spectrometer and compared with the spectra derived from authentic compounds or with published spectra.

RESULTS AND DISCUSSION

The compounds isolated and their relative abundance in cinnamon leaf, stem bark, and root bark oils are shown in Table I. Of the compounds identified, eight have not previously been reported in cinnamon oils. The new compounds were the monoterpene, terpinolene, the sesquiterpene, caryophyllene oxide, and six aromatic compounds (benzaldehyde, methyl chavicol, hydrocinnamaldehyde, 2-phenylethyl alcohol, 2-phenylpropyl acetate, and coumarin). A further 24 compounds that have not been previously found in cinnamon oils were tentatively identified and included ten monoterpenes (myrcene, *cis*-ocimene, *trans*-ocimene, fenchone, *trans*-linalool oxide furanoid form, linalyl acetate, bornyl acetate, borneol, geraniol, and geranyl acetate), three sesquiterpenes (β-selanene, γ-cadenene, and farnesol), two aliphatic compounds (hexanol and nonanal), and nine aromatic compounds (2-phenylacetaldehyde, 2-phenylethyl acetate, benzyl alcohol, phenol, methyleugenol, methylisoeugenol, 2-vinylphenol, vanillin, and 2-phenylethyl benzoate). Final identification of these volatiles awaits mass spectral analysis.

Most compounds were found in all three oils but the composition of the oils was quite different. The major component in leaf oil was eugenol (~70%) with caryophyllene, linalool, and benzyl benzoate also of some quantitative importance. Cinnamaldehyde (75%) was the major component in stem bark oil with a contribution also from cinnamyl acetate and caryophyllene, while camphor (56%) was the major component of root bark oil with cineole, α-terpineol, α-pinene, and limonene also of importance.

LITERATURE CITED

Angmor, J. E., Dicks, D. M., Evans, W. C., Santra, D. K., *Planta Med.* **21**, 416 (1972).
Edwards, R. A., Fagerson, I. S., *Anal. Chem.* **37**, 1630 (1965).
Hedin, P. A., Thompson, A. C., Gueldner, R. C., *J. Agric. Food Chem.* **23**, 698 (1975).
Senanayake, U. M., Day, W. R., Lee, T. H., submitted for publication in *J. Agric. Food Chem.* (1978).
Stahl, E., Jork, H., in "Thin Layer Chromatography", Stahl, E., Ed., Springer, Berlin, 1969.
Wijesekera, R. O. B., Jayewardene, A. L., Rajapakse, L. S., *J. Sci. Food Agric.* **25**, 1211 (1974).

Received for review July 14, 1977. Accepted January 13, 1978.

Comparative Study of Ashing Techniques for the Digestion of Horticultural Plant Samples

Munoo Prasad* and Michael Spiers

Three methods of digestion of plant material involving two dry ashing techniques and a wet ashing technique using $H_2SO_4 + H_2O_2$ were compared to a $HNO_3 + HClO_4$ method for the determination of Fe, Mn, Zn, Cu, Ca, and Mg by atomic absorption and K by flame emission in seven horticultural crops. The results obtained led to further determinations of these elements using a dry ashing method and the $HNO_3 + HClO_4$ method for a further eight horticultural crops. For all crops and elements investigated, the dry ashing procedure involving 0.5-h treatment of ashed material with HCl gave comparable results to both the $HNO_3 + HClO_4$ digestion and to the dry ash treatment involving a lengthy steam bath treatment of the ash. The $H_2SO_4 + H_2O_2$ method gave a high percentage of unsatisfactory Fe values and occasional unsatisfactory Ca and Zn values.

