Table 1. Effect of 0 or 10 ppm on chlorophyllase activity and chlorophyll content in calamondin fruit peel treated with 0 or 10 ppm CHI. Fruit were held at 30°C for 24 hr.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll degradation (%)</th>
<th>Increase in chlorophyllase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ethylene</td>
<td>11</td>
<td>195</td>
</tr>
<tr>
<td>Ethylene + CHI</td>
<td>2</td>
<td>81</td>
</tr>
</tbody>
</table>

Avg of 4 replications.

Table 2. Chlorophyllase activity in calamondin fruit peel as a function of time of exposure to ethylene. Fruit were treated with 10 ppm ethylene at 30°C.

<table>
<thead>
<tr>
<th>C2H4 exposure time (hr)</th>
<th>Increase in chlorophyllase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>51</td>
</tr>
<tr>
<td>18</td>
<td>81</td>
</tr>
<tr>
<td>24</td>
<td>137</td>
</tr>
</tbody>
</table>

Avg of 3 replications.

Ethylene consistently produced at the onset of the climacteric (3). A time-course study of ethylene vs. chlorophyllase demonstrated that a 6- to 12-hr ethylene exposure was required before measurable increased activity was attained (Table 2).

It should be possible to reduce the effect of ethylene on both chlorophyll degradation and chlorophyllase synthesis with the protein inhibitor CHI if chlorophyllase is one of the enzymes involved in chlorophyll degradation. CHI applied immediately prior to the ethylene treatment reduced the effect of ethylene on chlorophyll degradation by approx 80% and on chlorophyllase synthesis by 60% (Table 1). Inhibition of chlorophyllase degradation in citrus peel by CHI has also been reported by Goldschmidt (7). Incomplete inhibition of chlorophyllase synthesis probably indicates that all sites capable of synthesizing chlorophyllase were not exposed to CHI, even with vacuum infiltration. The CHI effect, even though not complete, indicates that ethylene can stimulate de novo synthesis or activation of this enzyme. Similar effects have been reported with other enzymes (6).

Comparative Wet-digestion and Dry-ashing of Orange and Schefflera Leaves for Nutrient Determinations

C. K. Labanauskas and M. F. Handy

University of California, Riverside

Abstract. Using wet-digestion as a standard, leaf samples of orange (Citrus sinensis (L.) Osbeck cv. Valencia) and Schefflera actinophylla Harms were dry-ashed at 500° and 700°C to demonstrate analytical sensitivity of the Schefflera nutrient elements to

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Literature Cited


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Dry-ashing of plant tissues for nutrient determinations has been widely used because of the convenience of this method in removing the large amounts of organic compounds (1, 2, 3). This procedure, however, has some disadvantages: (a) loss of analytical accuracy in some elemental values due to volatility at higher temp; (b) retention of some elements by residual carbon due to low ashing temp; or (c) retention of some elements by silicates present in the plant material. Although wet-digestion procedures are not overly cumbersome, they require some degree of caution (1, 3).

Plants generally known to be high in Ca and low in silica may be analyzed for the various elements of interest using a dry-ashing procedure without

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incurred substantial error. Serious errors have not been found when samples of citrus or avocado leaves were dry-ashed for analysis at 550°C (6). However, in a routine diagnostic check analysis of dry-ashed leaf samples of Schefflera actinophylla it was shown that large differences occurred in elemental concn even in duplicate analysis of the same well-mixed samples. These observations prompted an investigation of the major aspects of the dry-ashing procedure, including extreme temperature variation (700°C); one slightly lower temp (500°C); and wet digestion to evaluate the cause of the discrepancies in the Schefflera samples. A citrus sample, which has been used as a standard for many years, was run simultaneously with the Schefflera samples for additional comparative evaluation.

