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In situ measurement of acetaldehyde in senescing apple fruits.

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in situ MEASUREMENT OF ACETALDEHYDE IN SENESCING APPLE FRUITS

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By

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INTRODUCTION

Acetaldehyde is a naturally occurring plant substance that can result from the decarboxylation of pyruvate. Since the reaction is practically irreversible the enzyme is regarded as a decarboxylase. Pyruvic decarboxylase requires the cofactor diphosphothiamine (thiamine pyrophosphate, cocarboxylase), which is thought to be bound to the apoenzyme by magnesium ions. There is good evidence that in the decarboxylation of pyruvate the active acetaldehyde is linked to the thiazolium ring of the coenzyme (40). If NADH is present, the resulting acetaldehyde is reduced by alcohol dehydrogenase to ethanol. In yeast, it has been shown that another enzyme, pyruvic oxidase, can transfer the active acetaldehyde to lipoic acid, but this reaction has not been shown in higher plant tissue.

Under ambient conditions, in higher plants pyruvate is converted to acetyl-CoA through oxidation catalyzed by the pyruvate dehydrogenase system (40). Acetaldehyde and ethanol generally occur in only trace amounts due to relatively low decarboxylase activity, but should pyruvate accumulate, then acetaldehyde and ethanol may also begin to accumulate (2). When low oxygen or high carbon dioxide concentrations exist within plant tissues acetaldehyde and ethanol are known to accumulate, and their increase is often taken as an indication of fermentation.

In 1925 Thomas (66) showed that environments conducive to acetaldehyde accumulation produced cellular disorganization and browning of apples. The toxicity of acetaldehyde has been confirmed by subjecting apples to acetaldehyde through injection (10) or through vapor diffusion (61). Subsequently, acetaldehyde has been implicated in the development of several post-harvest physiological disorders of apples, such as low temperature injury (43), low O_2 injury (62), CO_2 injury (60,66), internal breakdown (61), and soft scald (47, 68). Several of these disorders involve tissue disorganization and are related to maturity and senescence, and evidence suggests that acetaldehyde accumulates during senescence (18,23).

Despite the interesting relationship between acetaldehyde and tissue disorganization that was demonstrated by Thomas in 1925, the methods of analysis he, and subsequently others, used have been suspect owing to their lack of sensitivity and specificity. Methods now available appear to offer more reliable results. This work was undertaken to study the changes in acetaldehyde content of apple tissue during ripening and senescence using the more specific and sensitive methods that are currently available.

REVIEW OF LITERATURE

A. Methods of Acetaldehyde Determination

I. Steam distillation

Interest in the acetaldehyde content of plant tissue developed from the work of Power and Chestnut in 1920 (54). Examining the odorous constituents of apple tissue, they extracted the aldehyde from crab apple parings by steam distillation, separated the aldehydes from the distillate by addition of saturating amounts of sodium bisulfite, and after 5 days extracted the nonaldehydic compounds with aldehyde-free ether. The bisulfite solution was made alkaline with sodium carbonate and distilled in a current of steam, producing a distillate containing the aldehyde fraction. After repeated extraction in a current of steam, the concentrated distillate was subjected to special tests. The presence of aldehydes was indicated by the rapid reduction of a cold ammoniacal solution of silver oxide, producing a metallic mirror; and by giving an immediate red coloration with Schiff's reagent. A test for formaldehyde with phenylhydrazine hydrochloride gave a negative result. On the other hand, the distillate gave the specific reaction for acetaldehyde which consisted of adding one or 2 drops of a 33% solution of dimethylamine and subsequently about 5 drops of freshly prepared 1% aqueous solution of sodium nitroprusside, whereby an indigo-blue color was produced which changed to

brown or yellow. The blue color was formed with a solution of 1:10,000 (w/w) acetaldehyde:H_2O, but was not formed by formaldehyde, the higher fatty aldehydes such as n-heptylic aldehyde, or by aliphatic terpene aldehydes such as citral, which gave only a reddish color.

The aldehyde in the distillates was further identified by Power and Chestnut (54) by formation of the silver salt of the aldehyde and by analyzing the precipitated silver salts for its silver content. The calculated per cent of silver in the silver salt of acetaldehyde is 64.6, and their findings ranged from 64.0 to 64.3, indicating the aldehyde from apple tissue was nearly pure acetaldehyde with possibly a trace of a higher homolog. From the weight of the silver salt obtained, they estimated that the amount of acetaldehyde in crab apple parings was about 0.001% of fresh weight.

The real accomplishment of Power and Chestnut (54) was not in this crude estimation of the amount of acetaldehyde in apple tissue, but rather in showing that acetaldehyde could be detected in the odorous emanation from the fruit. They accomplished this by placing apples in a large copper vessel having a tight-fitting cover and a small opening at the top and bottom so a current of air could be slowly aspirated through it. The air leaving the vessel was passed through a saturated solution of sodium bisulfite. Air was continuously drawn through the system for 7 or 8 days by means of a filter pump. The bisulfite solution was made

alkaline and distilled to collect the aldehydes. The concentrated distillate had the characteristic odor of acetaldehyde, immediately reduced an ammoniacal solution of silver oxide, and gave within a few seconds a deep pink coloration with Schiff's reagent. It also gave with dimethylamine and sodium nitroprusside the deep indigo-blue color which is characteristic of acetaldehyde.

In 1925 Thomas (66) confirmed that acetaldehyde was produced by intact apples. He passed air through a desiccator containing apples and bubbled the emerging gas through aqueous acid phloroglucinol (1% phloroglucinol in H_2SO_4), trapping the aldehydes as their sparingly soluble phloroglucides. Finding that with excess phloroglucinol the amount of precipitation was roughly proportional to the amount of aldehyde present, he estimated the amounts of acetaldehyde emanating from the apples under different environments by the weight of the precipitate formed. However, Thomas conceded that "although the method for several reasons is not recommended for work requiring a high degree of accuracy it has proved most useful in the experiments of comparison described in this paper." His data indicated that quantities of acetaldehyde greater than 0.015 g could be determined in this manner.