There is a great deal of controversy regarding the suitability of dry ashing vs. wet ashing techniques for the digestion of plant material prior to the determination of nutrients (Jones and Steyn, 1973; Koirtyohann and Pickett, 1975). Wet ashing of plant samples using $HNO_3 + HClO_4$ has proved satisfactory for the determination of micronutrients (Gorsuch, 1959, 1970; Isaac and Johnson, 1975; Koirtyohann and Pickett, 1975) and macronutrients (Gorsuch, 1970; Isaac and Johnson, 1975), while with dry ashing lower values have occasionally been reported for some micronutrients due to incomplete recovery (Basson and Böhmer, 1972; Gorsuch, 1970). Nevertheless, the dry ashing technique is widely used because of convenience and because the wet digestion method using perchloric acid can be hazardous. The choice of the dry ashing technique should, however, be dictated by the type of plants and elements to be determined, and it would be unwise to generalize regarding its suitability for all types of plants (Gorsuch, 1970). Recently, a wet ashing technique using $H_2SO_4 + H_2O_2$ has been found suitable for the determination of macronutrients (including N) and micronutrients in plant material associated with ecological studies (Parkinson and Allen, 1975). The objective of this investigation was, therefore, to assess two dry ashing techniques and the wet ashing technique using $H_2SO_4 + H_2O_2$ for the determination of macro- and micronutrients in a wide range of horticultural plant samples differing considerably in nutrient levels. These techniques were compared with the $HNO_3 + HClO_4$ wet digestion technique which we have used as a "standard" method since it receives universal application and is accepted as giving satisfactory results.

Levin Horticultural Research Centre, Research Division, Ministry of Agriculture and Fisheries, Private Bag, Levin, New Zealand.

MATERIALS AND METHODS

Plant Samples. Leaf samples of carrot cv. Topweight, freesia (Diploid), lettuce cv. Yateslake, peach cv. Golden Queen, rhododendron cv. Tallyho, and strawberry cv. Tioga and asparagus spear cv. Mary Washington were ashed by two dry ashing techniques, by a wet ashing technique, and by the standard $HNO_3 + HClO_4$ digestion technique. One of the dry ashing techniques was again

0021-8561/78/1426-0824$01.00/0

Table I. Coefficients of Variation of Four Ashing Methods for the Determination of Macro- and Micronutrients in a Range of Horticultural Plant Material

Method	No. of crops	Fe	Mn	Zn	Cu	Ca	K	Mg
1	7	3.5	4.0	2.5	8.3	6.9	1.9	5.6
2	7	3.6	8.7	2.0	9.8	2.4	2.0	2.0
3	7	4.6	2.1	3.8	10.7	4.9	2.1	2.1
4	7	13.1	4.0	5.0	6.1	3.5	1.5	1.5
1	15	3.2	3.6	2.3	8.2	4.7	1.8	4.3
3	15	5.2	4.3	6.3	7.3	6.8	3.1	3.8

compared with the standard HNO_3 + $HClO_4$ technique using leaves of a further eight crops, namely, cabbage cv. Golden Acre, celery cv. Utah 527OH, Chinese gooseberry cv. Hayward, dwarf bean cv. Processor, garlic cv. Californian Early, potato cv. Rua, red beet cv. Detroit Dark Red, and squash cv. Buttercup Bush. All plant samples were from trials conducted by the Levin Horticultural Research Centre.

Plant samples were dried at 85 to 90 °C for 24 h and ground with a Wiley mill to pass through a 1-mm sieve and redried immediately before weighing for the various ashing techniques.

Ashing Procedures. Method 1 consisted of taking 1 g of ground leaf sample in a tall-form 30-mL silica crucible with lid and ashing it at 475 °C for 4 h in a stainless steel lined muffle furnace. During muffling the crucibles were placed on a stainless steel stand so that there was no contact between the crucibles and the heated floor and walls. The sample after ashing was cooled, and the ash dissolved in 5 mL of 2 N HCl by heating it on a hot plate for 30 min. The volume was made up to 50 mL and filtered through a Whatman No. 2 filter paper. This method is similar to that reported by Isaac and Kerber (1971).

In method 2, the same initial ashing procedure was used. The ash was then treated with 5 mL of 6 N HCl and slowly taken to dryness on a water bath. This operation was repeated with a further 5 mL of 6 N HCl. The residue was dissolved in 20 mL of 0.1 N HCl, made up to 50 mL, and filtered through a Whatman No. 2 filter paper. This method is similar to that reported by Allan (1971).

