Table IV. Ranking of Cooked Rice Samples in Terms of Those Having the Greatest Popcorn-like Aroma (at the Top of the Table) to Those Having the Least (at the Bottom of the Table)

Malagkit Sungsong	↑ greatest popcorn aroma
Milagrosa	
Khao Dawk Mali 105	
IR841-76-1	
Basmati 370	
Seratus Malam	↓ least popcorn aroma
Azucena	
Hieri	
Calrose	
Texas Long Grain	

to that with the least. The order found is that shown in Table IV. It can be seen that this order is in general agreement with the concentrations of 2-acetyl-1-pyrroline found in these varieties shown in Table I. Malagkit Sungsong was ranked as having the most popcorn-like aroma and Texas Long Grain the least.

In a third type of quality evaluation the two varieties of rice, Calrose and Malagkit Sungsong, were compared in a difference test. One variety of cooked rice (2 × 50 mL) was placed in two opaque identical flasks (100-mL stoppered Erlenmeyer). One of these flasks was labeled "control". The other variety of cooked rice (50 mL) was placed in a third identical opaque flask. The three coded flasks were placed in the panel booth side by side. The judges' tasks were to smell each flask and match one of the two coded unknown samples with the sample marked control. With 41 total judgements, the correct sample was matched 83% of the time. This is highly significant data that the panel can tell the difference between the odor of the two cooked rice varieties. The two rice varieties were next compared by using the same test except that 25 mL of a 0.05-ppm solution of 2-acetyl-1-pyrroline was added to each Calrose sample (25 mL of odor-free water was added to each Malagkit Sungsong sample). In this case, in 40 judgements, the correct sample was matched only 62% of the time. This is only slightly better than pure chance where the correct sample would be matched 50% of the time. These results support the fact that the main difference between the odors of the two varieties is the much greater concentration of 2-acetyl-1-pyrroline in the Malagkit Sungsong variety.

It might be noted that Malagkit Sungsong (a waxy rice variety) is used in the Philippines for the preparation of flattened parboiled brown rice wherein this popcorn-like odor has been synonymous to good quality.

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Registry No. 2-Acetyl-1-pyrroline, 85213-22-5; 2-acetylpyrrole, 1072-83-9; 2-(1-hydroxyethyl)pyrrolidine, 63848-93-1.

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A Direct Titrimetric Method for the Rapid Estimation of Water-Extractable Sulfur Dioxide in Corn Grain

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A method is presented for the rapid measurement of water-extractable sulfur dioxide in corn. The method differs from standard alkali or distillation procedures in that bound SO_2 is not measured. Sulfur dioxide in ground corn samples is extracted in a buffer solution and titrated with iodine directly, without filtration. Average standard deviation of the method was 21.3 ppm, as determined from samples containing SO_2 levels ranging from 162.3 to 1197.0 ppm (weight basis). The method requires no specialized equipment and is suitable for control or research purposes. Direct comparison was made with the distillation procedure of the Manufacturing Confectioners' Alliance and the FMF (Pearson, 1977).

Treatment of high-moisture shelled corn with small amounts of sulfur dioxide inhibits microbial growth during low-temperature grain drying (Eckhoff et al., 1980; Van Cawenberge et al., 1982). The procedure, called the trickle-SO_2 procedure, involves the intermittent injection of SO_2 into the drying air which is carried into the bin where it acts upon the indigenous microflora. In order to develop appropriate SO_2 application procedures and to

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relate SO_2 levels to microbial inhibitory capabilities, it was necessary to determine the amount of mycocidally active SO_2 in the corn. Distillation procedures (Nichols and Reed, 1932; Schroeter, 1966) and direct titrimetric methods using cold alkali digestion (Ponting and Johnson, 1945; Nury et al., 1959) measure total SO_2 including bound SO_2 which is unavailable for microbial inhibition (King et al., 1981; Ingram, 1948). Hearne and Tapsfield (1956) cited the unpublished research results of E. F. Williams, which indicated that the level of SO_2 determined by distillation procedures is useless as a criterion for determining the protective power of the SO_2. Williams' results indicated that it is essential to know the level of "free" SO_2 when evaluating potential efficacy.

