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A Rapid Colormetric Test for Alcohol and Aldehyde Concentrations in Peanuts[1]

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ABSTRACT

The acidic potassium dichromate-silver nitrate reagent has been evaluated as a rapid colormetric test for alcohol and aldehyde concentrations in peanuts. Increased levels of alcohols and aldehydes have previously been related to off-flavor in peanuts. The acidic potassium dichromate-silver nitrate colormetric test was found to give a linear response for peanut samples which had been spiked with known ethanol concentrations between 10 and 100 nL/g. Peanut samples (200 g) may be assayed in seven minutes thus the method is applicable to the rapid analysis of large numbers of samples. Forty-four samples from commerical lots of peanuts were analyzed and 20 samples were determined to have above background concentrations of alcohols and aldehydes. Aroma response analysis and subsequent statistical analysis showed a curvilinear relationship between alcohol-aldehyde concentrations and aroma response and thus confirms the previously published reports on the alcohol-off-flavor relationship. The application of this method to quality control in peanut samples could be of significant value in improving the quality of peanuts being marketed and thus those being processed into consumer products.

Key Words: Off-flavor, quality, *Arachis hypogaea*, groundnut, marketing peanuts, alcohols, aldehydes.

Although extraneous and objectionable flavors in peanuts can arise from many sources, a major source of off-flavors in farmers stock peanuts is improper curing. Improper curing would be any set of conditions which allowed anaerobic respiration to become the predominant metabolic process. Whitaker and Dickens (9) found a high correlation between amounts of off-flavor in peanuts and the amount of anaerobic respiration. Anaerobic respiration becomes the predominate process as a result of the insufficiency of diffused oxygen to meet the increased aerobic respiration demands which result from the increased curing temperature. Anaerobic respiration produces ethanol and acetaldehyde as the principle products. In analyzing the volatile compounds from high-temperature cured peanuts, Pattee et al. (5) found ethanol and acetaldehyde to be the major compounds present. Singleton et al. (7), studying the influence of curing temperature on volatile compounds of peanuts, found ethanol to be the component which increased most rapidly with an increase in curing temperature. They postulated that acetaldehyde, ethanol, and ethyl acetate were primarily responsible for the off-flavor in high-temperature cured peanuts. Using Clerscored raw peanut samples and volatile profile analysis, Brown et al. (2) showed a highly significant ethanol to flavor relationship. Lovegren et al. (4) have also suggested that increases in alcohols and aldehydes are indicative of the presence of off-flavors in raw peanuts. The above studies all have in common the suggestion or conclusion that increased levels of alcohols and aldehydes in raw peanut samples are indicative of the presence of off-flavors.

Although gas chromatographic profiling of the volatiles in peanuts has been suggested as a method of ascertaining the quality of raw peanuts, this method has not been generally accepted because of the cost and time factors involved (6). The correlation of increased levels of alcohols and aldehydes to the presence of off-flavors suggests that a chemical colormetric test for alcohols and aldehydes might be sensitive enough to distinguish between acceptable-flavor and off-flavored samples. Such a test could be the photometric analysis for alcohols and aldehydes by the acidic potassium dichromate-silver nitrate reagent ($K_2Cr_2O_7$-$AgNO_3$) (1,3). The proposed test utilizes potassium dichromate in sulfuric acid solution as a reagent for oxidizing volatile components in peanuts such as ethanol and acetaldehyde, which have been related to increased off-flavor in peanuts. Ethanol is oxidized as shown in Equation 1. The reaction is accompanied by a color change from the yellow of the potassium dichromate to the green of the chromic sulfate, and is catalyzed by the presence of silver nitrate in the reagent. The amount of color change in the reagent is measured using a spectrophotometer and is proportional to the amount of alcohol delivered to a given quantity of reagent.

$$\underset{\text{(yellow)}}{2K_2Cr_2O_7} + 8H_2SO_4 + 3C_2H_5OH \xrightarrow[AgNO_3]{} \underset{\text{(green)}}{2Cr_2(SO_4)_3} + 2K_2SO_4 + 3CH_3COOH + 11H_2O \quad (1)$$

The objectives of this study were to establish a chemical test for peanuts using the acidic $K_2Cr_2O_7$ -$AgNO_3$ reagent and to determine if this test could distinguish between raw peanut samples with acceptable flavor and those with off-flavor.

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Methods and Materials

Analysis Method

Reagent: The acidic $K_2Cr_2O_7$ - $AgNO_3$ solution is made by dissolving potassium dichromate, 0.025% (w/v) and silver nitrate, 0.025% (w-v) in 50% reagent grade sulfuric acid (v/v).