Schefflera leaves were collected from commercial nurseries located in San Diego, California. These samples were prepared for leaf tissue analysis according to a method described earlier (4), as was the citrus leaf standard material. Dry-ashed 2 g samples of Schefflera and citrus leaf were simultaneously analyzed by the same procedure (5). In addition to regular ashing and dissolution of the Schefflera and citrus leaf ash, the residue left on the filter paper was reashed and solubilized to determine the amount of the elements in the residue. The residue, which was substantial in Schefflera and absent in the 700°C citrus ash, and the filter papers were put back into the crucibles and reashed in the muffle furnace at 700°C for 2 hr to burn off the filter paper. Ten ml of a mixture 3HCl:1HNO₃ (v/v) was added to the crucibles, heated to boiling, and taken to near dryness. Ten ml of distilled water and 5 ml of 3N HCl were added to each crucible, heated to boiling, filtered directly into 50 ml volumetric flasks, made to volume, and analyzed for the appropriate elements by the method previously mentioned (5).

Wet-digestion of the Schefflera and citrus leaves consisted of predigestion of the samples with 10 ml HNO₃ overnight at ambient laboratory temp to oxidize the bulk of the organic compounds, followed by digestion at the boiling point with 2 ml HClO₄ to remove the remainder of the carbon (1, 3). The samples were cooled, filtered, and diluted to 50 ml volume. The remainder of the analytical procedure was the same as described previously (5).

The wet-digestion sample analyses for Schefflera and citrus were considered to represent the true analytical values since no carbon remained in the digested samples, and silica is normally desorbed and consequently non-absorbing (7). The samples were analyzed for K, Ca, Mg, Na, Zn, Mn, Cu, and Fe.

Analytical values obtained from the standard dry-ashing and wet-digestion procedures of the citrus leaf material showed relatively small differences (Table 1). The analytical losses in samples ashed at 500°C were: Cu—20% loss with no recovery from the residue probably due to detection limitation; Fe—13% loss with 8% recovery from the residue; and Na—14% loss with no recovery from the residue. The analytical losses of nutrients ashed at 700°C were as follows: Zn—19% loss with only 6% recovery from the residue; K—8% loss with only 2% recovery from the residue; Fe—6% loss with 2% recovery from the residue.

Copper and Fe losses from the citrus sample filtrate of the 500°C ashing were associated with adsorption on the carbon particles. The analytical losses of K and Zn, 8 and 19%, respectively, from the leaves ashed at 700°C were presumed to be associated with volatilization of these elements because only a small fraction of these losses was recovered from the residue (Table 1). The data obtained from the Schefflera samples ashed at 500°C revealed that even the macronutrient concn did not differ significantly from those values obtained from wet-digestion (Table 1). The concn of micronutrients found in those leaves ashed at 500°C did differ substantially from those values obtained from wet digestion (Table 1). The loss of Cu was 12% with no recovery from the residue; Fe loss was 8% with 6% recovery from the residue; and Mn loss was 7% with 5% recovery.

Substantial analytical losses of all of the elements analyzed were found in Schefflera leaves ashed at 700°C (Table 1). The % of analytical losses of various elements in the samples and recovery from the residues, respectively, were: K—22 and 17, Ca—96% and 83, Mg—99 and 94, and Na—35 and 35. The micronutrient % analytical losses and recovery from the residues, respectively, were: Zn—99 and 90, Cu—100 and 100, Mn—96 and 90, Fe—100 and 90. All percentage loss and recovery calculations were based on the wet-digested sample values.

The data presented in this paper clearly show that substantial error may be introduced into leaf tissue analysis through reliance on a set standard dry-ashing procedure for elemental analysis of all plant materials. It is important to have adequate and precise temp controls on the ashing furnace. Too low or too high ashing temp may affect the determined nutrient concn in the leaf material. Citrus and avocado plants growing under natural field conditions were not affected as drastically by ashing temps as those of the Schefflera samples coming from commercial nurseries. The reason may be that citrus and avocado samples contain substantially higher concn of Ca than Schefflera leaves. Calcium may act as a stabilizer component in dry-ashing when silica is present.

If a dry-ashing procedure is to be used for nutrient determinations in plant tissue materials without having previous knowledge of the nutrient concn in the sample, a wet-digestion procedure should be carried out first, or along with dry-ashing to establish true values of nutrient concn in the material in question.

### Table 1. Comparison of nutrient analytical values on filtrate and residue by 2 ashing temperatures and wet-digestion methods on standard 'Valencia' orange leaf and Schefflera actinophylla leaf samples.