The identity of these aldehydic metabolic products had to be established by methods other than melting points since phloroglucides are amorphous in nature, having ill-defined melting points. Thomas (66) repeated and confirmed the experiment

of Power and Chestnut (54) and showed that the phloroglucide precipitate was at least in part due to the presence of acetaldehyde in the living apples. To establish the identity of the aldehyde he prepared a steam distillate of apple tissue and formed crystalline derivatives with p-nitrophenylhydrazine and dimethylhydroresorcinol. The melting points were identical with those of acetaldehyde p-nitophenylhydrazone and acetaldehyde dimethylhydroresorcide synthesized in the laboratory. To estimate the acetaldehyde content of apples he used the method of Ripper (57). The acetaldehyde was expelled from the sample by steam distillation into a series of receiving flasks containing sodium bisulfite. After 2 hours of steam distillation, the acetaldehyde content of the apple tissue was determined by titrating the receiving and a control flask against standard iodine, using starch solution as an indicator. Thomas stated that "experience showed, first, that acetaldehyde only rarely escaped to the second flask, and then only in traces, and second, that after 2 hours' steam distillation all significant acetaldehyde was expelled from apple pulp."

Working with Bartlett pears, Harley and Fisher in 1927 (29) determined in a manner similar to that of Power and Chestnut (54) that acetaldehyde was normally produced in living pear tissue. Using the same qualitative tests as Power and Chestnut (54), they found acetaldehyde to be the only aldehyde produced under their experimental conditions.

The quantitative method used in their experiments was essentially that of Thomas (66), except that the tissue was cut into smaller pieces and the distillation stopped after one liter of distillate was collected. This usually required about 90 minutes, after which it was found that all significant traces of acetaldehyde had been expelled from the pear tissue.

Gerhardt and Ezell (26) in 1939 noted that the odorous constituents of fruits were all derivatives of 3 large classes of organic compounds, namely, the aldehydes, alcohols, and esters of the fatty acid series; the olefins; and the heterogenous group of essential oils, many of which contain unsaturated hydrocarbons. These compounds are all chemically reactive and are easily oxidized. They determined the total oxidizable volatile constituents emanating from stored fruit by absorbing these materials in concentrated H_2SO_4 rather than employing the bisulfite addition reaction. The absorbed substances were oxidized with ceric sulfate and measured in terms of ceric sulfate reduced. Experiments showed that stoichiometric relations were not involved and the reaction could not be expressed by equation. However, working with acetaldehyde under standard conditions of temperature (98°C) and molarity of $Ce(SO_4)_2$ (0.1M), they found the reduction-oxidation ratio $Ce(SO_4)_2/CH_3CHO$ to be constant after 2 hours of oxidation. A quantitative study of the absorption of acetaldehyde in concentrated H_2SO_4 and its ultimate determination by oxidation with ceric sulfate resulted in an

average recovery of 97.2%. The acetaldehyde for this study was released from solutions held at 18°C by aeration with aldehyde-free air at the rate of 10 liters/hour for a period of 4 days. The amount of acetaldehyde liberated was determined by standardization of the original solution before and after aeration, following the method of Sutton (64). This indicated that volatiles such as acetaldehyde may be quantitatively removed from an air stream of 10 liters/hour, and may be oxidized directly in the original absorption flask with ceric sulfate.

In 1942, Gerhardt (23) reasoned that if odorous constituents are liberated from the surface of intact fruits, they must exist in larger amounts in the internal atmosphere and cell contents of such fruits. Using steam distillation for the removal of these volatiles he devised a method for measuring simultaneously the total oxidizable volatiles and the CO_2 present in the internal atmosphere of fruits and vegetables. This method involved the refluxing of the plant tissue in a stream of CO_2-free air, absorption of organic volatiles in concentrated H_2SO_4 and of CO_2 in 0.1N $Ba(OH)_2$, and the oxidation of the volatiles with $Ce(SO_4)_2$.

In determining the effect that length of extraction had on the yield of volatiles and CO_2, Gerhardt (23) found that after an initial 2 hours of extraction the additional amount removed grew progressively less with each 2-hour period of extraction. The reduction in the quantities of

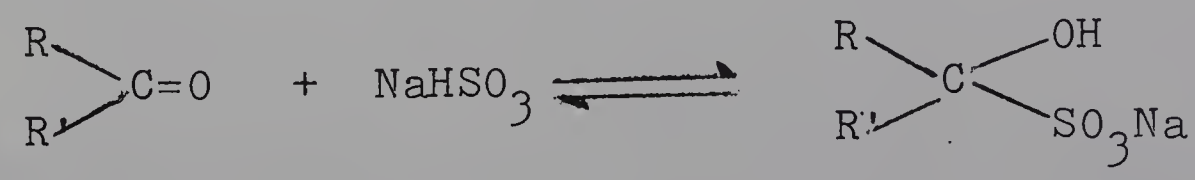
volatiles removed after the second hour of extraction was substantiated by Wardlaw and Leonard (73) and also by Willaman and Brown (74) in their removal of CO_2 from plant tissues by aspiration in boiling alcohol. An asymptotic rate of volatile liberation was suggested by Gerhardt (23), but was not substantiated by his limited data.

The accuracy of Gerhardt's technique was tested by recovery of known amounts of acetaldehyde added to the distillation flasks and extracted for 2 hours. Equivalent amounts of acetaldehyde were calculated from the amount of $Ce(SO_4)_2$ reduced, based on a reduction-oxidation ratio of $Ce(SO_4)_2/CH_3CHO$ of 51:1 found earlier (26). The results indicated that approximately 91% of the total acetaldehyde was recovered. Assuming that the ratio of removal of volatiles from tissue, like removal from aqueous solution, is asymptotic in nature, further extraction would hardly be practical. A comparison of tissue sampling technique (mincing in a food chopper vs. removal of a cylinder of tissue by a 3/4 inch cork borer) showed considerably greater loss of CO_2 but not of total volatiles when the tissue was minced.

Employing the steam distillation-bisulfite addition method, Gerhardt (23) determined acetaldehyde simultaneously with total volatiles. While acetaldehyde made up only 12.3% of the total oxidizable volatiles at the beginning of storage, with time it accumulated in greater amounts than other odorous constituents and after 225 days at 2°C it accounted for ap-

proximately 50% of the total volatiles. Acetaldehyde apparently increased from 0.16 mg/100 g FW at the beginning of storage, to 4.97 mg/100 g FW after 225 days.

There are several objections to the preceding approaches to quantitative analysis for acetaldehyde. (1) Enzymatic changes may be introduced as the fruit tissue is injured by slicing. Thomas (66) used 6 apples for each sample and cut each apple into 8 pieces before dropping them onto a support of perforated zinc in the distillation flask, where they stayed for 2 hours during the extraction. (2) Non-enzymatic changes could be brought about by the high temperatures employed. (3) Aldehydes other than acetaldehyde may undergo the bisulfite (39) or sulfuric acid (23) addition reaction thus giving inaccurate results. Carbonyl compounds generally react with sodium bisulfite with the formation of addition products,



where R is an alkyl group, and R' is a hydrogen atom in the case of aldehydes. Almost all aldehydes react with sodium bisulfite, but ordinarily only the cyclic ketones and methyl-ketones react. The addition-product formation is a reversible reaction, and the distribution at equilibrium is dependent on the nature of the carbonyl, the pH, temperature, solution concentration, and the excess of bisulfite (39).