A schematic drawing of the analysis system is given in Figure 1. Nitrogen gas is used as the volatile carrier at a flow rate of 190 mL/min and room temperature (ca.24C). The amount of alcohol and aldehydes delivered to the reagent is dependent upon the concentration of alcohols and aldehydes in the comminuted sample, the amount of sample, rate of perfusion by the carrier gas (N_2), the time of perfusion and the rate of diffusion of alcohols and aldehydes through the peanut particles. The rate of diffusion is dependent upon particle size, temperature and other characteristics of the peanut material. A flow rate of 190 mL/min for 5 min. was selected to prevent excessive bubbling of the reagent and to allow an approximate two volume gas exchange in the sample container (2x500 mL). The sample size of 200-g at room temperature was found to be suitable for the test, although the fritted tube and gas washing bottle arrangement (Figure 1) did not assure that the entire 200 g sample was perfused by N_2 flowing from bottom to top. At the end of the 5 min. period, the reacted acidic $K_2Cr_2O_7$ - $AgNO_3$ solution is transferred to a curvette and read at 440 nm in a Model 635D Varian spectrophotometer using unreacted acidic $K_2Cr_2O_7$ - $AgNO_3$ solution as the reference. Because the reaction produces a color decrease, the normal reference and sample positions are reversed to produce a positive absorbance reading. The reference solution is stable for 45 min. to 1 hr. in the spectrophotometer. The acidic $K_2Cr_2O_7$ - $AgNO_3$ solution stored in a sealed, amber glass container is stable for over two months.

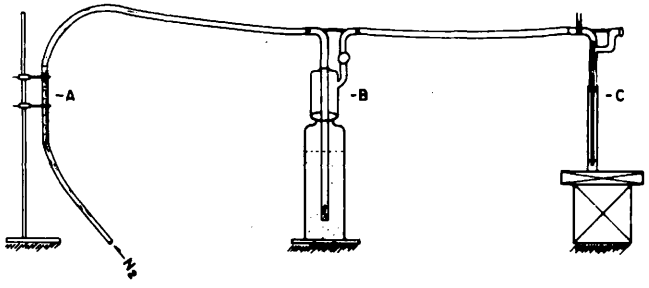

Fig. 1. Schematic diagram of the acidic potassium dichromate-silver nitrate-volatile analysis system. A. Roger Gilmont Instruments, Inc. Flowmeter, size no. 1. B. Sample container-500 mL gas washing bottle w/coarse fritted cyclinder. C. Reaction tube w/5 mL potassium dichromate-silver nitrate reagent.

Sample Sources

Large-seeded virginia type peanuts from the 1981 crop, obtained from a commercial source and evaluated to have a normal, acceptable flavor by a 10-member professional taste panel, Department of Food Science, North Carolina State University, were used as the standard peanut source. These peanuts were stored at 2-5C and 60-70% relative humidity (RH) from the time of purchase until analyzed.

Standard source peanuts spiked with known ethanol levels were used for the development of the standard curve. This was done by bringing the desired volume of ethanol to 10 mL with water using a volumetric flask, and applying the 10 mL volume to a 500 g peanut sample in a 1.057 L (quart) Mason jar. The spiked sample was equilibrated at 2-4C for 24 hr. and shaken twice during the first 3 hrs. to aid moisture distribution. After addition of the ethanol solution, the peanuts contained approximately 8.3% moisture (wet basis). The sample was then ground in a Waring Blender for 10 sec., thoroughly mixed, and subdivided into two 200 g subsamples. The subsamples were sealed in plastic bags, which were then sealed in a Mason jar for overnight equilibration. When a large number of samples were required at the same ethanol level, several 500 g samples were mixed together after grinding and subdivided into 200 g subsamples.

To obtain virginia type peanuts with a known curing treatment, NC 6 peanuts from the Peanut Belt Research Station, Lewiston, NC were cured in controlled environment chambers. The peanuts were harvested on October 17 and 30, 1983. The various curing conditions used were 32C, 50% RH; 40C, 70% RH: 47C, 70% RH: and 52C, 70% RH. The samples were rated for aroma intensity by three individuals with experience in organoleptic evaluations of peanuts with high-temperature curing off-flavor. The aroma rating was zero for no off-flavor and four for a high level of off-flavor. One is acceptable, two is marginally acceptable and three is an unacceptable level of off-flavor. Forty-four 1983 commercial source samples of unknown history, which weighed at least 90 g each, were obtained from Dr. T. H. Sanders, National Peanut Research Laboratory, Agricultural Research Service, USDA, Dawson, Georgia. Following the alcohol evaluation, the ground sample was sealed in an amber jar and stored at 2-4C until aroma rated. Aroma rating was conducted on room-temperature equilibrated samples by four panelists familiar with peanut off-flavors.