Method 3, which was the "standard" method, consisted of digestion of dried leaf sample with HNO_3 + $HClO_4$ (Johnson and Ulrich, 1959). The 1-g sample was left overnight with 10 mL of concentrated HNO_3 in a 50-mL calibrated test tube. The sample was then heated gently on a Technicon block digester (Model BD-40) in order to prevent excessive frothing. The temperature was then slowly brought to 210 °C. Heating was continued until the volume of HNO_3 was reduced to 5 mL. It was cooled, 2 mL of $HClO_4$ (72%) was added, and the heating was resumed. Heating was continued for 0.5 h after dense white fumes of $HClO_4$ appeared in the test tube. The sample, after cooling, was made up to 50 mL. Often a white precipitate formed on dilution, which was dissolved by warming the sample with hot water (75–80 °C).

Method 4 consisted of digestion of 1 g of the dried sample in the block digester with 10 mL of digestion solution consisting of H_2SO_4, H_2O_2, $LiSO_4$, and Se powder for 2 to 3 h (Parkinson and Allen, 1975). Excessive frothing occurred when the digestion solution was added even without heating, and constant operator attention was required during the early part of the digestion. Occasional precipitation in the digest on dilution to 50 mL was overcome as in method 3.

Each ashing technique was carried out in triplicate for all plant samples.

Analytical Methods. Iron, Mn, Zn, and Cu were determined directly in the extract using a Pye Unicam SP 1900 double-beam atomic absorption spectrophotometer. Dilution was required before Mn could be determined in rhododendron. The samples were diluted 1:50 or 1:100 using automatic diluters with an $LaCl_3$ solution containing 0.2% La as a releasing agent and ionization buffer before Ca and Mg were determined by atomic absorption and K by flame emission. The instrument was used according to manufacturer's specifications (Pye Unicam, 1975). Separate standards were made for the four methods so that the acidity of the standards conformed with that of the sample. Lanthanum chloride was added to the standards of Ca, K, and Mg. Distilled deionized water was used throughout the experiment.

RESULTS

The coefficients of variation (CV) for the four methods over seven crops, and for methods 1 and 3 over 15 crops are given in Table I. Method 4 gave a high CV for Fe. The CV for Cu was high for all methods. The coefficients of variation for methods 1 and 3 were very similar for each element over 15 crops.

Methods 1 and 2 gave similar results for all elements except Cu (Table II and IV). This is reflected in the regression coefficient being close to unity (except for Cu) and in the high correlation coefficients. The extra acid treatment (method 2) of ashed material led to small increases for Fe in carrot and strawberry, for Mn in carrot, lettuce, and peach, for Zn in strawberry, for Cu in peach and strawberry, and for Mg in asparagus. It is interesting to note, however, that method 1 gave results on these samples which were closer to those by method 3, the "standard" method, with the exception of Fe in carrot, Cu in peach, and Mg in asparagus.

Generally, the nutrient values by method 4 were similar to method 3 except for Fe values by method 4 for asparagus, carrot, freesia, and peach which were lower (Table II). In addition, Ca values for peach by method 4 and Zn values for rhododendron were somewhat low. Their lower values were also reflected in the regression coefficient (Table IV). The correlation coefficient between method 4 and method 3 for Fe is also much lower.

Values obtained by method 1 for all elements for the 15 crops were numerically similar to method 3 (Tables II, III, and IV). However, somewhat higher values for K were obtained for cabbage, carrot, celery, lettuce, and strawberry by method 1. Similarly, slightly higher values were obtained for Mg in some crops, particularly peach, by method 1. In contrast, the Cu values by method 1 were marginally lower for most crops (Tables II and III), and this is reflected in the regression coefficients and correlation coefficients (Table IV).

DISCUSSION

Precision was good for all methods (except Fe by method 4) and compared favorably with other studies (Jones and Isacc, 1969; Smith and Schrenk, 1972). The relatively higher CV for Cu by all methods is due to low values of Cu in most crops tested and its determination by atomic absorption close to detection limits.

Agreement between the results by methods 1 and 2 was good and would be acceptable for most experimental work.