<table>
<thead>
<tr>
<th>Element</th>
<th>Schefflera</th>
<th>Citrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet-Dig.</td>
<td>500°C</td>
</tr>
<tr>
<td>K</td>
<td>0.60</td>
<td>0.61</td>
</tr>
<tr>
<td>Ca</td>
<td>4.56</td>
<td>4.64</td>
</tr>
<tr>
<td>Mg</td>
<td>0.353</td>
<td>0.357</td>
</tr>
<tr>
<td>Na</td>
<td>0.030</td>
<td>0.035</td>
</tr>
<tr>
<td>Zn</td>
<td>20.1</td>
<td>21</td>
</tr>
<tr>
<td>Mn</td>
<td>32.1</td>
<td>34</td>
</tr>
<tr>
<td>Cu</td>
<td>4.0</td>
<td>5</td>
</tr>
<tr>
<td>Fe</td>
<td>104</td>
<td>120</td>
</tr>
</tbody>
</table>

Each value is a mean of 5 individual determinations.

### Literature Cited


Contaminants Present in Materials Commonly Used to Purify Plant Extracts for Hormone Analysis

George C. Martin, Frank G. Dennis², Paul Gaskin³, and Jake MacMillan³

University of California, Davis

Abstract. Sensitive analytical techniques such as gas chromatography or combined gas chromatography-mass spectrometry, detect not only plant hormones, but chemical contaminants as well. Thorough cleaning of solvents and chromatographic materials used for extracting plants as well as identification of the contaminants present will assist in resolving the contaminants from hormones present in the same extract.

Bioassay of extracts permits the detection of submicrogram quantities of plant hormones. Contaminants present in solvents or chromatographic supports are seldom a problem as they are usually biologically inactive at the concs encountered, although exceptions have been reported (2). When gas chromatography (GC) is used for analysis, however, contaminants can be readily detected and thus can present problems. For example, the plasticizer acetyl tri-n-butyl citrate is often detected in plant extracts (1). Further, n-alkanes can be introduced into extracts when Parafilm is used to cover glassware containing plant samples (3). In this report we indicate the wide-spread occurrence of other artifacts, and identify several of the most abundant.

Standard commercially available solvents were redistilled on a fractional distillation column prior to use and stored in glass bottles with Teflon-lined lids. The following chromatographic materials were used: “Silicar” TLC 7G (Mallinckrodt Chemical Co.); 0.25 mm precoated, aluminum-backed TLC plates of silica gel 60 without fluorescent indicator (Merck and Co.); polyvinylpyrrolidone (PVP) polyclar AT (GAF Corporation); and instant thin-layer chromatography (ITLC) (Gelman Products). The solvents and chromatographic materials were analytical grade and were used in the same manner as when purifying plant extracts.

On evaporating the solvent, the residues were dissolved in methanol and methylated with ethereal diazomethane. The methylated (Me) samples were either dissolved in ethyl acetate and analyzed by GC directly, or dissolved in dry pyridine and silylated by adding hexamethyldisilazane:trimethylchlorosilane:pyridine (2:1:2, v/v/v), resulting in methyl ester trimethylsilyl ether (MeTMS) derivatives for GC.

The Me and MeTMS derivatives were chromatographed on a Pye 104 gas chromatograph using 2% SE 33 on Gas Chrom Q (80-100 mesh) packed in 153.4 cm x 3.5 mm i.d. glass columns. Column temp was programmed from 180°C to 225°C at 3°C/min, with a nitrogen flow rate of 60 ml per min. Gas chromatography-mass spectrometry (GC-MS) of MeTMS derivatives was done on an A.E.I. MS30 coupled to a Pye 104 GC through a silicone membrane which prevents helium from entering the MS. The 213.4 cm x 2 mm i.d. glass column for GC-MS was packed with 2% SE33 on Gas Chrom Q 980-100 mesh), and the helium flow rate was 25 ml per min. Mass spectra were obtained at 24 eV with a source temp of 210°C and a separator temp of 190°C. The MS were recorded at 6.5 sec per