Sample Preparation

The peanut sample was ground for 10 sec. in a Waring Blender using a 1.057 L container and transferred to a 500 mL gas washing bottle with a coarse fritted cylinder.

Results and Discussion

Preliminary experiments established that the procedure described in this study could measure ethanol from spiked ethanol samples which ranged in concentration from 10 nL of ethanol per g of peanuts to above 100 nL/g. To establish a standard curve for the acidic $K_2Cr_2O_7$ - $AgNO_3$ test and to determine the variation among measurements with the method analyses were made on samples which contained each of six different spiked ethanol concentrations. Fourteen samples were analysed for each concentration. The method is linear between 10 and 100 nL of alcohol per g of peanut (Figure 2). The 95% confidence limits indicate that a single observation has a variation range of ± 15 nL/g for estimating the alcohol concentration from an absorbance reading. A standard $K_2Cr_2O_7$ - $AgNO_3$ absorbance response curve for ethanol, obtained by the direct addition of known quantities of ethanol to 5 mL of reagent, is given in Figure 3 and is the average of five observations. The response values for added ethanol compared to the spiked ethanol response values suggest that only about 1% of the spiked ethanol was diffused over to the reaction mixture by the N_2 gas. No attempt has been made to correct the analysis results from the commercial samples which follow, however, the term - pL dif-

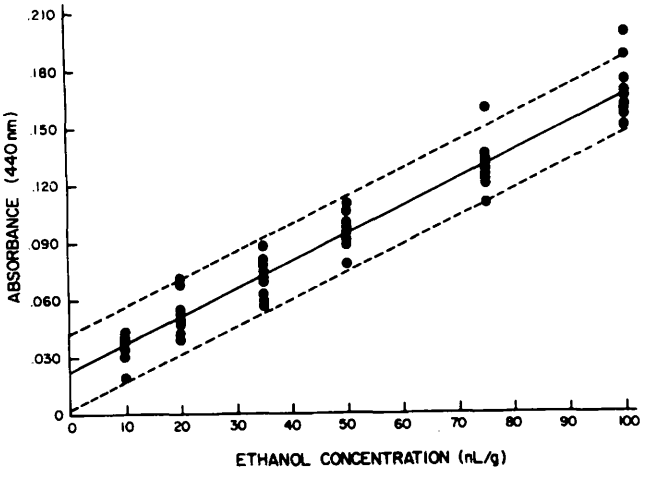

Fig. 2. Standard curve for acidic potassium dichromate-silver nitrate spiked ethanol analysis with 95% confidence limits. Note-standard reference and sample cell positions are reversed to produce positive response curve.