Table II. Results for the Elemental Analysis of Seven Plant Materials by Four Methods

Crop	Method	Ppm				%		
		Fe	Mn	Zn	Cu	Ca	K	Mg
Asparagus	1	103.7	10.3	8.3	5.0	0.50	0.59	0.100
	2	93.7	9.0	8.7	5.6	0.52	0.55	0.115
	3	115.0	8.3	5.7	5.5	0.52	0.54	0.109
	4	42.3	10.0	4.7	4.1	0.49	0.60	0.105
Carrot	1	196.7	80.7	51.0	10.6	1.42	5.69	0.167
	2	213.3	87.0	52.3	10.5	1.44	5.32	0.169
	3	220.0	78.3	52.0	12.6	1.49	5.19	0.174
	4	132.1	80.0	50.7	9.7	1.49	5.10	0.167
Freesia	1	134.3	163.7	86.0	8.8	0.52	4.47	0.199
	2	137.0	172.0	87.3	8.8	0.51	4.45	0.204
	3	145.1	156.7	85.7	9.6	0.50	4.43	0.200
	4	95.3	161.7	79.3	8.2	0.50	4.39	0.192
Lettuce	1	52.7	36.7	39.0	5.1	1.33	5.55	0.189
	2	52.0	40.0	40.7	4.5	1.34	5.28	0.187
	3	51.7	35.0	42.3	5.0	1.31	5.22	0.181
	4	48.3	40.7	35.0	4.2	1.28	5.06	0.171
Peach	1	121.0	253.3	188.0	12.6	3.59	3.30	0.535
	2	122.0	267.0	186.0	13.7	3.50	3.29	0.545
	3	123.0	250.0	181.3	13.9	3.60	3.25	0.470
	4	107.2	243.0	169.0	13.3	2.95	3.24	0.497
Rhododendron	1	46.6	781.0	18.7	3.2	0.95	0.86	0.378
	2	46.6	765.3	20.0	3.3	0.94	0.82	0.389
	3	39.0	759.3	18.0	3.4	1.05	0.75	0.349
	4	37.0	779.0	13.3	2.9	0.94	0.68	0.315
Strawberry	1	45.3	77.0	26.7	19.6	0.85	2.30	0.219
	2	49.7	81.0	31.3	21.8	0.84	2.34	0.218
	3	44.3	73.3	25.0	19.3	0.83	2.38	0.210
	4	41.4	84.1	25.0	20.9	0.83	2.47	0.193

Table III. Results for the Elemental Analysis of a Further Eight Materials by Two Methods

Crop	Method	Ppm				%		
		Fe	Mn	Zn	Cu	Ca	K	Mg
Celery	1	58.7	72.7	34.3	20.3	4.02	3.47	0.286
	3	55.7	70.0	35.0	24.0	4.29	3.16	0.252
Cabbage	1	77.3	35.3	28.3	7.9	2.22	4.92	0.145
	3	75.7	35.0	31.3	9.0	2.42	4.55	0.147
Chinese gooseberry	1	70.0	126.0	18.3	8.9	4.03	3.38	0.191
	3	68.7	126.0	18.3	9.5	4.39	3.18	0.179
Dwarf bean	1	86.0	311.0	215.3	9.2	1.41	6.66	0.539
	3	81.0	310.3	218.3	10.9	1.57	6.53	0.492
Garlic	1	52.0	149.3	30.0	13.7	1.32	4.87	0.165
	3	48.3	156.0	31.7	14.7	1.46	4.90	0.168
Potato	1	117.0	466.0	58.6	13.7	1.82	6.31	0.361
	3	116.7	460.1	63.0	16.2	1.80	5.95	0.340
Red beet	1	109.0	77.0	18.7	7.7	5.01	3.76	1.05
	3	106.0	84.3	20.0	8.1	5.40	3.63	1.06
Squash	1	144.0	93.3	64.7	13.7	7.85	3.81	0.626
	3	153.0	92.0	63.3	15.0	8.03	3.62	0.598

Table IV. Coefficients of Regression[a] and Correlation Coefficients[a] (in brackets) of One Method on Another