II. Aroma concentration

A different approach to the study of volatile constituents of fruits arose from the development by Milleville and Eskew (48) in 1944 of a commercial process for recovering the entire volatile fraction from apple juice. The volatile fraction was concentrated 150-fold through the following steps: (1) superheating the juice; (2) flash-vaporizing the superheated juice at atmospheric pressure; (3) mechanically separating the vapors from the unvaporized juice; and (4) fractionating the vapors to obtain a more concentrated flavor.

A systematic chemical examination of the volatile apple concentrate was made by White (74) in 1950 after preliminary examination of the concentrate gave results differing widely from those of Power and Chestnut (54). The concentrate was fractionally distilled and each fraction was treated by appropriate identification methods. The carbonyl compounds in a juice concentrate were identified by preparing 2,4-dinitrophenylhydrazones and subjecting them to chromatographic separation on a bentonite column, and identifying them by mixed melting points and by elemental analysis. The concentration of acetaldehyde found in the 150-fold essence was 260 ppm, but more meaningful was the identification, along with acetaldehyde, of other carbonyls such as acetone (8 ppm), caproaldehyde (90 ppm), and 2-hexanal (64 ppm). These substances may have been measured as acetaldehyde in the earlier

procedures.

The data presented "gives a rough approximation of the composition of apple volatiles", reporting the compounds identified and the calculated amount of each compound in the 150-fold volatile apple concentrate. White (74) pointed out that while the data may be converted to the original apple juice basis by dividing by 150, it would be somewhat misleading since no effort was made to extract all the juice from the original apples or to recover the volatiles from the press cake. In addition, the procedure employed for removing the volatiles (crushing in a hammer mill followed by cold pressing) may have resulted in enzymatically catalyzed changes altering the proportion of the constituents, and some volatiles undoubtedly were lost to the atmosphere during the extraction.

Formation of 2,4-DNP-hydrazones, used by White (74), was later utlized in studies of the emanations from living apples in storage (69,70). Huelin (34) in 1952 used the collection method of Power and Chestnut (54) (passing air over whole apples and absorbing the products in bisulfite) and identified, in addition to acetaldehyde, acetone and propanal by chromatographing the 2,4-DNP-hydrazones on paper. The identity of the acetone derivative was confirmed by a spectral absorption curve and that of the aldehyde derivative by conversion to hydroxamic acids which were separated by paper chromatography.

Henze, et al. (32) in 1954 obtained a much more complex mixture of carbonyl compounds from commercial apple storage

volatiles than White (74) had obtained from volatiles distilled from apple juice, or Huelin (34) had obtained from volatile emanations of apples. They collected volatiles for a 7-month period using activated coconut shell carbon in a commercial air purification unit. The sodium bisulfite-extractable carbonyl compounds collected on the activated carbon were analyzed as their 2,4-DNP-hydrazones. Acetone, propanal and acetaldehyde were identified by co-chromatography with authentic compounds on columns of 2 different adsorbents, by comparison of ultraviolet spectral absorption curves and (for acetone) by melting point determination. Numerous smaller fractions were separated, 7 of which appeared by their absorption curves to be a closely related series of dienal or trienal carbonyl compounds. One compound might have been 2-hexanal. They suggested that collection of apple volatiles during a 7-month storage period may have resulted in accumulation of trace carbonyl compounds not detectable in short-term experiments. The complexity might, on the other hand, indicate a more efficient method of collection or that chemical changes occurred while the compounds were adsorbed on the carbon surface.

Meigh (46) in 1956 analyzed the volatile aldehydes and ketones present in the air surrounding gas-stored apples. Air was drawn through gas-tight storage cabinets for 6 days, volatiles were collected in a series of cold traps, and these were subsequently converted to 2,4-DNP-hydrazones which were

separated by column or paper chromatography. The analysis was at first regarded as qualitative, but later it was considered approximately quantitative after an efficiency study showed 81 to 110% recovery of acetone and 31 to 76% recovery of acetaldehyde from the air stream during a 6-day period. Using bulk samples of derivatives of apple volatiles accumulated from a number of experiments, Meigh (46) made qualitative determinations employing column and paper chromatography, melting point determinations, and spectral absorption curves. The main constituent found was acetone, with smaller amounts of acetaldehyde, n-butanal, propanal, ethylmethylketone, and isobutanal, with traces of methylpropylketone and, tentatively, isovaleraldehyde.

A vital point illustrated by these aroma determinations is that apples produce a number of carbonyl compounds and the indiscriminate analytical procedures employed earlier may be subject to considerable error.

III. Headspace analysis

The development of gas-liquid chromatography (GLC) made possible the separation and measurement of individual components in complex volatile mixtures. For this purpose, however, boiling, steam distillation, nitrogen stripping, or evacuation followed by cryogenic trapping have often been used to obtain a concentrate for GLC analysis. Large amounts of fruits and lengthy procedures have usually been involved. The elevated temperatures used in distillation

and the prolonged period of handling during sample preparation may lead to chemical changes or losses, and quantitative transfers of trapped volatiles are difficult. Consequently, work of this type has been qualitative, involving basic flavor studies, or has been concerned only with relative comparisons and not absolute quantities of volatiles in the product.

Grevers and Doesburg (27) used this approach to study proportionate changes of apple volatiles during storage and ripening. To collect volatiles, purified air was passed over fruits in containers and volatiles in the effluent air stream were adsorbed on carbon. Monthly samples were taken, and collected volatiles were removed from the carbon by high vacuum desorption. The total amount of volatiles produced was determined gravimetrically, and proportionate changes of the individual components were determined by visually comparing GLC chromatograms. Acetaldehyde was not specifically identified.

A vapor concentration technique was later used by Smagula, et al. (61) in a study of the volatile accumulation within apples affected with the physiological disorder "watercore". Sliced apple pulp was frozen in liquid N_2 and ground in a Waring blendor, and 30 g of the frozen powdered tissue were placed in a 200-ml Mason jar containing 150 ml of a 15% trichloroacetic acid solution to inactivate enzymes. After mixing for 10 seconds, the slurry was transferred to a 1000-ml flask, attached to a trapping apparatus, and evacuated for 15

minutes; the volatiles removed from the solution were collected in 2 liquid N_2 traps. By GLC analysis of the trapped substances, 3 major compounds were identified as acetaldehyde, ethanol, and ethyl acetate using cochromatography with known standards and comparison of retention times at 2 different temperatures. Peak areas were assumed to represent the concentrations of substances present in the extracts, but the actual concentrations in the tissue were not determined.

