thickness, stomatal size and frequency, and trichome frequency and structure may all have an influence on water loss (13). We have undertaken microscopic studies to determine if these anatomical factors are involved. The precocious yellow gene *B* in 'Multipik' is known to be highly pleiotropic (11, 12). A number of negative secondary effects have been reported. These include leaf-yellowing, lower yields, and poor seed production (11, 12). These secondary effects may or may not be expressed depending on the genetic background. Paris *et al.* (9) reported that a precocious yellow zucchini cultivar 'Goldy' was capable of producing the yield and yield quality (grade) of green-fruited zucchini cultivars. Schaffer and Boyer (11) found little effect of gene *B* in fruit development in 'Early Prolific' background. Our results limit us to discussion of between-cultivar differences. A comparison of isogenic lines would be worthwhile to determine the possible effects of gene *B* on water loss and storability.

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CALCIUM DETERMINATION IN CITRUS PULP WASH—A COMPARISON OF A COLORIMETRIC PROCEDURE WITH ATOMIC ABSORPTION SPECTROPHOTOMETRY

RICHARD L. COLEMAN, MARY S. SAUNDERS AND ROBERT A. BAKER
U. S. Citrus & Subtropical Products Laboratory[2]
P. O. Box 1909
Winter Haven, Florida 33883-1909

Abstract. **Two methods of calcium determination, a commercial colorimetric procedure and atomic absorption, were evaluated for routine laboratory use on citrus products and other materials. Samples tested included commercial calcium standards, well and process water, and serial dilutions of citrus pulp wash. Satisfactory results on citrus pulp wash samples could not be obtained with atomic absorption without the additional steps of ashing and redissolving in dilute acid. The colorimetric procedure cost less than the atomic absorption method and gave results which were as accurate.**

Some citrus and other drink processors need to carry out a few calcium determinations on various samples intermittently. A comparison of available calcium determination methods was undertaken to find the most practical means for determining calcium content in citrus products. Two methods were selected as least laborious and least costly, yet reasonably accurate. These were atomic absorption spectrophotometry and a colorimetric procedure and they were compared systematically (3). Our experience and results comparing these methods are the subject of this report.

Materials and Methods

The atomic absorption spectrophotometer (AA) was a Perkin Elmer Model 372 using acetylene and air. Samples (3 ml) were prepared by the following methods: 1) unaltered samples were aspirated directly into the AA flame; 2) lanthanum was added to reduce interference from phosphates, and samples were aspirated into the AA flame; 3) samples were ashed at 500°C, extracted with HCl, and treated with lanthanum before aspiration; or 4) samples were ashed at 900°C, extracted with HCl, and treated with lanthanum before aspiration. All AA samples were prepared and analyzed in a commercial analytical laboratory. (Applied Agricultural Research, Inc., Lakeland, Fla.).

Colorimetric determination of total calcium was carried out using a commercial analytical kit (Calcium Determination Kit No. 586, Sigma Chemical Co., St. Louis, Mo.). This determination is based on calcium's reaction with cresolphthalein complexone to form a purple color with a maximum absorption at 575 nm. A Bausch & Lomb Spectronic 20 with standardized test tubes was used to read color intensity as absorbence at 575 nm. Double deionized water, prepared by use of two deionization systems (Mitco Water Laboratories, Inc., Auburndale, Fl., and Cole-Parmer Instrument Co., Chicago, Il.), was used in all standards and dilutions.

[1]Private Consultant. 1102 East Magnolia Drive, Tallahassee, FL 32301
[2]South Atlantic Area, U. S. Dept. Agr., Agr. Res. Serv.
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Frozen concentrated pulp wash [water extracted soluble orange solids (WESOS)] was obtained from a local processor where there were problems with an undesirable precipitate in the product. As part of an investigation into the composition and cause of this precipitate, it was desirable to determine calcium concentrations in this product and in the waters used for elution.

Dilutions of 1/5, 1/10, and 1/20 were made volumetrically after allowing the WESOS concentrate to equilibrate at room temperature. Transfer pipettes were repeatedly rinsed to ensure that all material had been transferred. Aliquots of these samples were sent for AA analysis, and subjected to colorimetric analysis for total calcium.

