germ damage determined in bleached and unbleached grain samples, plotted as ordered pairs (bleached, unbleached) for 87 observations.

II data is believed to result from using only the federal grain laboratory and increased proficiency with the method.

Figure 4 shows the graphic results of 87 observations for comparing the two methods. The slope of the regression line indicates that damage percentages were higher in the bleached samples than the unbleached samples. The germ-damage percentages ranged from 0 to 87%, with the greatest concentrations for points below 30%. Evaluation of grain samples in the low germ-damage range was considered more applicable to actual grading situations because numbered grain grades are determined by relatively low germ-damage levels.

Germination and GADA Correlation Study

Results of regression analysis comparing the bleaching procedure and the present grading technique with germination and GADA are shown in Tables III and IV. Germination percentages ranged from 0 to 97%, whereas graded damage never fully reflected these low germination values. Correlations between grading and viability tests were higher for wheat than for sorghum. In all data sets, higher r-values resulted from the comparison of germ damage found in bleached than in unbleached grain.

CONCLUSION

Treating grain with 6% NaOCl makes detection of germ damage, heat damage, and sorghum pigment easier. Germ-damage determination by the bleaching method reduces grading times for 30 g. of grain sorghum, wheat, and rye by 23, 9, and 59 min., respectively. Correlation of the current grading method with the bleaching method is significant for wheat, sorghum, rye, and corn. Percentages of germ damage found in bleached grain correlated to a higher degree with germination and GADA than did percentages found in unbleached grain. Thus, the bleaching method

TABLE III. REGRESSION ANALYSIS FOR PERCENT GERMINATION AND GADA ON TEN SAMPLES OF WHEAT

Values Compared	r	r^2	Regression Coefficient B
Bleached, unbleached	0.99***	0.97***	0.895***
Bleached, germination	−0.97***	0.95***	−1.132***
Bleached, GADA	−0.96***	0.92***	−0.009***
Unbleached, germination	−0.95***	0.90***	−1.217***
Unbleached, GADA	−0.90***	0.90***	−0.010***

TABLE IV. REGRESSION ANALYSIS FOR PERCENT GERMINATION AND GADA ON TEN SAMPLES OF GRAIN SORGHUM

Values Compared	r	r^2	Regression Coefficient B
Bleached, unbleached	0.90***	0.80***	0.39***
Bleached, germination	−0.89***	0.79***	−1.604***
Bleached, GADA	−0.79***	0.63**	−0.006**
Unbleached, germination	−0.64*	0.42*	−2.658*
Unbleached, GADA	−0.56	0.32	−0.010

has been shown to be a faster and a better index of germ condition than the current method.

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Literature Cited

1. TRUMBO, D. A., ADAMS, C. K., MILNER, M., and SCHIPPER, L. Reliability and accuracy in the inspection of hard red winter wheat. Cereal Sci. Today 7: 62 (1962).
2. BAKER, D., NEUSTADT, M. H., and ZELENY, L. Application of the fat acidity test as an index of grain deterioration. Cereal Chem. 34: 226 (1957).
3. BAKER, D. A colorimetric method for determining fat acidity in grain. Cereal Chem. 38: 47 (1961).
4. COLE, E. W., and MILNER, M. Colorimetric and fluorometric properties of wheat in relation to germ damage. Cereal Chem. 30: 378 (1953).
5. BAIRD, P. D., MacMASTERS, M. M., and RIST, C. E. Studies on a rapid test for the viability of corn for industrial use. Cereal Chem. 27: 508 (1950).
6. GLASS, R. L., and GEDDES, W. F. Grain storage studies. XXVII. Inorganic phosphorus content of deteriorating wheat. Cereal Chem. 36: 186 (1959).
7. LINKO, P. Quality of stored wheat. Simple and rapid manometric method to determine glutamic acid decarboxylose activity as quality index of wheat. J. Agr. Food Chem. 9: 310 (1961).
8. CHRISTENSEN, C. M., and QASEM, S. A. Note on a rapid method of detecting germ damage in wheat and corn. Cereal Chem. 36: 461 (1959).
9. FELLER, K., and BUCHHOLZ, K. Eine einfache Schnellmethode zur Bestimmung von Keimschäden und gebräunten Körnern. Getreidemuhle 6: 66 (1962).
10. FRYER, H. C. Concepts and methods of experimental statistics. Allyn and Bacon, Inc.: Boston (1968).
11. YAMANE, T. Statistics: An introductory analysis (2nd ed.). Harper and Row: New York (1967).

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