Free SO_2, which includes sulfite ions, bisulfite ions, and the microbially active sulfurous acid, is often measured in liquids such as wine and fruit juices by direct titration with iodine (Bennett and Donovan, 1943; Joslyn, 1955). In solid products the free SO_2 must be extracted from the solid prior to titration. Ross and Treadway (1960) measured the water-extractable SO_2 (free SO_2) in precut potatoes by mixing the potatoes with a water–buffer solution in a blender for 2 min, vacuum filtering the slurry, and then titrating aliquots of the filtrate with iodine. Adaptation of their procedure to corn proved unsatisfactory due to excessive loss of SO_2 during vacuum filtration. The procedure was slow and cumbersome because of the amount of handling required in the blending and filtration steps. These shortcomings prompted the development of a simplified water-extractable SO_2 procedure using nonagitated extraction and direct titration of the slurry. The procedure requires less time per analysis and has an accuracy comparable to that of standard total SO_2 procedures.

Although the procedure was developed for the analysis of corn samples taken from the trickle-SO_2 process, it is suitable for the analysis of corn samples from the wet milling process, from sulfited sweet corn, or from any other process where SO_2 is added to corn. The procedure is directly applicable to other cereal products and with slight modification to legumes. In this paper the analytical method is outlined in detail with a discussion of pertinent tests and results used in the development of the procedure and in the determination of the analytical specifications.

EXPERIMENTAL SECTION

Special Reagents. *Buffer Solution.* Dissolve 35 g of citric acid monohydrate and 65 g of disodium phosphate heptahydrate in distilled water. Add 0.5 mL of toluene as a preservative and dilute to 1 L (Ross and Treadway, 1960).

Iodine, Standard Solution. Dissolve 12 g of iodine and 22 g of potassium iodine in 500 mL of hot distilled water. Diluted with distilled water, 10 to 1, to provide a 0.016 N solution. Standardize against sodium thiosulfate according to the AOAC Method (Association of Official Agricultural Chemists, 1955). Store in a dark bottle.

Starch Indicator Solution, 1% (Used in Determining End Point of Sulfite–Iodine Reaction). Mix 10 g of soluble starch, 25 g of potassium iodine, 10 g of sodium bicarbonate, and 0.01 g of mercuric iodine in hot distilled water and dilute to 1 L with hot water. Mix for 4 min in a blender (Ross and Treadway, 1960).

Procedure. (1) One hundred milliliters of distilled water and 25 mL of buffer solution were measured into a 500-mL Erlenmeyer flask. (2) The corn sample to be analyzed (no prior preparation necessary) was ground by using a Waring-type coffee grinder or similar grinder, approximately 10.0 g of the ground corn was weighed out, this was put in the Erlenmeyer. Seal the flask with a rubber stopper. (3) The sample was allowed to sit 30 min. Agitation of the flask is not necessary for good results. (4) Two milliliters of starch indicator solution was added, and the corn slurry was titrated rapidly and continuously with iodine to a medium blue which persists for at least 20 s while stirring with a magnetic stirrer. (5) A blank is prepared by taking a similar sample of corn, grinding, and putting it in an Erlenmeyer flask with 100 mL of distilled water, 25 mL of buffer solution, 2.5 mL of HCl, and 10 mL of formaldehyde (28% solution). The formaldehyde forms a stable complex with the free sulfite in the acified solution (Potter and Hendel, 1951) which does not react with iodine. Allow the mixture to sit 10 min and titrate to a light purple. (6) The SO_2 content of the sample, on a wet basis, is determined by multiplying the corrected milliliters of iodine (titrated value minus blank value), the iodine normality, and 0.03203, which converts the amount of iodine used into gram equivalents of sulfur dioxide, and by dividing by the sample weight (Ross and Treadway, 1960, 1972; Pratner et al., 1944; Reifer and Mangan, 1945).

$$\text{ppm of } SO_2 \text{ (wt basis)} = \text{cor mL of } I_2 \times 0.03203 \times 10^6 \times \text{normality of } I_2/\text{sample wt}$$

Determination of the end point proved to be the most difficult part of the procedure due to the fleeting end point and the particulates in the slurry. Use of a high-intensity lamp to illuminate the flask during titration aided in end point determination.