Results and Discussion

The colorimetric kit costs $25.00 for enough working solution and standards to run 95 tests. Only small (50µl) samples of solutions were necessary and these were reliably transferred with a pipettor. The resulting purple color intensity was immediately readable at 575 nm. The indicated reading was stable; refrigerated samples have been reread the next day with no significant change. In the instruction manual, with the colorimetric kits, a coefficient of variation was reported on sera samples of 0.4% to 1.2%. Eight samples, tested in triplicate, consisted of three standard solutions, well water, processing water, and three dilutions of WESOS concentrate. The resulting coefficients of variation from 0 to 11.9% are shown on Table 1. The range of coefficients of variation for well water and processing water was from 0 to 7.8%. Whole samples containing various dilutions of WESOS had a range of 0-11.9%.

The colorimetric kit instructions advise "for most instruments the procedure is linear up to a level of 15 mg/dl". In this experiment, we found this to be the case for standards and water samples. However, in 2 of the 3 tests carried out on WESOS samples we found lower responses than expected, based on the dilution ratios (Table 2). Using the dilutions of 1/20, 1/10, and 1/5, the ppm of Ca measured should approximately double from 1/20 to 1/10 and again from 1/10 to 1/5. Higher concentrations of WESOS tended to be lower in ppm Ca than expected from the dilution ratios. Other tests run on concentrated (65° Brix) WESOS (data not shown) indicated approximately half of the calcium known to be present. Even though the ranges of Ca concentration recommended for use of the kit had been exceeded in the concentrate samples, there remained an indication of some interference occurring in 1:5 dilutions of WESOS.

The Atomic Absorption Analysis costs $2.00 per sample if digestion of the sample is not required. We found digestion of WESOS samples to be necessary due to both viscosity and interference from sugar. This would increase the cost/sample to $3.00. Initial ashing at 500°C left incompletely ashed samples and some spattering occurred in samples ashed at 900°C.

Ashing, extracting, dilution and interference (despite the addition of lanthanum) seemed to cause unreasonably high coefficients of variation in these AA results (Table 1).

Table 1. Coefficients of variation (%).

Materials	Colorimetric			AA
Std. 50 ppm Ca	0.4	2.7	4.7	0.0
Std. 100 ppm Ca	0.7	1.8	2.3	3.8
Std. 150 ppm Ca	0.0	1.5	1.9	1.2
Well water	1.9	5.4	7.2	3.5
Processing water	0.0	7.8	3.5	0.0
WESOS 1/20	4.2	3.1	7.9	out
WESOS 1/10	2.9	1.9	11.9	12.4
WESOS 1/5	0.0	5.5	9.2	6.1

Table 2. Average ppm of calcium.

Materials	Colorimetric			AA
Std. 50 ppm Ca	50	50	50	48
Std. 100 ppm Ca	100	101	100	91
Std. 150 ppm Ca	150	150	149	142
Well water	47	47	40	49
Processing water	50	44	44	51
WESOS 1/20	32	41	29	out
WESOS 1/10	57	67	60	86
WESOS 1/5	103	124	107	113

Table 2 shows the average ppm of calcium found in eight tests each run in triplicate on different days. The 13th Edition of A.O.A.C. (1) reports coefficients of variation for calcium determination by emission spectroscopy to range from 3 to 7%. The coefficients of variation shown under "AA" in Table l were exceptionally high and did not improve in four separate attempts at AA determination of calcium in WESOS samples.

One problem common to both AA and the colorimetric procedure in the analysis of WESOS was caused by viscosity. This is no new problem to those who have handled frozen concentrated orange juice (FCOJ), but variations in viscosity can affect readings from AA equipment which uses small diameter tubing and aspiration to transport samples into the flame. As viscosity increases the rate of flow through the AA's sample tube was slower and consequently the spectrophotometer readings for more viscous samples were lower.

Particles present two types of problems in both analytical procedures: they may cause physical blockage of tiny orifices in pipettes and aspirators and they also may contain concentrations of materials which may affect indicator solutions in turn causing variations in data.

In summary, preliminary comparison of the colorimetric calcium determination with a commercial AA determination indicates that the colorimetric procedure was less costly, required fewer steps for analysis of a citrus juice product, and was more convenient while providing comparable data.

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