tokinin. Caffeine concentrations of 10^{-5} M and less with kinetin in the media did not affect the yield of soybean callus over that growing on media containing no caffeine. However, when 10^{-4} M of caffeine was used, yields were reduced and the 10^{-2} M rate completely inhibited growth. Thus, the increased callus growth of the soybean bioassay from citrus extracts in my original observations was not due to the caffeine.

In recent studies, Nathanson (1984) pointed out that caffeine may be a naturally occurring pesticide. He found that relatively low concentrations of methylxanthines were potent synergists when combined with certain other compounds. One of these synergistic compounds was octopamine, which we have reported previously to occur in citrus and other plants (Stewart and Wheaton, 1964; Wheaton and Stewart, 1970). It is tempting to speculate that caffeine might function as a deterrent to herbivores in reproductive buds and flowers.

In plants, caffeine sprays have been reported to increase flowering of potatoes and olives whereas xanthine was shown to increase flowering of grapefruit seedlings (Kessler and Bak, 1959). Other researchers have investigated some of the more basic properties of caffeine and related xanthine derivatives with cell division in plants. These compounds were found to prevent cytokinesis and induce formation of binucleate cells in plants (Kihlman, 1955; Kihlman and Levan, 1949). Binucleate cells induced with caffeine may be held in the G_1 stage for extended periods (Davidson, 1983). This stage is prior to the S phases where DNA synthesis occurs. Davidson (1983) has suggested that caffeine may be chemically similar to the naturally occurring cell regulator that controls mitosis and/or differentiation. If this hypothesis can be supported, it may be that caffeine or similar methylxanthines are synthesized in many plants or at least in juvenile tissues of woody plants and that these compounds may function as growth regulators.

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Use of a Standard-Addition Bromide-Selective Electrode Technique To Determine Bromide and Trace Its Migration in Peaches

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A bromide-selective electrode (BrSE), coupled with a standard-addition procedure, was successful in detecting known levels of bromide ion in peach (*Prunus persica* (L.) Batsch) extract. Bromide, added as 0, 1, 5, 10, 25, 50, or 100 mg/L of deionized H_2O (bromide standard solution) to ground peaches, was detected with a mean recovery of 103 ± 2%. BrSE analysis yielded similar results as X-ray fluorescence and thiosulfate titration when bromide content was measured in MeBr-fumigated peaches or in peach puree fortified with NaBr. The concentrations of bromide in the peel and flesh of peaches that had been fumigated with MeBr at 32 g/m^3 for 3.5 h averaged 11 and 4 mg/kg, respectively, both 1 and 7 days after treatment. A standard-addition BrSE method can be useful for determining residues when fruit has to be fumigated for quarantine purposes.

Methyl bromide (MeBr), when absorbed in many plant tissues, decomposes to yield a methylated protein product and bromide ions (Winteringham et al., 1955). Bromide may be measured by thiosulfate titration (Shrader, 1942), X-ray fluorescence (Getzendaner, 1961), and ion-selective electrode potentiometry (Pflaum et al., 1962). Potentiometric analyses of bromide have usually been direct estimations of bromide concentrations utilizing a calibration curve of electrical potential vs. bromide content.

Some researchers have used BrSE to analyze vegetables and fruit grown in soil fortified with KBr or fumigated with MeBr for bromide (Abdalla and Lear, 1975; Basile and Lamberti, 1981). Others have analyzed MeBr-fumigated fruits and cereals with a BrSE (Banks et al., 1976; Gnanasunderam and Triggs, 1983). In some studies, ashing and drying of the commodity preceded extracting of the bromide. Only Banks et al. reported recovery data or comparisons with established analyses of bromide determination on fruits and vegetables with residues below 3000 mg/kg of bromide. Their bromide recoveries ranged from 94 to 118% for wheat fortified with KBr at 25–75 mg/kg and from 95 to 112% for maize, copra, sorghum, and soybeans fortified with KBr at 50 mg/kg. Their results with BrSE were similar to those with neutron activation analysis, Volhard titration after exchange chromatography, and X-ray fluorescence for a concentration range of 20–53 mg/kg.