In addition to the vapor concentration procedures, direct vapor or headspace analysis can be accomplished (44, 65) and this offers several advantages to investigators of the volatile make-up of a food sample (20). The technique is relatively fast, requiring little sample preparation other than a period of equilibration at some standard temperature. Because no extraction and concentration steps are involved, the likelihood of artifact introduction or sample change is greatly minimized. However, only the sample's more volatile components are examined and the physical structure of the sample greatly influences vapor-analytical results. For example, some volatiles may be locally concentrated or contained within elements such as air sacs or seeds, and so may appear at disproportionately low concentrations in the headspace analysis of a whole fruit. They may be released by crushing the fruit, but disrupting the fruit structure introduces the possibility of enzymatically produced artifacts (17).

Despite these problems, direct vapor analysis has been

used frequently with fruit. Volatiles from apples were related to variety, maturity and ripeness by Brown, et al. (7) using this approach. Samples of 5 to 6 apples were placed in 1-gallon jars at 20°C. Air flowing through the jars was analyzed for CO_2 and ethylene. Other volatiles were determined on a 10-ml aliquot of headspace after stopping the air flow through the jars for 50 minutes. GLC data were expressed quantitatively by multiplying retention times in minutes by peak heights in millivolts. The quantitative data were plotted against time to form curves depicting the production patterns of the volatiles during the 30 to 40 days of the tests. Some of the peaks were tentatively identified by retention-time comparison to known compounds. However, acetaldehyde was not one of these tentatively identified compounds.

Romani and Ku (58) directly sampled volatiles emanating from ripening pears by daily sealing jars containing 1.5 kg of fruit for 30 minutes. Five ml of vapor were then withdrawn and analyzed by GLC. Quantitative estimates of the volatile components were made by measuring the peak area and expressing the results in square centimeters/kg fruit. Volatiles were tentatively identified by cochromatography with known standards. Acetaldehyde concentration in the emanations was shown to rise after 3 days at 20°C, following several weeks of storage at 0°C.

Working with intact oranges, Norman and Craft (52)

chromatographically measured endogenous volatiles emanating during and after storage of single fruit samples in 100% N_2. Jars were sealed for one hour, after which 5 ml of vapor were analyzed by GLC. They identified acetaldehyde, ethanol, ethyl acetate, and methanol as major volatile substances produced during and after nitrogen storage.

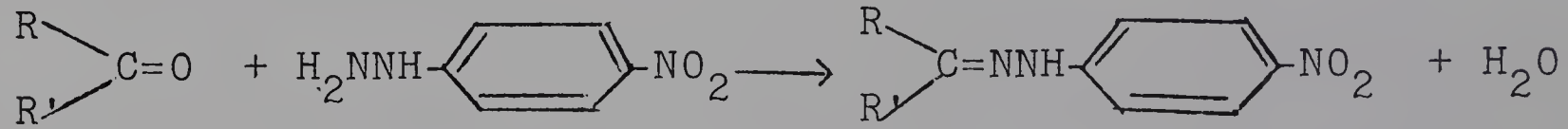
A gas chromatographic method used for the determination of alcohol in citrus juice by headspace GLC analysis (12) was later adapted to follow acetaldehyde changes in citrus fruits during controlled-atmosphere storage (13). A 10-ml portion of composite juice from 10 fruits was placed in tightly stoppered bottles which were kept at 33°C for one hour, after which 2-ml headspace samples were analyzed by GLC. The chromatograms showed only 3 peaks: acetaldehyde, ethanol and limonene.

Flath, et al. (20) compared fruit varieties and fruit products using high resolution vapor analysis, but cautioned against the use of vapor analytical results for estimating the relative concentrations of volatile compounds within a food sample. They pointed out that very few if any samples can be dealt with as ideal homogeneous solutions, with the equilibrated vapor above the sample constituents acting according to Raoult's law. Furthermore, variables affecting the composition of headspace at a given temperature have been discussed by Nawar (49,50). They include vapor pressure of the compound, type of medium in which it is distributed,

degree of solubility in the medium, concentration of the compound in the liquid phase, its miscibility with other organic compounds in the mixture, and the presence of salts and sugars. Therefore, despite the convenience of its application, direct headspace analysis has limited usefulness in attempting to determine the physiological roles played by fruit volatiles, since endogenous concentrations cannot be unequivocally determined.

IV. Formation of 2,4-DNP-hydrazones

The reagent 2,4-dinitrophenylhydrazine (2,4-DNPH) readily forms water-insoluble derivatives with carbonyl compounds. As shown by the equation (39),



where R is an alkyl group, and R' is a hydrogen atom in the case of aldehydes, the reaction is irreversible. Formation of 2,4-DNP-hydrazones was used in some (32,34,46,69,70,74) of the papers cited earlier as a means of identifying carbonyls. Ralls (55) carried this technique further when he combined steam distillation extraction and 2,4-DNP-hydrazone formation with GLC analysis to study the potential flavor components of peas. The 2,4-DNP-hydrazone derivatives of carbonyl compounds were regenerated by a rapid exchange reaction with α-ketoglutaric acid and volatilized directly into a gas chromatograph for separation and identification.

Mason, et al. (45) used a modification of this re-

generation procedure in studying the components of roasted peanuts. Since the bicarbonyl and other compounds such as pyrazines are not regenerated from their 2,4-DNP-hydrazone derivatives, the monocarbonyl compounds could be analyzed separately. Six to 8 mg of the mixture of the 2,4-DNP-hydrazone derivatives were weighed, thoroughly mixed with 3 times their weight of α-ketoglutaric acid, and transferred to regeneration tubes. After subjecting the sample to 250°C for 30 seconds, the carbonyl vapors were collected in a gas-tight syringe and injected into a gas chromatograph-mass spectrophotometer unit. This work was qualitative in nature, using GLC, combined GLC-mass spectrophotometry, thin-layer chromatography (TLC), and visible spectral analysis to separate and identify components. The determination of volatile constituents by formation of 2,4-DNP-hydrazone derivatives has not previously been attempted on fruits, however.