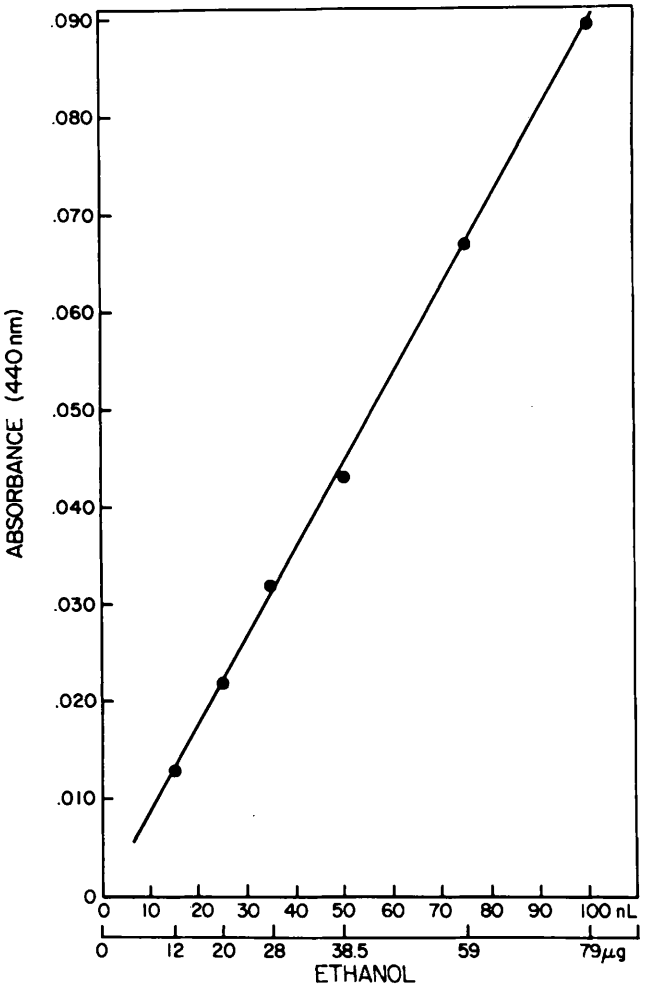

Fig. 3. Standard absorbance (440 nm) response curve from acidic potassium dichromate-silver nitrate reagent (5 mL volume) reaction with known ethanol levels. Note-standard reference and sample cell positions are reversed to produce positive response curve.

fused alcohol per g (pL DA/g)-is used to indicate the relative alcohol concentrations in the samples.

Alcohol and aldehyde levels in peanuts from commercial sources which had been evaluated for flavor by a professional taste panel as having no off-flavors, gave concentration values of 0 to 40 pL DA/g. Peanuts from the 1983 crop cured at 32, 40, 47, and 52C gave concentration values of 155, 40, 275, and 270 pL DA/g and preference ranking of 2, 1, 4, and 3, respectively. Concentration values are an average of triplicate samples. These data suggest that different levels of alcohols and aldehydes in peanut samples can be detected by the acidic $K_2Cr_2O_7$ - $AgNO_3$ test.

To determine if peanuts were being marketed with high concentrations of alcohols and aldehydes, the 44 commercial samples of unknown history, were tested. Twenty-four of the samples had values between 0 and 30 pL DA/g. Thirteen gave values between 66 and 138 pL DA/g and 7 samples had values above 160 pL DA/g. The 20 samples with values of 66 pL DA/g and above plus 7 random samples from the 0 - 30 pL DA/g group were aroma evaluated. The averaged pL DA/g values for aroma response levels of 0 through 4 were 43 pL DA/g, 81 pL DA/g, 129 pL DA/g, 159 pL DA/g, and 342 pL DA/g, respectively. The close agreement between aroma and flavor has been reported by Syarief (8).

The relationships established between off-flavor and alcohol level as indicated by $K_2Cr_2O_7$ - $AgNO_3$ values confirms the observations previously published (2, 4, 5, 6). This study has demonstrated that the $K_2Cr_2O_7$ - $AgNO_3$ test is sensitive enough to differentiate among peanut samples according to their alcohol-aldehyde concentration and that the method is rapid enough, 7 min. total time, to be applicable to large numbers of samples such as would be analyzed at peanut buying stations.

Literature Cited

1. Borkenstein, R. S. and H. W. Smith, The breath analyzer and its applications. Med. Sci. and the Law. 2:13-22.
2. Brown, M. L., J. T. Wadsworth, H. P. Dupuy and R. W. Mozingo. 1977. Correlation of volatile components of raw peanuts with flavor score. Peanut Sci. 4:54-56.
3. Dubowski, K. 1970. Measurement of ethanol in breath. *In*: F. W. Sunderman and F. W. Sunderman, Jr., Eds. Laboratory Diagnosis of Diseases Caused by Toxic Agents. Warren Green Inc., St. Louis, MO 63105.
4. Lovegren, N. V., C. H. Vinnett, and A. J. St. Angelo. 1982. Gas chromatographic profiles of good quality raw peanuts. Peanut Sci. 9:93-96.
5. Pattee, H. E., E. O. Beasley, and J. A. Singleton. 1965. Isolation and identification of volatile components from high-temperature-cured off-flavor peanuts. J. Food Sci. 30:388-392.
6. Sanders, T. H., A. M. Schubert, and H. E. Pattee. 1982. Maturity Methodology and Postharvest Physiology. *In*: H. E. Pattee and C. T. Young, Eds. Peanut Science and Technology. Amer. Peanut Res. & Educ. Soc., Yoakum, TX 77995, pp. 640-641.
7. Singleton, J. A., H. E. Pattee, and E. B. Johns. 1971. Influence of curing temperature on the volatile components of peanuts. J. Agric. Food Chem. 19:130-133.
8. Syarief, H. 1983. Statistical evaluation of flavor and texture profile methods for describing food products. Ph.D. thesis. Department of Food Science, North Carolina State University, Raleigh, NC. 167 pp.
9. Whitaker, T. B. and J. W. Dickens. 1964. The effects of curing on respiration and off-flavor in peanuts. Proceedings Third Natl. Peanut Res. Conf., Auburn, AL. pp 71-80.

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