Each of the above studies was conducted utilizing a calibration curve to estimate bromide concentration. An alternative to the use of a calibration curve is the use of

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a standard-addition technique (Cammann, 1979) in which the sample and standard have an identical background composition. This procedure aids in overcoming problems with background interference and complexing ligands.

We conducted this research to determine whether a BrSE standard-addition technique can reliably be used to measure bromide ion residues in crude aqueous peach extracts at levels commonly observed in fruits and vegetables fumigated with MeBr. The diffusion of bromide residues between peel and flesh of peaches in cold storage was also examined.

EXPERIMENTAL SECTION

Recovery of Bromide from Peach Extract. A total of 10–14 peaches were finely divided without additional liquid in a blender and separated into 25-g portions. Samples (100 mL) were prepared by adding to each 25-g portion 0 (blank), 1, 5, 10, 25, 50, or 100 mg/L of bromide solution consisting of KBr (Orion bromide standard solution, Orion Research Inc., Cambridge, MA), 0.01 mol of $NaNO_3$ crystals, and water. After mixing, each sample was centrifuged at 1000*g* and the supernatant decanted into Erlenmeyer flasks.

Samples consisting of 50.0-mL aliquots of the supernatant were analyzed for bromide with a BrSE (Beckman Instruments Inc., Fullerton, CA). Either a fiber junction calomel electrode (Corning Scientific Instruments, Medfield, MA) or a double-junction sleeve electrode (Orion) served as reference electrode on an Altex Select Ion 5000 ion analyzer (Beckman). We used standard-addition analysis and added 0.5 mL of aqueous bromide standard to the sample after having taken an initial measurement of the potential (mV) across the sample. Standards of 20, 100, 500, 1000, 2500, 5000, or 8000 mg/L served as the addition solutions for the 0, 1, 5, 10, 25, 50, or 100 mg/L bromide samples, respectively. The resulting data were statistically analyzed by Student's t-test. Before peach extracts were analyzed, 50.0-mL aliquots of deionized water with 0 (blank), 1, 5, 10, 25, 50, and 100 mg/L bromide were analyzed in a procedure similar to that used for the peach extracts. Between usage periods, the electrode was stored alone in 0.01 M bromide standard solution to reduce the possibility of interfering ions exchanging with bromide on the electrode membrane, thereby reducing its selectivity for bromide.

A value for slope of the electrode response curve was required to calculate the bromide concentration. The procedure for making slope estimates using standard addition is similar to that for making concentration estimates, except that, for the former, 0.5 mL of an addition standard is added to a blank, followed by recording of the potential reading and another addition of the same concentration. The concentration of the 50-mL aliquot after each addition is known, and the slope is the variable. These estimates were made for each level of addition standard in quadruplicate with each reference electrode.

The estimation of recovery of bromide from peach extract was calculated from the mean of observed bromide concentrations at each addition level, including the blank, and applying a formula suggested by Banks et al. (1976). We corrected for the water content of the peaches (85.0%), because this source of water diluted the added bromide. We calculated the mean and standard deviation of the recovery value for all levels combined. Separate mean recovery values were obtained for each reference electrode.

Each of three trials (A–C) consisted of four replications at each level of expected bromide concentration for each reference electrode. We used the initial (i.e., prior to the addition step) potential readings of each sample in two trials to derive a calibration curve for the third trial. We used estimates interpolated from these calibration curves to calculate recoveries to permit comparison of the estimates of bromide concentration obtained by a curve and by the standard-addition procedure. The resulting data were statistically analyzed by using an analysis of variance (ANOVA).