DISCUSSION

The length of time required for extraction was determined by measuring the water-extractable SO_2 (WE-SO_2) content of corn (28.5% moisture) held at various extraction times. Two tests were performed with five replicates at each extraction time. The first test compared extraction times of 10 min, 30 min, 1 h, 2 h, and 24 h for corn containing approximately 720 ppm. The standard deviation between replicates at a given extraction time ranged from 10.8 to 23.0 ppm. Analysis of variance on the data showed statistical differences at the 0.01% level. A Duncan multiple range test revealed the 24-h and the 30-min extraction times were different from the other three. The 30-min extraction time yielded 25.7 ppm higher values than the 10-min or 1-h extraction times. The 24-h extraction time yielded 228 ppm less than the 30-min extraction time.

Based on the results of the first test, a second test was performed on 28.5% moisture corn containing approximately 650 ppm of free sulfite using extraction times of 10, 20, 30, 40, and 50 min. The standard deviation between the five replicates at each extraction time varied from 11.8 to 20.2 ppm. Analysis of variance on the data showed no statistical difference at the 0.01% level. A standard extraction time of 30 min was chosen as a mean value although the analysis does not appear to be sensitive to a 20-min deviation in extraction time. Subsequent tests with samples containing SO_2 levels ranging from 500 to 2500 ppm showed a similar optimal extraction time of between 10 and 50 min.

Whole kernel extraction was unsatisfactory. Half of a sample of treated corn was ground and extracted in the water–buffer solution for various lengths of time ranging from 10 min to 24 h. The other half of the corn sample was left whole and the SO_2 extracted similarly. The whole kernel extraction showed the highest SO_2 yield after 24 h; however, the yield was only 55% of the value determined from the ground sample after only 30 min of extraction.

The accuracy of the procedure was determined by the recovery of known amounts of sodium bisulfite (Fisher

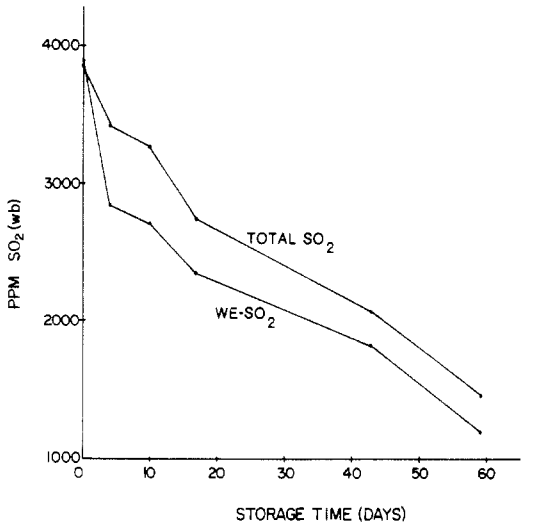

Figure 1. Comparison of water-extractable SO_2 (WE-SO_2) and total SO_2 values for 28.5% moisture corn treated with 1.0% SO_2 and stored at 20 °C.

Certified ACS) and sodium thiosulfate (0.1 N, Fisher Certified). The bisulfite or thiosulfate was added to flasks containing the buffer solution and untreated corn (28.5% moisture) prior to the 30-min holding time. The bisulfite was added to give an SO_2 level of 450 ppm and the thiosulfate a level of 1585 ppm. Bisulfite recovery was 96.11% with a standard deviation of 5.6 ppm and thiosulfate recovery was 98.92% with a standard deviation of 5.7 ppm, which is comparable to the recovery reported for total SO_2 procedures (Thrasher, 1962; Thompson and Toy, 1945; Nichols and Reed, 1932). The precision of the WE-SO_2 procedure was determined on 10 samples of treated corn, the moisture content ranging from 15% to 35%, containing SO_2 levels ranging from 162.3 to 1197.0 ppm. Standard deviations ranged from 11.2 to 32.6 ppm with an average standard deviation of 21.3 ppm. For samples with low SO_2 contents dilution of the iodine solution increases the precision slightly but much of the variance appears to be due to sampling.

