A COLORIMETRIC METHOD FOR DETERMINING FAT ACIDITY IN GRAIN[1]

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ABSTRACT

A rapid colorimetric method for determining fat acidity in grain has been developed. The method is based upon the reaction of the fatty acids in benzene solution with aqueous cupric acetate to form soaps. The copper soaps are soluble in the benzene solution and the intensity of the resulting blue color of the solution is measured by a colorimeter. The intensity of the blue color measured as percent transmittance is compared with fat acidity values expressed as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in 100 grams of dry grain. For samples of wheat and corn the relationship between fat acidity values and percent transmittance is linear in the range of fat acidity values 20 to 100. The coefficient of correlation is −0.988 and −0.983, respectively, with standard deviations from regression of 2.47 and 3.42. The time required for the entire test is 10 minutes.

The fat acidity of grain is determined by extraction of the oil from the grain followed by titration of the free fatty acids present in the oil. In the method generally used for this determination (1), the oil is extracted from the grain with petroleum ether in a Soxhlet extractor and the titration is carried out in a solution of benzene-alcohol with standard aqueous potassium hydroxide solution. A rapid method (2) of similar principle has been proposed in which the extraction of the oil is simplified by using a grinder-extractor (Stein mill).[3] In both methods the fat acidity value is expressed as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in 100 g. of dry grain.

The new colorimetric method is based upon the reaction of fatty acids to form metallic soaps. Kaufman and Budwig (3) used cupric acetate to detect the spots of fatty acids on paper chromatograms. This paper reports an investigation of the reaction of cupric acetate with fatty acids dissolved in benzene. An aqueous solution of cupric acetate when shaken with fatty acids dissolved in benzene reacts with the fatty acids to form benzene-soluble copper salts which impart a blue color to the benzene solution. The reagent is sensitive enough to detect differences in the amounts of free fatty acids pres-

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[3]The mention of specific instruments is made for the purpose of identification and does not imply any endorsement by the United States Government.

ent in the oil from damaged grain. The differences in the smaller amounts of free fatty acids present in sound grain, whose fat acidity values are generally below 20, are not so easily detected. The intensity of the blue color in the benzene solution can be measured with an appropriate instrument. A comparative study was made of this colorimetric method with the usual procedure involving titration with standard potassium hydroxide solution.

Materials and Methods

Fat acidity was determined by the colorimetric method on 85 samples of wheat and 79 samples of corn. The transmittance readings were compared to the fat acidity values as determined by the method of the Association of Official Agricultural Chemists (1).

The percent transmittance of the solutions was measured with a Bausch & Lomb "Spectronic 20" spectrophotometer. As the fat acidity value increased, the intensity of the blue color increased and the percent transmittance decreased. Fatty acid solutions containing 10, 20, 30, 50, 80, and 100 mg. of fatty acids in 5 ml. benzene were treated with cupric acetate and transmittance curves from 350 to 700 mμ were made. From these curves the wave length of 640 mμ was chosen for measuring the percent transmittance of the copper soap solutions. At this wave length the transmittance of wheat and corn oil solutions in benzene is 98 to 100%.

A sample of grain weighing 40 g. is ground for 1 minute in a grinder-extractor (Stein mill). Then 50 ml. benzene are added to the ground sample and the oil is extracted by running the mill 4 minutes. The sample is filtered and 10 ml. of the filtrate measured into a test tube containing 2 ml. of 5% cupric acetate solution. The tube is stoppered and shaken by inverting rapidly 50 times by hand. After the solution has separated into two layers, the top benzene layer is decanted through a fluted filter into a colorimeter tube and the percent transmittance read at 640 mμ with a colorimeter or spectrophotometer. Benzene is used as the blank for adjusting the instrument.

Results and Discussion

Graphs of percent transmittance (Y) vs. fat acidity values (X) show a linear relationship in the range of fat acidity values 20 to 100. (See Figs. 1 and 2.) Above and below this range the relationship becomes curvilinear; therefore statistical analyses were confined to the samples within the range 20 to 100. Coefficients of regression and correlation for 60 samples of corn were -0.830 and -0.983, respective-

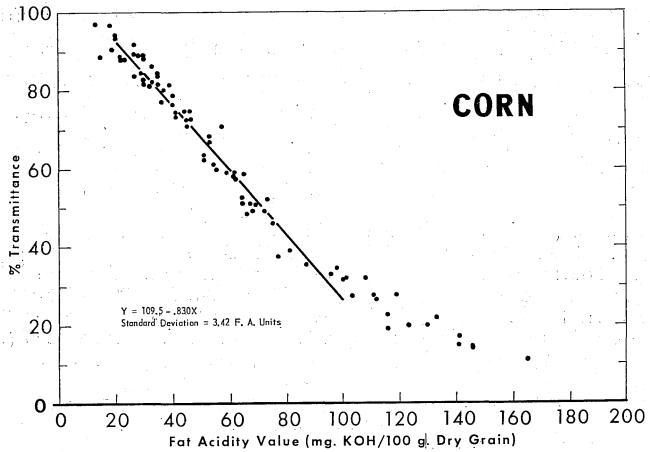

Fig. 1. Relation between fat acidity determined by the AOAC method and the percent transmittance at 640 mμ of benzene extracts of 79 corn samples after reaction with aqueous cupric acetate. (Y = 109.5—.830X; Standard Deviation = 3.42 F.A. units.)

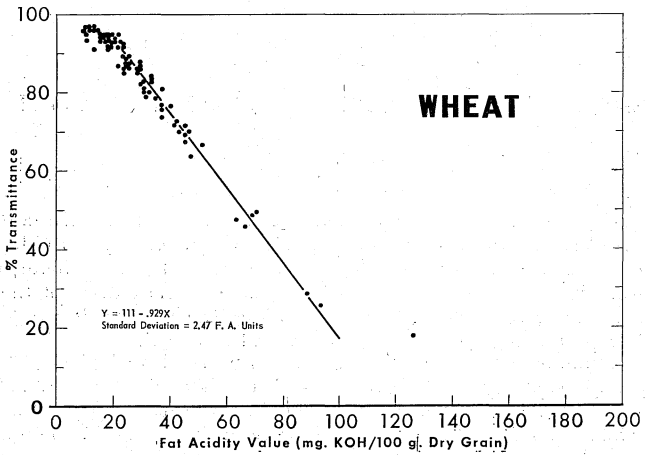

Fig. 2. Relation between fat acidity determined by the AOAC method and the percent transmittance at 640 mμ of benzene extracts of 85 wheat samples after reaction with aqueous cupric acetate. (Y = 111—.929X; Standard Deviation = 2.47 F.A. units.)

ly, with sample standard deviation from regression of 3.42 fat acidity units. For 54 samples of wheat, coefficients of regression and correlation were –0.929 and –0.988, respectively, with sample standard deviation from regression of 2.47 fat acidity units.

Since fat acidity values between 20 and 100 are significant in defining grain condition, the colorimetric method would be useful

where a rapid determination of fat acidity is needed. The entire test can be completed within 10 minutes. The regression lines of Figs. 1 and 2 may be used for converting transmittance readings to fat acidity values or for setting up a scale of transmittance readings indicating degrees of deterioration.

Literature Cited

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