Table I. Recovery of Bromide Using a Standard-Addition Technique

	rec of Br,[a] %			
	water		peach extract	
concn of Br, samples, mg/L	calomel	dbl junction	calomel	dbl junction
1	98.3	95.6	104.5	100.6
5	106.3	102.7	100.4	103.6
10	110.8	101.2	104.2	103.2
25	105.9	102.9	100.9	102.9
50	106.4	104.8	103.8	105.2
100	104.9	103.9	100.8	100.8
mean	105 (4)	101 (3)	102 (4)	103 (2)

[a] Each observation reported with standard deviation on last reported digit obtained from mean of 12 replications among three trial runs.

Finally, peaches were either fumigated with 32 g of MeBr/m^3 for 3.5 h at 20 °C or ground and fortified with NaBr crystals at the rate of 10 mg/kg in the fruit. Quintuplicate samples of these fruits were analyzed for bromide by X-ray fluorescence (Getzendaner, 1961), thiosulfate titration (Conacher, 1974), and BrSE potentiometry using a standard-addition procedure. The resulting data were statistically analyzed by using ANOVA.

Location and Diffusion of Bromide in MeBr-Fumigated Peaches. Peaches were fumigated with 32 g of MeBr/m^3 for 3.5 h at 20 °C. Untreated peaches were used as controls. After the fumigated peaches had been aerated for 1 h, they and the controls were stored at 2.5 °C. Sections of 25–30 of the fumigated and control peaches, representing the peels only, the flesh only, and the flesh with peels intact (wholes), were separated and weighed after 1 and 7 days of storage. All samples were homogenized with a 0.1 N solution of $NaNO_3$, mixed for 1 h on a shaker, and centrifuged at 1000*g*. The supernatant of each sample was then analyzed in quadruplicate for bromide by use of a standard-addition method, with a 500 mg/L standard used for the addition. The value of the slope of the BrSE was determined in quadruplicate on peach samples without added bromide. The calomel fiber junction reference was used for the determinations. The resulting data were statistically analyzed by ANOVA.

Statistical Analysis. Means from statistically analyzed data were considered significant when the corresponding F values yielded a $p < 0.05$.

RESULTS AND DISCUSSION

Analysis of Bromide in Peach Extract and in Water. The mean recovery values of bromide in peach extract using standard addition were approximately 100% (Table I). The differences in mean recovery values appeared insignificant, whether the comparison was made between reference electrodes (double junction vs. calomel), sample matrices (water vs. peach extract), or bromide concentration levels.

Both electrodes used in this experiment had an internal filling solution of KCl, but the double-junction electrode also contained an outer filling solution of a nitrate or acetate salt into which the internal filling solution seeped. This seepage occurred at a lower rate than that of the external filling solution into the sample, thereby mini-

Table II. Recovery of Bromide in Spiked Peach Extract Samples as Estimated by a Calibration Curve and a Standard-Addition Technique

	recovery,[a] %			
	calomel		dbl junction	
trial	std addn	calibrn curve	std addn	calibrn curve
A	100 (3)	103 (10)	101 (2)	120 (10)
B	103 (3)	110 (10)	102 (2)	112 (7)
C	104 (5)	132 (7)	105 (3)	110 (10)

[a] Each observation reported with (standard deviation) on last reported digits obtained from the mean of separate recovery values at concentrations of 1, 5, 10, 25, 50, or 100 mg/L. Each recovery value, in turn, was obtained from the mean concentration estimates of four replications. Standard error of mean was 8.

mizing the chloride flow into the samples. Reference electrodes constructed with ground-sleeve diaphragms have provided the most accurate measurements for cells with liquid junction (Cammann, 1979). This accuracy is due to a larger, steadier stream of internal filling solution flowing from the double-junction sleeve electrode to the sample (Koryta, 1975; Cammann, 1979). Our results suggest that any advantages gained in the use of a double-junction electrode over a calomel electrode in peach extract are small.