A comparison between the WE-SO_2 procedure and the downward distillation procedure described by Pearson (1977) is shown in Figure 1. The corn initially at 28.5% moisture was treated in a polyethylene bag with 1.0% SO_2 and held for 1 h before being opened and transferred to 100-mL test tubes which were then sealed and stored at 20 °C. Periodically two tubes were removed from storage and the corn was analyzed for WE-SO_2 and total SO_2. At the time of transfer to the tubes the WE-SO_2 and total SO_2 values were similar but at all other sampling times less water-extractable SO_2 was measured than total SO_2. This is consistent with Joslyn (1955) and Bennett and Donovan (1943) in that the distillation procedure measures both the free SO_2 (includes sulfite ions, bisulfite ions, and sulfurous acid) and the bound SO_2, the SO_2 that forms addition products with other compounds such as acetaldehyde and glucose. According to Nelson (1959), Ingram (1948), Carr et al. (1976), King et al. (1981), and Schmiz (1980), only the free SO_2 (primarily sulfurous acid) is active in controlling microbial growth. Since the water-extractable procedure presented herein measures only free SO_2, it is a more accurate measure of the amount of SO_2 available for microbial inhibition. This difference between procedures can be important when the level of SO_2 is used as an indicator to predict future storability of the product. For example, two samples of high-moisture corn (28.5% moisture) treated with SO_2 and held at room temperature for 2 months had total SO_2 contents of 578 and 655 ppm, respectively, while their WE-SO_2 contents were only 162 and 181 ppm. This is the same magnitude of difference between the total SO_2 and WE-SO_2 shown in Figure 1 (240–600 ppm). This shows that the amount bound, as determined by this difference, is not an equilibrium phenomena as described by Ingram and Vas (1950) but may relate to a nonreversible phenomena with the magnitude determined by initial SO_2 levels. This causes the difference between the two values to be proportionally larger at lower levels of SO_2. With other biological materials this difference can be even larger as was found for whole corn silage where 33.1% moisture silage treated with 0.5% SO_2 had a total SO_2 level of 3657.8 ppm and a WE-SO_2 level of 2630.9 ppm for a difference of over 1000 ppm. Similarly, sweet sorghum (70% moisture) treated with 3.0% SO_2 had a total SO_2 level of 10625 ppm and a WE-SO_2 level of 6764 ppm for a difference of almost 4000 ppm. Determining inhibitory levels based on the WE-SO_2 level is more readily transferable from product to product than if determined by total SO_2 levels.

Other commodities can be measured by using this procedure with only slight adaptation. Products that can be ground by using a coffee grinder, or similar grinder, can be extracted similarly as corn. It was found that for forage-type crops such as whole corn silage and sweet sorghum, a blender can be used to chop and extract the SO_2 from the plant material similar to the procedure of Ross and Treadway (1960). In such cases it was found that a standard Ball-type jar on which the blender blades could be attached (Oster Model 848-36K) was easier and quicker to work with than the standard blender container. The blades could easily be screwed off the jar following the 2 min of blending, and the sample could be titrated in the Ball-type jar without having to transfer the slurry.

CONCLUSIONS

Water-extractable SO_2 is an accurate measure of the free SO_2 in corn. The procedure can be performed by personnel with only minimal training, requires no specialized equipment, and has an accuracy and precision that is satisfactory for research and control purposes. The procedure is applicable to other food products and commodities.

Registry No. Sulfur dioxide, 7446-09-5; iodine, 7553-56-2.