Method	Method	No. of crops[c]	Zn	Mn	Fe	Cu	Ca	Mg	K
1	2	7	1.021 (0.999)	1.021 (0.998)	0.932 (0.991)	0.869 (0.989)	1.034 (0.998)	0.989 (0.995)	1.050 (0.998)
4	3	7	0.938 (0.998)	1.019 (0.999)	0.520 (0.869)	1.067 (0.967)	0.790 (0.990)	1.125 (0.988)	0.961 (0.999)
1	3	15	1.004 (0.997)	1.023 (0.999)	0.994 (0.986)	0.871 (0.971)	0.991 (0.996)	1.105 (0.994)	1.052 (0.999)

[a] Coefficients are for the regression of the data obtained by the first named method on that of the second named method. [b] All correlation coefficients are highly significant $p = 0.001$. [c] $n = 21$ where 7 crops and $n = 45$ where 15 crops.

There were indications that for some elements the extra treatment of ashed material led to marginal increases in a few crops. Since, however, no real advantage was gained, method 2 was not tested further for the other eight crops.

In spite of some fairly obvious differences between the results obtained by methods 3 and 4, most results by method 4 would be acceptable for routine diagnostic work. However, Fe values of many plant samples by method 4 are unacceptably low. This could be due to the formation of anhydrous ferric sulfate which is insoluble in anhydrous sulfuric acid. Diluting the acid after digestion apparently did not quantitatively dissolve the ferric sulfate (Gorsuch, 1970). In addition, the Zn value for rhododendron and the Ca value for peach are unacceptably low. A possible explanation for the low Ca value for peach by method 4 is the precipitation of part of the Ca as calcium sulfate due to the high content of Ca in peach (Gorsuch, 1970). There is no obvious explanation for the low Zn value.

Method 1 gave comparable results to method 3 for all elements for the 15 crops, and the values from either

method would be acceptble for diagnostic and most experimental work. Isaac and Johnson (1975) and Smith and Schrenk (1972) found no difference between dry ashing and wet ashing for micronutrients in alfalfa, citrus, orchard, pecan, and tomato leaves, and in alfalfa, corn, sorghum, and wheat leaves, respectively. Baker and Smith (1974) also reported no differences for micronutrients in cabbage leaves but reported lower value for Cu in corn cob by dry ashing relative to wet ashing. Our experience has been that for nonleaf samples, such as dried strawberry fruit and wheat flour, lower recoveries occur for Cu by dry ashing. The slightly lower recovery of Cu on a number of crops by method 1 relative to method 3 could be due to retention of this element by Si on the crucible, or by Si in the sample (Allen, 1971). An increase in ashing temperature from 475 to 500 °C may have given slightly higher Cu values because Cu appears to be retained on unoxidized carbonaceous residue (Baker and Smith, 1974). Although temperatures above 500 °C may give better Cu values, significant losses of K and Mn could occur (Gorsuch, 1970; Isaac and Jones, 1972).

The higher values for K by method 1 compared to method 3 on some crops could be partly explained as a result of precipitation of $KClO_4$ when the digest was being diluted in method 3. Apparently the warming of the solution did not quantitatively dissolve the precipitate. Precipitation of K as $KClO_4$ is likely if the K levels are high and the sample weight is also high (Johnson and Ulrich, 1959). There is no obvious explanation for the slightly higher Mg values by method 1. Variation of some replicates for Mg was greater than normal and the possibility of contamination cannot be ruled out.

From this investigation the following can be concluded: (i) A dry ashing procedure which gave comparable results to wet ashing for Fe, Mn, Zn, Cu, Ca, and Mg using atomic absorption and for K using flame emission has been found acceptable for a wide variety of horticultural crops. (ii) No advantage was gained by digesting the ash in 6 N HCl and bringing it to dryness twice; the more simple and rapid method of warming the ash for 0.5 h with 2 N HCl gave comparable results. (iii) Wet ashing using $H_2SO_4 + H_2O_2$ gave a high percentage of unsatisfactory Fe values and occasional unsatisfactory Ca and Zn values. This method, therefore, has a limited application.