In the experiment of standard addition vs. calibration curve involving trials A–C, estimates derived from standard addition were statistically different from those derived from calibration curves (Table II). Since the known addition estimates are closer to 100%, they can be said to be more accurate. Recovery differed statistically among the trials, but not between the electrodes. Thus, it would appear that the initial potentials of the samples were sufficiently different to prevent calibration curves of the trials from being consistent with each other. Standard-addition analyses are much less affected by differences in potentials of complex matrices, because the standard is added to the identical matrix that is affecting the potential of the sample.

The three slopes from trials A–C values were very similar for a given reference electrode. The composite calibration curves, using the data of all three trials together, were $y = -53.1x + 125.6$ with $r^2 = 0.998$ and $y = -55.2x + 101.2$ with $r^2 = 0.990$ using the double-junction and the calomel references, respectively; y has units of millivolts and x of log (concentration); the concentration range is 1–100 mg/L.

The choice of slope value for estimating a standard-addition concentration value is important. A variation in the slope measurement of ±1 mV/decade could mean a difference of 0.6–0.7 mg/L in the result for a sample at 25 mg/L of bromide. The concentration change represented by a change in the potential upon addition standard increases according to (Durst, 1969)

$$C_x = \Delta C(10^{\Delta E/S} - 1)^{-1} \quad (1)$$

where C_x is the concentration of sample before addition, ΔC is the change in concentration upon adding the addition standard, ΔE is the change in potential upon adding the addition standard, and S is the response of the electrode with concentration (slope). A range of slope values of −53 to −59 mV results in a range of calculated concentration values from 23.1 to 26.8 mg/L, given a 17-mV potential shift between original sample and sample with a 2500 mg/L addition standard. Slope estimtes at any concentration level can be produced from standard-addition techniques (Table III). However, using slope values derived from a calibration curve would have resulted in less accurate recoveries of bromide from known samples

Table III. Estimates of Slope Near Preaddition Concentration Levels of Bromide in H_2O

concn range, mg/L		slope,[a] mV/decade	
before addn	after addn	calomel	dbl junction
0.2	0.4	−41 (6)	−42 (2)
1	2	−55 (2)	−54 (2)
5	10	−57 (1)	−57.1 (7)
10	20	−57 (1)	−57 (1)
25	50	−58 (2)	−57.5 (9)
50	100	−57 (2)	−58 (1)
80	160	−57 (1)	−57.6 (8)

[a] Each observation reported obtained from mean of 12 replications among three trial runs. Shown with standard deviation for last reported digit.

Table IV. Comparison of BrSE Potentiometry, X-ray Fluorescence, and Thiosulfate Titration in Determining Inorganic Bromide in Peaches

	obsd Br concn,[a] mg/kg		
Br source	BrSE potentiometry	X-ray fluoresc	thiosulfate titration
added Br from MeBr	8.0 (2)	9.0 (5)	10 (2)
added Br from NaBr	8.8 (2)	8.6 (6)	8 (4)

[a] Each value is mean of five replications shown with standard deviation on last reported digit. Standard error of mean was 0.3.

Table V. Concentration of Bromide in Peach Tissues after CH_3Br Fumigation and Storage at 2.5 °C

incubn period,		mean Br concn,[a] mg/kg		
days	treatment	wholes	peel	flesh
1	control	0.7 (2)	1.5 (7)	0.5 (2)
	fumigated	5 (1)	11 (3)	4 (1)
7	control	0.6 (1)	1.3 (4)	0.50 (8)
	fumigated	5 (2)	11 (3)	4 (2)

[a] Each mean reported obtained from 16 replications and shown with standard deviations on last reported digit. Standard error of mean was 0.3.

than those given in Table I. Thus, the two means of slope determination do not appear to be interchangeable. In fact, the slopes at concentration levels above 1 mg/L are very similar to the calibration curve slope obtained in water (−57.6). This seems to indicate that the standard-addition estimate corrects for interferences in the peach extract matrix.

Determination of bromide concentrations by use of standard addition and BrSE on separate lots of peaches fumigated with MeBr or fortified with NaBr yielded statistically insignificant differences from results obtained by use of X-ray fluorescence or thiosulfate titration (Table IV). The BrSE method demonstrated noticeably less variability than either of the other two techniques.

