and its abundance accounts for the water solubility of this fraction. The most volatile fractions from both orange and grapefruit essence oils were not water soluble and had D-limonene as the main component in each case (Coleman and Shaw, 1971; Coleman *et al.*, 1972). Lack of water solubility for these fractions had prevented meaningful taste evaluation of them in single strength citrus juices.

The taste threshold of the sample of water-soluble volatile components from tangerine essence oil and its flavor quality were determined in single-strength orange juice. Triangular taste tests were employed for taste threshold determinations with an initial level sufficiently high (83 ppm) for panel members to become acquainted with the flavor being evaluated. The concentration was presented in successive tests at 41, 25, 8, and finally 17 ppm. The lowest level at which a significant difference was detected was 25 ppm (16 correct of 24 judgments or >99% significance; Krum, 1955). Flavor quality was then evaluated using a paired comparison test with the experimental sample containing 25 ppm of the water-soluble volatiles. Panelists experienced in tasting aqueous orange essence added to orange juice were employed in this study. The panel judged these tangerine essence oil volatiles to have a desirable essence-like flavor when added to single-strength orange juice (10 correct of 12 judgments or 95% significance; Krum, 1955). Thus, a potent water-soluble fraction of volatile components with a desirable essence-like aroma and flavor can be separated from a citrus essence oil and used to impart a fresher flavor to orange juice.

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Determination of Sulfur in Plant Material Using a Leco Sulfur Analyzer

A method is described for the determination of sulfur in plant material using a Leco Sulfur Analyzer. A 0.05-g finely ground dried (at 80°) plant tissue sample is weighed into a combustion cup containing a small amount of iron accelerator. Magnesium oxide is added to cover the tissue sample. The combustion cup containing the sample is muffled at 500° for 1 hr. Iron and tin accelerator are added in layers and the combustion cup is placed into the induction furnace of the Leco Sulfur Analyzer. The combustion gases are passed over antimony before entering the titration chamber of the titrator. Sulfur determinations were made on five different plant tissues and compared with results obtained by other laboratories. The precision of the method was determined (σ 0.0088) by repeated analyses of NBS Standard 1571, Orchard Leaves.

Sulfur is becoming increasingly important in the production of various field and vegetable crops in Georgia. Analysis of plant tissue from suspected sulfur-deficient plants offers a means of verifying the deficiency. The Georgia Soil Testing and Plant Analysis laboratory purchased a Leco Sulfur Analyzer in hopes of using the instrument to determine the sulfur in plant tissue on a routine basis. A number of procedures were considered based on the review of methods for the determination of sulfur in agricultural samples prepared by Beaton *et al.* (1968). Of all the procedures available, the Leco Sulfur Analyzer offered the best alternative based on simplicity of operation and speed. Personal experiences from several who had successfully used the Leco Sulfur Analyzer for plant tissue analyses were encouraging (Castenson, 1970; Ferrara, 1969; Trowbridge, 1969). However, the results obtained with a Leco Sulfur Analyzer for total sulfur analyses in soils had not proven to be entirely acceptable (Bremner and Tabatabai, 1971). The problem seems to be related to sample preparation. Although a Leco method for the determination of total sulfur in soil has been published (Tabatabai and Bremner, 1970), there is no published procedure using the Leco Sulfur Analyzer for plant material.

A sulfur analysis procedure for plant material has been developed in this laboratory which gives sulfur results comparable to those determined by other laboratories and methods.

APPARATUS

The sulfur analyzer is manufactured by the Leco Laboratory Equipment Corp., St. Joseph, Mich., and consists of: an induction furnace, (Model 521-500), with the "L" modification, combustion tube (Leco No. 519-4), and ignitor (Leco No. 519-5); sulfur titrator, (Model 532-000); gas purifying train; source of pure oxygen, and sample crucibles (Leco No. 528-14). The gas train is modified to pass the combustion gases over antimony prior to entering the reaction chamber of the titrator. The principle of the combustion method is given in detail in Leco Form 100(10-66) (Leco Laboratory Equipment Corp., 1966).

MATERIALS AND METHODS

Sample Preparation. Plant tissue is dried at 80° for 24 hr, ground to pass a 20-mesh screen. Finer grinding has not been found necessary. A 0.05-g aliquot is weighed into a combustion crucible containing 0.2 g of iron accelerator. A scoop (Leco No. 503-32) is used to dispense the various substances into the combustion crucible. The approximate weight for each scoop addition is given for information purposes only, as additions are made by scooping rather than weighing. Two scoops (0.5 g) of magnesium oxide are placed over the sample. The crucible is placed in a cool muffle furnace and muffled at 500° for 1 hr. After cooling, one scoop (1.3 g) of iron accelerator (Leco No. 501-77) and one scoop (1.1 g) of tin accelerator (Leco No. 501-76) is placed in the combustion crucible.

Reagents. An Arrowroot starch solution is prepared by mixing 2 g of arrowstarch (Matheson Coleman and Bell) in 50 ml of deionized water. The suspension is poured with stirring into 150 ml of boiling deionized water. After boiling for 2 min, the solution is allowed to cool to room temperature and 6 g of potassium iodide is added. This solution is stored in the refrigerator and should be renewed every 7 days.

A potassium iodate–iodide stock solution is prepared by dissolving 0.444 g of potassium iodate and 5.0 g of potassium iodide in 1 l. of deionized water. The working solution is prepared by diluting 200 ml of the stock solution to 1 l. with deionized water.