V. Other methods

Nelson and Hoff (51) investigated the effect of variety, processing and storage time on tomato volatiles. The volatiles were recovered in pure, water-free form by partitioning the volatiles into oil, then stripping with inert gas and trapping at low temperature. The volatiles were transferred to a vapor tube, and vapor aliquots were taken for qualitative and quantitative GLC analysis. Volatiles were identified by subjecting vapor samples to functional

group classification reagents (33) and by comparison of mass spectral data with those of authentic compounds. The quantities recovered were determined from peak heights and/or areas obtained with GLC. An internal standard, isobutyl acetate, was added prior to extraction of each sample to compensate for minor variations in the operating conditions of the chromatograph.

Cobb (11) developed a technique for quantitation of flavor components which could be applied to virtually any food system. This technique dealt with the addition of radioactively labeled flavor compounds to a food system prior to reduced pressure distillation. To show its applicability, he added radioactively labeled benzaldehyde to peanut butter. In the distillate, the benzaldehyde was converted to its corresponding 2,4-DNP-hydrazone and subsequently separated by TLC. Recovered material was measured quantitatively using ultraviolet spectroscopy and isotope monitoring. The native (unlabeled) aldehyde was calculated via isotope dilution.

The real advantage of isotope dilution lies in the fact that once a known quantity of pure isotope is added to a system, native and labeled compound can be expected to behave similarly. The labeled compound should be very pure and must be completely separated from other materials. However, quantitative isolation of the labeled additive is not necessary and weighable quantities are not needed if an

indirect method of determining its mass, e.g., spectrophotometry, can be used.

B. Acetaldehyde Concentration in Fruit Tissues

Most of the purportedly quantitative determinations of acetaldehyde in fruits have employed steam distillation and bisulfite iodometry. A comparison of determinations made by investigators using different apple varieties under various storage conditions appears in Table 1. Although data have been published in various units, they have all been converted to microgram/gram fresh weight (ug/g FW) for comparison.

Miller (47) showed that the acetaldehyde concentration varied with the portion of the apple sampled. The peel, which is the most biologically active part of the apple, consistently contained the highest amount of acetaldehyde. Also, individual varieties differed considerably in their acetaldehyde concentration. As seen in Table 1, however, at harvest or shortly afterwards the concentration of acetaldehyde in the tissue has consistently been found to be relatively low. With ripening at both low and high temperatures the concentration increased markedly, regardless of variety. Fidler (18) showed that increasing time at low temperature greatly enhanced the rise in acetaldehyde after transfer to high temperature.

Pears are closely related to apples and are susceptible to similar postharvest disorders. Values for acetaldehyde

Table 1. Acetaldehyde content of apple tissue as determined by steam distillation and bisulfite iodometry.

Apple variety		Acetaldehyde concentration (ug/g FW)	Storage conditions	Reference
Newtown Wonder		3.0	0 days, -0.6°C	Thomas, 1925
		7.0	150 days, -0.6°C	
Newtown Wonder		5.0	0 days, 4°C	Fidler, 1933
		28.0	240 days, 4°C	
		18.0	0 days, 4°C 100 hours, 23°C	
		32.0	240 days, 4°C 100 hours, 23°C	
Bramley's Seedling		2.0	0 days, 4°C	
		26.0	120 days, 4°C	
		5.0	0 days, 4°C 100 hours, 23°C	
		47.0	120 days, 4°C 100 hours, 23°C	
Jonathan	Peel	10.0	6-10 days, 2°C 120 days, 0°C	Miller, 1936
	Pulp	0.0		
	Core	3.0		
Grimes Golden				
	Peel	62.0	6-10 days, 2°C 150 days, 0°C	
	Pulp	28.0		
	Core	0.0		

Continuation - Table 1.

Apple variety	Acetaldehyde concentration (ug/g FW)	Storage conditions	Reference
Golden Delicious	2.2	41 days, -0.6°C	Gerhardt, 1942
	2.9	41 days, -0.6°C 7 days, 18°C	
Winesap	1.9	38 days, -0.6°C	
McIntosh	2.1	45 days, -0.6°C	
Delicious	1.6	0 days, -0.6°C	
	5.8	180 days, -0.6°C	
	11.4	225 days, -0.6°C	
	23.7	180 days, 2°C	
	49.7	225 days, 2°C	

concentration in pear tissue have been assembled for comparison in Table 2, and it can be seen that these values are generally higher than those reported for apple tissue. It may be noted that pears are generally more perishable than apples. As seen with apples, acetaldehyde appears to have consistently increased sharply with time at both low and high temperatures.

For additional comparison, acetaldehyde concentration reported for other fruits are shown in Table 3. Assuming that comparison among different procedures is valid, oranges and tomatoes have a relatively low acetaldehyde content whereas pineapples contain exceptionally high concentrations of this substance. It is interesting to note that seeds may also contain significant amounts of acetaldehyde. Ralls (55) found that blanched, frozen peas contained 0.56-2.4 ug acetaldehyde/g fresh weight.

C. Association of Volatiles with Physiological Disorders

Brooks, et al. (4) in 1919 were among the first to suggest an association between volatiles produced by fruit and the occurrence of a physiological disorder within the fruit. They considered the accumulation of certain metabolites as a possible origin of apple scald. In their investigation they found evidence suggesting that the disorder was due to volatile or gaseous substances, other than CO_2, which were produced in the metabolism of the fruit and which could be carried away by air currents or

Table 2. Acetaldehyde content of pear tissue as determined by steam distillation and bisulfite iodometry.

Pear variety	Acetaldehyde concentration (ug/g FW)	Storage conditions	Reference
Bartlett	3.0	0 days, 15-18°C	Harley and Fisher, 1927
	18.0	7 days, 15-18°C	
Bartlett	2.7	5 days, 0°C	Gerhardt and Ezell, 1938
	5.9	58 days, 0°C	
	6.7	68 days, 0°C	
Bartlett	3.1	106 days, -0.6°C	Gerhardt, 1942
	57.6	106 days, -0.6°C 7 days, 18°C	
Bartlett	28.0	0 days, 14°C	Reyneke and Pearse, 1945
	240.0	7 days, 14°C	
Comice	10.0	40 days, 0°C	Gerhardt and Ezell, 1932
	110.0	220 days, 0°C	

Table 3. Acetaldehyde content of various plant tissue.