LITERATURE CITED

Allan, J. E., "The Preparation of Agricultural Samples for Analysis by Atomic Absorption Spectroscopy", Varian Techtron, Springvale, Australia, 1971, pp 1–15.
Baker, A. S., Smith, R. L., *J. Agric. Food Chem.* **22**, 103 (1974).
Basson, W. D., Böhmer, R. G., *Analyst (London)* **97**, 482 (1972).
Gorsuch, T. T., "The Destruction of Organic Matter", Pergamon Press, Oxford, New York, Toronto, Braunschweig, 1970.
Gorsuch, T. T., *Analyst (London)* **84**, 135 (1959).
Isaac, R. A., Kerber, J. D., "Instrumental Methods for Analysis of Soils and Plant Tissue", Soil Sci. Soc. Amer. Inc., Madison, Wis., 1971 pp 17–37.
Isaac, R. A., Johnson, W. C., *J. Assoc. Off. Agric. Chem.* **58**, 437 (1975).
Johnson, C. M., Ulrich, A., *Calif. Agric. Exp. Sta., Bull.* **766**, 26–32 (1959).
Jones, J. B., Isaac, R. A., *Agron. J.* **61**, 393 (1969).
Jones, J. B., Steyn, W. J. A., in "Soil Testing and Plant Analysis", Walsh, L. M., Beaton, J. D., Ed., Soil Sci. Soc. Amer. Inc., Madison, Wis., 1973, pp 249–270.
Koirtyohann, S. R., Pickett, E. E., in "Flame Emission and Atomic Absorption Spectroscopy", Vol. 3, Dean, J. A., Rains, T. C., Ed., Marcel Dekker, New York, N.Y., 1975, pp 344–366.
Parkinson, J. A., Allen, S. E., *Commun. Soil Sci. Plant Anal.* **6**, 1 (1975).
Pye Unicam Ltd, "Atomic Absorption Spectrophotometry with Pye Unican SP 1900 and SP 190 Series Instruments", Cambridge, England, 1975, pp 1–32.
Smith, D. L., Schrenk, W. G., *J. Assoc. Off. Agric. Chem.* **55**, 669 (1972).

Received for review November 15, 1977. Accepted February 27, 1978.

Celery Leaf Juice: Evaluation and Utilization of a Product from Harvest Debris

Hans A. Buttkus

Celery trimmings, primarily leaves, from Fraser Valley and Okanagen vegetable packinghouses, which are normally not utilized for human consumption, yielded upon macerating and pressing 70–80% of a dark-green, bitter-tasting juice. Removal of the chloroplast fraction by low centrifugal fields resulted in a clear, yellow, celery-flavored juice, of acceptable taste. This refined celery leaf juice contained 5–6% of total solids, 0.8% of total crude protein, 0.1% of heat-precipitable proteins and amino acids, peptides, and proteins not preciptitated by heat. It also contained the vitamins, niacin, riboflavin, thiamin, and ascorbic acid at 0.45, 0.05, 0.01, and 2 mg/100 g, respectively. The major mineral constituents were Ca, 0.30%; Na, 0.20%; 0.15%; Mg, 0.02%; P, 0.01%; trace amounts of Fe, Cu, Mn, Al, Ni, Cr, Ba, and Sr were also present. Most of the lipid material, which contained some thiodan I, II, and malathion from agricultural practices, was removed from the juice with the chloroplast fraction during centrifugation. Rat feeding trials with the refined celery leaf juice solids (LJS) over a period of 8 weeks showed no growth inhibition or gross abnormalities of the major organs. Taste panels preferred tomato–celery leaf juice blends over commercially available V-8 type vegetable juices.

Relatively few of the plant leaves growing on earth are used directly in the human diet. Edible leafy plants representative of different plant families in the northern hemisphere such as spinach, lettuce, cabbage, asparagus, parsley, onion, and bamboo shoot have been selected over many years for their low bitterness, low fiber content, and for the absence of toxic constituents. The inherent objectionable bitterness and astringent character are major quality aspects which make most of the plant leaves unsuitable for human consumption.

Agriculture Canada Research Station, Summerland, Bristish Columbia, V0H 1Z0.

0021-8561/78/1426-0827$01.00/0