Procedure. The Leco Sulfur Analyzer is operated as specified by the manufacturer in Leco Form 1101C (Leco Laboratory Equipment Corp., 1964). A blank titration and that for a known standard (NBS-133A containing 0.3% sulfur is used in this laboratory) are made. The furnace constant (F value) is determined as follows: F = (% S content of standard) (weight of standard in g)/(standard titration − blank titration, ml). The analyzer is now ready for plant tissue analyses. Care must be exercised to prepare the blanks and standards in the same manner as the samples. For best results, the F value should be determined every fourth to seventh sample, with the larger sample run between F value determinations for samples of similar composition. The percent sulfur in plant tissue is determined as follows: % S in sample = $F \times$ (sample titration − blank titration) sample weight in g.

Experimental Procedures. The crucible containing the prepared plant tissue is covered with crucible covers (Leco No. 528-42) and set in place in the induction furnace. The time for analysis is set at 5 min. The oxygen flow is 1 l. per minute. The time required for complete reaction is 5 min and should not be shortened. For best performance, the grid switch on the induction furnace is set on "high" and the plate current should reach 400 to 450 mA during the burn. Incomplete combustion occurs at lesser plate amperages. If the titration empties the burette, the sample must be rerun. Either the sample size is reduced or the strength of the titrant is increased. Experience has shown that a reduction in sample size gives better results. For samples containing 1.0% sulfur or more, the potassium iodide–iodate solution can be used at full strength rather than the $^1/_5$ dilution.

It was found that if the plant tissue samples are not muffled prior to analysis, erratic results are obtained. As soon as unmuffled plant tissue begins to ash in the induction furnace, the starch, potassium iodide, and iodate solution in the reaction chamber of the titrator turns dark blue in color. The color lightens in a few minutes and the titration proceeds normally. However, the initial reaction will affect the final titration, giving rise to erratic and erroneous results. The nature of the substance or substances causing this color reaction on initial combustion of unmuffled plant tissues is not known. Muffling the samples prior to analysis eliminates the initial color reaction. There is no indication of loss of sulfur during muffling. Plant tissue is always muffled in uncovered combustion crucibles with the iron accelerator added and the tissue covered with magnesium oxide. A 100-mm U tube containing small lumps of antimony is placed in the combustion train to remove interfering chlorine and other halogen gases (Bremanis *et al.*, 1967).

Table I. Percent Sulfur in Plant Material

Plant material	Percent sulfur[a] Mean concentration ± std dev	Leco method
Alfalfa tops	0.23 ± 0.02	0.23, 0.24
Corn leaves, center section	0.14 ± 0.02	0.12, 0.12
Cotton leaves	0.93 ± 0.07	0.89, 0.90
Pecan leaves	0.16 ± 0.04	0.15, 0.15
Wheat tops	0.16 ± 0.01	0.16, 0.16
Orchard leaves, NBS Standard 1571	0.14 ± 0.02	0.12, 0.12

[a] Mean of six determinations.

DISCUSSION

There are no known plant tissue standards with a certified sulfur content. The U. S. National Bureau of Standards is preparing a series of plant tissues for release in the near future (Meinke, 1971). The first of these was issued in 1970, Standard Reference Material 1571, Orchard Leaves. However, sulfur was not included in the elements certified.

In order to determine the accuracy of the Leco method, six different plant materials were given to six laboratories for analysis using their own sulfur procedures in a collaborative study. One of the six plant tissues was the Standard Reference Material 1571, Orchard Leaves. The other plant tissues were prepared in this laboratory. Three laboratories wet digested the plant material with a mixture of nitric and perchloric acids. The sulfate content in the digest was then determined by either gravimetric or turbidity procedures. One laboratory used an X-ray fluorescence technique. Two laboratories used Leco Sulfur Analyzers. The average sulfur contents determined by the six laboratories were compared to the results obtained by the Leco method described in this paper. Table I lists duplicate analyses obtained by the Leco method compared with average sulfur content determined by the six laboratories.

The Leco method results compared favorably with the mean results determined by the other six laboratories. The Leco method tended to give slightly lower sulfur results for most of the materials. However, the differences were not sufficient to invalidate the method. The variances are well within expected differences to be found for plant tissue analyses (Jones, 1969).

A known amount of sulfur in the form of the NBS Steel Reference Sample No. 133A containing 0.33% sulfur was added to 0.05-g aliquots of the six plant tissue samples used in the collaborative study. In order to reduce the amount of titrant used to that contained in one burette, the sample size for cotton was cut to 0.25 g and the amount of the spike was cut to one-half that added to the other five tissues. The analysis procedures were conducted as described in this paper

Table II. Percent Recovery of a Sulfur Spike Added to Plant Material

Plant material	Mg sulfur added	Mg sulfur found	% Recovery
Alfalfa tops	0.165	0.164	99.0
Corn leaves, center section	0.165	0.170	101.5
Cotton leaves	0.0825	0.0790	95.5
Pecan leaves	0.165	0.162	98.0
Wheat tops	0.165	0.164	99.0
Orchard leaves, NBS Standard 1571	0.165	0.162	98.0

and the percent recovery was determined based on the amount of sulfur added as the NBS Steel Reference Sample. The results are given in Table II.

The recoveries were within 2% of the amount added except for the cotton leaf sample. Repeated analyses tended to give the same consistently lower results as reported in Table II. It was concluded that the natural heterogeneity of plant tissue and the high sulfur content in the cotton leaf tissue sample were contributing to the low recovery. As was recommended earlier in this paper, the recovery test should have been done with the titrant used at full strength for the cotton leaf sample, since the percent sulfur of the sample plus the spike exceeded 1.0%.

The precision of the method was determined by repeated analyses of NBS Standard 1571, Orchard Leaves. The standard deviation of ten analyses selected at random was 0.0088.

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