Fruit material	Methodology	Quantitation	Acetaldehyde concentration (ug/g FW)	Condition of product	Reference
Pineapple	Vacuum distillation, cold trapping, fractionation	Formation of 2,4-DNPH derivatives (by weight)	61.0 135.0	Winter fruit Summer fruit	Haagen-Smit, et. al., 1945
Orange, Washington Navel	Headspace of whole fruit, 1 hour accumulation	Headspace analysis (GLC)	0.008 0.026	1 day, 7°C 20 hours, 3°C	Norman and Craft, 1971
Peas, Perfection	Steam distillation	Formation of 2,4-DNPH derivatives, regeneration into GLC	0.56-2.4	Blanched, frozen, thawed	Ralls, 1960
Tomato H-1350	Partitioning into oil, gas stripping, cold trapping	Vapor analysis (GLC)	0.92	1 day after picking	Nelson and Hoff, 1969
Roma			0.21		
Rutgers			0.48		

taken up by various absorbents. They produced effects resembling scald by exposing the apples to the vapors of various esters (4) and demonstrated that the disorder could be controlled by the use of oil-impregnated paper wrappers capable of absorbing emanations from the fruit (5).

Overholser, et al. (53) found that internal browning of Yellow Newtown apples was greatly reduced by ventilating the fruit and, during the early part of the storage season, was likewise reduced by wrapping the apples in oiled paper. They concluded that this disorder was due to the accumulation of essential oils or similar deleterious substances which were produced by the apples during the storage.

Since then, a number of workers have found an almost ubiquitous association of acetaldehyde with the occurrence of a number of disorders of apples and pears. Data from a number of experiments have been summarized in Tables 4 and 5 for comparison.

However, there is disagreement on the role played by the accumulation of acetaldehyde. Thomas (66) studied the formation of ethyl alcohol and acetaldehyde in apples and found that acetaldehyde was produced in large quantities in certain mixtures of CO_2 and O_2. High concentrations of acetaldehyde were often accompanied by browning of the cells. The fact that acetaldehyde occurred normally and could occur in relatively large quantities in apple tissue (54,66) suggested that it might be intimately related to certain

Table 4. Association of acetaldehyde content and physiological disorders of apples.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	LOW TEMPERATURE BREAKDOWN		
Bramley's Seedling	Sound	5.0	Thomas, 1931
	Slight low-temperature breakdown	20.0	
	Severe low-temperature breakdown	80.0	
	Sound tissue of apples showing low-temperature breakdown	6.0	
	CORE BROWNING		
Cleopatra	Sound	10.0	Thomas, 1931
	Severe core browning of recent occurrence	40.0	
	Sound tissue of apples showing core browning	8.0	

Continuation - Table 4.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	DEEP SCALD		
Jonathan	Whole apples suffering from severe deep scald	60.0	Thomas, 1931
	Apparently sound tissue of apples suffering from severe deep scald	20.0	
Jonathan	Normal, Peel	20.0	Miller, 1936
	Outer pulp	3.0	
	Inner pulp	6.5	
	Core	4.5	
	Soft scald, Peel	60.0	Miller, 1936
	Outer pulp	17.0	
	Inner pulp	23.0	
	Core	4.5	

Continuation - Table 4.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	SOGGY BREAKDOWN		
Grimes Golden	Normal, Peel	62.0	Miller, 1936
	Pulp	28.0	
	Core	0.0	
	Soggy breakdown, Peel	91.0	
	Pulp	24.0	
	Core	19.0	
	FREEZING INJURY		
Grimes Golden	0 hours, -8°C	1.0	
	18 hours, -8°C; 4 hours, room temperature	1.0	
	0 hours, -8°C; 24 hours, room temperature	3.0	
	18 hours, -8°C; 24 hours, room temperature	11.0	

Continuation - Table 4.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	CO_2 INJURY		
Grimes Golden	Normal peel	6.0	Miller, 1936
	CO_2-treated peel	22.0	
Newtown Wonder	50% CO_2 and 50% O_2		Thomas, 1929
	3 days, 1°C	100.0	
	7 days, 1°C, visible browning in the interior	200.0	
	15 days, 1°C, visible browning incident in epidermis and progresses inwards	300.0	
	23 days, 1°C, injury incident in epidermis and progresses inwards; injured tissue browns on subsequent exposure to O_2	400.0	
	38 days, 1°C, apples completely killed	400.0	

Continuation - Table 4.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
Newtown Wonder	70% CO_2 and 30% O_2		Thomas, 1929
	3 days, 15°C	50.0	
	5 days, 15°C, visible browning incident in epidermis and progresses inwards	100.0	
	11 days, 15°C, visible browning incident in epidermis and progresses inwards	300.0	
	20 days, 15°C, apples completely killed	---	
	100% CO_2		
	5 days, 15°C	50.0	
	11 days, 15°C	60.0	
	20 days, 15°C, injury incident in epidermis and progresses inwards; injured tissue browns on subsequent exposure to O_2	60.0	

Table 5. Association of acetaldehyde content and physiological disorders of pears.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	SUPERFICIAL SCALD		
Bartlett	No scald	79.0	Reyneke and Pearse, 1945
	Moderate scald	202.0	
	Severe scald	288.0	
	27.6% scald	61.0	
	88.0% scald	398.0	
	91.0% scald	390.0	
	0 days, 15-18°C - no scald	30.0	
	4 days, 15-18°C - 1% surface scald	119.0	
	4 days, 15-18°C - 10% surface scald	174.0	
	7 days, 15-18°C - no scald	18.0	
	7 days, 15-18°C - 5% surface scald	105.0	
	7 days, 15-18°C - 30% surface scald	195.0	
	7 days, 15-18°C - 100% surface scald	337.0	
	Parings from normal pears	13.0	
	Parings from scalded pears	181.0	
	Sound shite tissue of scalded and broken-down pears taken 5 mm beneath the skin	154.0	

Continuation - Table 5.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	CORE BREAKDOWN		
Bartlett	Following 58 days at 0°C		Gerhardt and Ezell, 1938
	1 day, 18°C, some yellow color	10.4	
	3 days, 18°C, full yellow, some softening	12.3	
	5 days, 18°C, off-flavor	52.9	
	7 days, 18°C, some surface scald and core breakdown	80.4	
	10 days, 18°C, severe scald and core breakdown	111.0	
	14 days, 18°C, tissue broken-down and discolored	41.8	
	Sound hard pears from cold storage	3.0	
	Soft ripe pears free from scald or breakdown	31.0	
	Pears free from scald but showing core breakdown	238.0	
	3 days, 20-22°C, sound, soft, ripe	38.0	
	6 days, 20-22°C, no scald, slight breakdown	141.0	
	9 days, 20-22°C, no scald, badly broken-down	281.0	

Continuation - Table 5.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	CORE BREAKDOWN		
Bartlett	Sound pears from cold storage	3.0	Harley and Fisher, 1927
	5 days, 20-22°C	73.0	
	8 days, 20-22°C	133.0	
	10 days, 20-22°C	237.0	
	25 days, 20-22°C, all tissues broken-down; protoplasm completely disorganized	100.0	
Bartlett	August 9 harvest		Harley, 1929
	0 days, 22-24°C	0.0	
	2 days, 22-24°C	3.0	
	10 days, 22-24°C	12.4	
	20 days, 22-24°C	116.6	
	24 days, 22-24°C, slight browning about vascular and core tissues	142.8	
	30 days, 22-24°C, browning about core tissues and extending into cortex; fruit wilted	132.2	

Continuation - Table 5.