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Enzymes of Carbohydrate Oxidation in Developing Wheat Grains

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Activities of key respiratory enzymes were monitored at different stages in developing grains of low (C-591) and high (WH-157) yielding wheat (*Triticum aestivum* L.) cultivars. Hexokinase activity increased up to day 35 of grain development and then remained almost constant, whereas phosphofructokinase and pyruvate kinase activities peaked at day 28. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase showed biphasic behavior with peaks at days 14 and 28 after anthesis. Activities of soluble as well as mitochondrial malate (NAD^+) and isocitrate ($NADP^+$) dehydrogenases again peaked at day 28. Though the patterns of enzyme activity were similar in the two cultivars, the level in each case, when expressed on a per grain basis, was significantly higher in WH-157 than in C-591 throughout the major period of grain maturation. However, when the results were expressed on a per milligram dry weight basis, the rates were almost identical and there was no substantial difference in the rates for two varieties, indicating that the respiratory processes yielding energy needed for biosynthesis of starch and other reserves in developing wheat grains are not limiting in the small-sized grain cultivar.

Respiration in an actively metabolizing tissue is concerned mainly with oxidation of substrates to provide energy and intermediates required for biosynthetic processes. In this process, the pathways responsible for degradation of respiratory substrates converge ultimately on the Krebs cycle and hence on to respiratory chain phosphorylation. Thus, in the respiration of any class of substrates, four main steps can be recognized: breakdown of polymers and oligomers to their constituents, conversion of these constituents to acetyl-CoA or an intermediate of the Krebs cycle, oxidation of the latter via the cycle, and the reoxidation of cofactors reduced in the above reactions (ap Rees, 1980). Since carbohydrates are generally the principal source of energy, glycolysis attains significance as a major pathway of carbohydrate degradation. However, the oxidative pentose phosphate pathway is an important alternative to glycolysis and is now well-known to supply NADPH for use in biosynthetic reactions in cytoplasm. Additionally, it also serves as a source of pentose phosphates, particularly ribose-5-P for nucleotide and nucleic acid synthesis and erythrose-4-P for synthesis of aromatic amino acids and many other secondary aromatic compounds (Kelly and Latzko, 1980). Glyceraldehyde-3-P released by the transketolase reaction of the above pathway may also be fed into glycolysis and the carbon is eventually released as CO_2 during mitochondrial respiration, thus producing energy. Hence, measurement of levels of key enzymes of glycolysis, the pentose phosphate pathway, and the Krebs cycle will elucidate their role in providing energy, reducing power, and various carbon intermediates needed for varied synthetic activities that occur in developing cereal grains.

Keeping the above in view, the present investigation was aimed at determining whether low- and high-yielding wheat cultivars differ with respect to levels of key enzymes of various respiratory pathways and to associate these parameters further with grain size/starch content. Such information could relate to our previous knowledge of the possible mechanism of starch deposition and physiological and/or biochemical constraints operating in the above process in developing wheat grains (Kumar and Singh, 1980, 1981).

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MATERIALS AND METHODS

Wheat cultivars, namely, WH-157 and C-591, differing in their final grain size (dry weight, 50 and 40 mg $grain^{-1}$, respectively) and starch content (41 and 32 mg $grain^{-1}$, respectively) representing high- and low-yielding cultivars, respectively, were grown in the field as described earlier (Kumar and Singh, 1980). About 500 earheads per replication (three replications in each case) were tagged just at the start of anthesis. Grains were first harvested at day 7 after anthesis and then at weekly intervals until complete maturity of crops. Samples from each replication were analyzed independently. Thus, each value in the figures is the average of three determinations.

Enzyme Extraction. Twenty-five earheads harvested randomly from each replication were brought to the laboratory in a polythene bag buried in an ice bucket. Three spikelets from the upper and three from the lower end of each earhead were discarded. For soluble enzymes, 1 g of grains (whole grains, including both embryo and pericarp) removed randomly from earheads were hand homogenized at 0 °C in a mortar and pestle with 0.02 M Tris–maleate buffer, pH 7.0. The homogenate was centrifuged at 10000*g* at 4 °C for 40 min and the supernatant decanted. The residue was washed once with extracting buffer and centrifuged as before. The combined extract made to a known volume and served as the preparation for soluble enzymes (enzymes of glycolysis, pentose phosphate pathway, and soluble malate and isocitrate dehydrogenases).

For mitochondrial enzymes, the mitochondria were isolated essentially by the method of Bonner (1967) as modified by Krasnook et al. (1979). All steps, unless stated

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