Location and Diffusion of Bromide in Peaches. There was no significant diffusion of bromide between peel and flesh of peaches during 1 or 7 days of storage at 2.5 °C following MeBr fumigation (Table V). Further, peels contained about 3 times as much bromide as the flesh. Differences in bromide levels between fumigated and control peaches were highly significant in each category of fruit tissue. Thus, BrSE can be used to detect differences in bromide levels in the tissues of peaches following MeBr fumigation.

Movement of bromide from the peel into the more water-laden flesh was expected to be spontaneous once the bromide ion was hydrated. However, more bromide was found in the peach peel than in the flesh, probably because the peel is more accessible to the fumigant and the peel

contains significantly more protein than the flesh (Hansen, 1970).

CONCLUSION

A standard-addition ion-selective electrode technique can be used to accurately and precisely determine bromide in the range of 1–100 mg/L in peach extract. With such a method we were able to detect bromide with a recovery slightly above 100% and with less variability than with a method based on a calibration curve. Bromide analysis in crude peach extract saves the time that was previously used in ashing, drying, and other preparatory processes. Use of BrSE costs about one-fifth that of neutron-activation analysis or X-ray fluorescence.

The detection limit in peach extract for a double-junction electrode coupled with the standard addition procedure appears to be below 0.2 mg/L. This is evidenced by a mean of 0.14 ± 0.06 mg/L for the peach extract blanks, where the mean is still more than double the standard deviation. However, the accuracy of this value was not determined. The concentration represented by the minimum potential unit of the potentiometer increases proportionately with C_x (eq 1). Therefore, one decimal place is lost from the accuracy of a concentration measurement in the standard-addition BrSE measurement of bromide as the concentration value increases 1 order of magnitude.

A BrSE can detect bromide in peaches resulting from MeBr fumigation. We also found that bromide does not significantly diffuse from skin to flesh under conditions that approximate those during storage or transport of peaches across the U.S.

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Isolation of Flavor Compounds in Model Systems by Countercurrent Continuous Dialysis

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A 40-m tubing type continuous dialyzer was designed in this study. The dialysis rate of flavor compounds was substantially increased over that of a batch process. The effect on dialysis efficiency of varying flow rates of sample and solvent was studied. The ultimate purpose of flavor isolation could be accomplished by manipulating these two operating parameters. The effects of solvent composition, polarity, molecular weight, and acidity of flavor components were also investigated. The more polar and smaller flavor compounds exhibited greater diffusivities. The addition of 2% (v/v) ammonium hydroxide or 1% (v/v) water in diethyl ether was found to be beneficial in isolation of basic flavor components.

INTRODUCTION

The selection of a flavor-isolation technique depends on the nature of the starting material, the flavor constituents of interest, the precision required, time available, and cost. The general methods of flavor isolation have been reviewed by several workers (Jennings and Rapp, 1983; Schreier, 1984; Reineccius, 1984; Reineccius and Anandaraman, 1984). These methods take advantage of the differences in volatilities and solubilities of the flavor compounds vs. the other constituents. For fatty foods, the flavor-isolation process is confronted with problems that are not present in fat-free systems. For example, the vapor pressure of flavor constituents was found to be up to 500 times less in the presence of fats than in totally aqueous systems (Buttery, 1973). Fats may undergo hydrolysis in the presence of water such as in steam distillation (Honkanen and Karvonen, 1966). Fats as well as flavors are extracted if solvent extraction is attempted, since they are mutually soluble (Arnold and Barnhart, 1972). The fats present in a flavor extract will stay in the injection port of a gas chromatograph or column and decompose by heat, thereby interfering with the analysis of the flavor profile (Chang et al., 1977).

To obtain fat-free flavor isolates and make solvent extractions applicable to fatty foods, Benkler and Reineccius (1979, 1980) explored a dialysis method to separate flavor

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