Variety	Treatment or condition	Acetaldehyde concentration (ug/g FW)	Reference
	CORE BREAKDOWN		
Bartlett	September 12 harvest		Harley, 1929
	0 days, 22-24°C	0.0	
	2 days, 22-24°C	6.6	
	7 days, 22-24°C, slight browning about vascular and core tissues	145.0	
	10 days, 22-24°C	148.0	
	15 days, 22-24°C, tissue badly broken-down; no wilting	154.0	
	17 days, 22-24°C, tissue badly broken-down	122.7	

nonparasitic disorders commonly found in stored fruits.

The occurrence of acetaldehyde and its relationship to scald and core breakdown of Bartlett pears was investigated by Harley and Fisher in 1927 (29). Acetaldehyde was always found in relatively large quantities in scalded and broken-down pears. No pears containing more than 14 mg of acetaldehyde in 100 g of fresh tissue were found which did not show scald and/or breakdown. Positive correlations existed between severity of both scald and breakdown and the concentration of acetaldehyde in the affected tissue. Furthermore, acetaldehyde was found in greater quantities in the scalded skin tissues than in the apparently normal white tissues beneath. The greatest quantity was found in the broken-down core areas. Harley and Fisher (29) interpreted these data to show a localized accumulation of acetaldehyde in two distinct areas, namely, the core and the outer surfaces. They suggested that browning of the tissue resulted from acetaldehyde concentrations reaching a point of toxicity in these areas. Their proposed sequence of scald formation was (a) acetaldehyde formation, (b) toxic action, (c) scald and core breakdown, and (d) water-logging of the tissue.

Gerhardt and Ezell (25) determined the volatile emanations from Bartlett pears during storage and ripening, and observed that acetaldehyde accumulation in the tissue roughly paralleled the emanation of total volatiles. An

increase in both acetaldehyde and total volatiles was associated with the appearance of scald or core breakdown. As the fruit ripened at 18°C, acetaldehyde accumulated rapidly and reached a maximum with the appearance of severe scald or core breakdown, after which it decreased rapidly with complete tissue disorganization. They interpreted the correlation between acetaldehyde accumulation and the development of scald and core breakdown as confirmatory evidence in support of the earlier conclusion of Harley and Fisher (29).

An alternative viewpoint was proposed by Trout (71) in 1930. From experiments on the storage of pears in artificial atmospheres, he concluded that scalding was not due simply to accumulation of acetaldehyde in the tissue. He suggested that the most likely chain of causation of scald was (a) breakdown, (b) water-logging of tissue, (c) anaerobic conditions with CO_2 accumulation, and (d) aldehyde formation.

This interpretation was supported by Thomas (68) following experiments with apples. He concluded that sizeable accumulation of ethyl alcohol and acetaldehyde did not precede breakdown, soft scald, or deep scald of apples. However, with the development of these disorders, these substances progressively accumulated in the tissues. He proposed that accumulation of alcohol and acetaldehyde was a secondary phenomenon accompanying cellular disruption in the development of these disorders, and showed that they rapidly ac-

cumulated after physiologically young, healthy apples suffered artificial injury by freezing or bruising.

Reyneke and Pearse (56) studied the relationship between respiration and physical condition of fruit as affected by oil treatments. They found that a high rate of respiration was associated with low acidity, high rate of aldehyde production, low juice content and a poor or rapidly deteriorating physical condition. Covering the fruit with an oil film was found to depress the respiration rate, reduce the rate of aldehyde production, and delay the occurrence of scald. Senescent scald appeared to be preceded by a certain amount of "breakdown", indicated by a low or reduced juice content. Their findings appeared to support the belief that this scald was preceded by senescent breakdown and that its appearance is accelerated by conditions which favor a relatively high rate of respiration and poor physical condition. Reyneke and Pearse (56) thus supported the theory of Trout (71) that the effect of acetaldehyde is probably of a secondary nature.

Internal breakdown is a disorder of apples that has been commonly considered to be a manifestation of advanced fruit senescence. However, internal breakdown also frequently follows the occurrence of watercore, another physiological disorder in apples (3,6,41,42). Watercore manifests itself as a watery, translucent area which begins development near the vascular bundles of the fruit and spreads outward

toward the skin as severity increases. These symptoms suggests movement of sap into the vascular area from adjacent areas causing localized flooding of tissues.

Smagula, et al. (61) studied the metabolic relationship between watercore and internal breakdown in Delicious apples. They found changes in the respiratory pattern of watercored tissue as well as accumulation of ethanol, acetaldehyde and ethyl acetate. This suggested that fermentation might be occurring within the watercored tissue. Severe watercore reduced O_2 uptake about 26% and resulted in a 13% rise in the respiratory quotient of this tissue. Acetaldehyde, ethanol and ethyl acetate were shown to be present in relatively high concentrations in extracts from watercored tissue. The concentrations of these volatiles were expressed and compared in terms of area under the recorded peaks. The peak areas for acetaldehyde, ethyl acetate, and ethanol recorded for severely watercored samples averaged 5, 10, and 12 times greater, respectively, than the peak areas recorded for normal tissue. However, since the volatiles were not completely removed from the tissue, the actual concentrations of these substances in the tissue were not determined.

In summary, it is evident that the literature contains many accounts of an association between acetaldehyde and various physiological disorders of apples and pears (Tables 4 and 5), yet it is not clear whether acetaldehyde is a causative agent or merely a consequence of the cellular disorganization

accompanying these disorders. Further study of this relationship will require an accurately quantitative analytical procedure to determine acetaldehyde levels in tissues undergoing changes. Accuracy of the steam distillation-bisulfite addition method of quantitatively determining acetaldehyde used extensively in earlier investigations is open to criticism, and existing alternative procedures also have inherent weaknesses. The occurrence of these fruit physiological disorders is of major economic importance, and further investigation into their cause is warranted. However, it appears that before more definitive experiments can be conducted, a more accurate and selective method of acetaldehyde analysis must be developed.

PROCEDURES AND RESULTS

A. Acetaldehyde Determination

I. Headspace analysis

To examine acetaldehyde determination by headspace analysis, 4 apples were placed in a sealed 9-liter desiccator and after 24 hours the accumulated volatile emanations were sampled. A 2 ml headspace sample was injected into an F & M Scientific Model 1609 Gas Chromatograph[1] equipped with a flame ionization detector. A 6ft x 1/8 in. copper column packed with 10% w/w Carbowax 20 M on Chromosorb W, 60-80 mesh, was used for this analysis. A representative chromatogram is depicted in Figure 1. Although a peak corresponding to that of authentic acetaldehyde was always present, varying the carrier gas flow rate and the column temperature could not bring about a consistently satisfactory separation of acetaldehyde from the other volatile substances.

Headspace of a juice sample was also analyzed according to the technique described by Davis and Chance (12). The pulp of one Delicious apple was macerated in 50 ml of distilled water. Ten ml of this macerate were transferred to a 50-ml Erlenmeyer flask, stoppered with a vaccine cap and placed in a 40°C water bath. After 30 minutes equilibration, a 2 ml headspace sample was taken and injected into

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[1] F & M Scientific Corporation, Avondale, Pennsylvania.

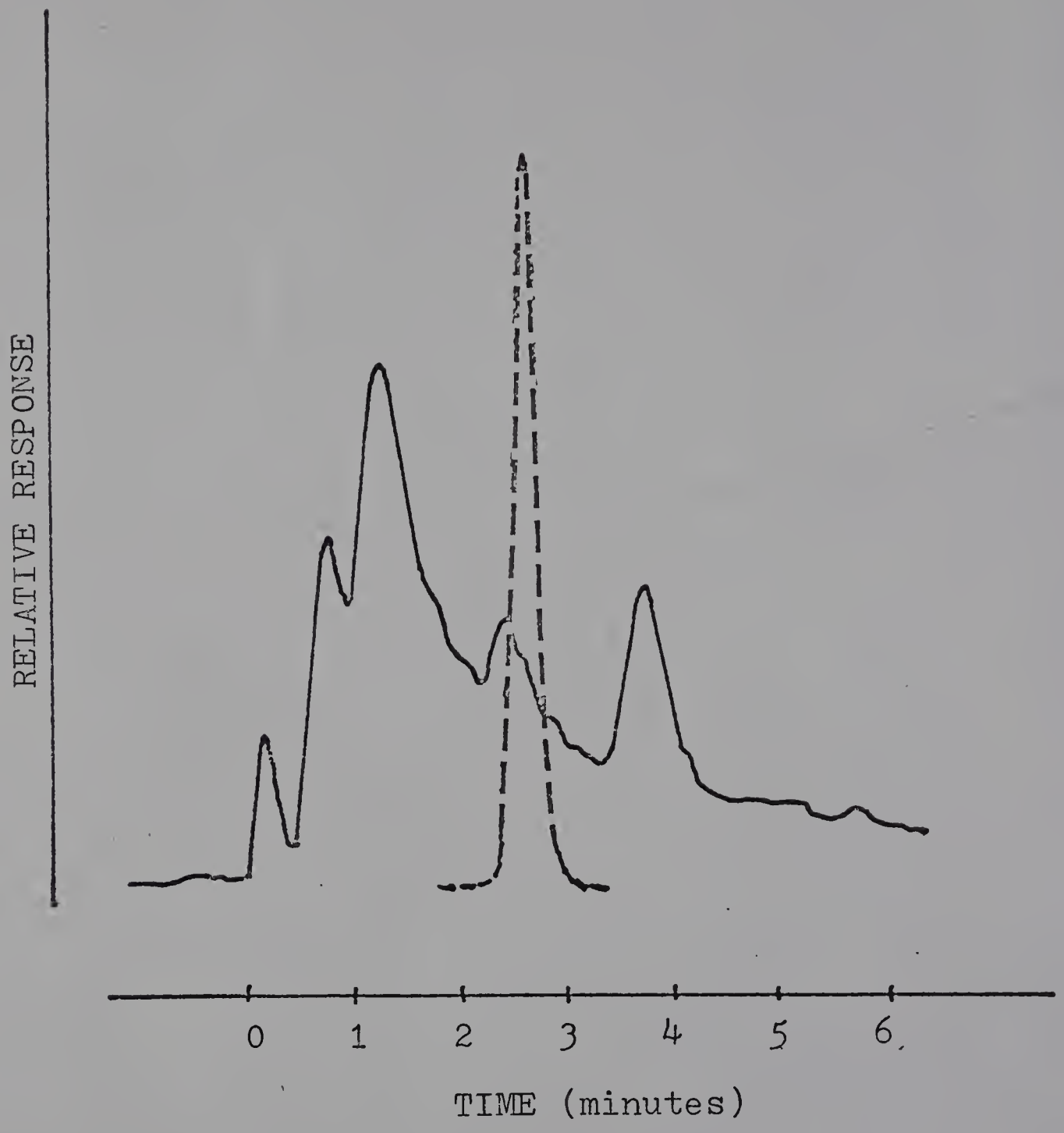

Figure 1. Typical chromatogram of a 2 ml headspace sample of Delicious apple volatiles accumulated in a sealed desiccator for 24 hours. The peak representing acetaldehyde is shown by dotted lines.

a Varian Aerograph Moduline Series 2700 Gas Chromatograph[2] equipped with a flame ionization detector. A 6ft x 1/8 in. Stainless steel column packed with 3% w/w Carbowax 20M on Chromosorb W (100-120 mesh) was employed. Satisfactory separation of acetaldehyde again was not achieved.

Davis and Chance (12) reported finding 3 major peaks by headspace analysis of orange juice. In our analysis of an apple macerate, we recorded 7 peaks (Figure 2). The presence of a number of different volatile substances makes the analysis of acetaldehyde in apple juice more difficult than in orange juice.

II. Vacuum distillation and cold trapping

Quantitative removal of the acetaldehyde from macerated tissue was attempted using vacuum distillation and the cold trapping apparatus described by Smagula, et al. (61). Tissue was frozen in liquid N_2, ground to a snowy consistency in a Waring blendor, and transferred to a 10% trichloroacetic acid solution (TCA). An Osterizer blendor was used for mixing the frozen slurry with TCA for protein precipitation. A vacuum was drawn for varying periods of time and the volatiles were trapped in 2 liquid N_2 traps.

To be suitable for the intended research, this system must completely remove acetaldehyde from the slurry. Further-

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[2] Varian Aerograph, Walnut Creek, California.

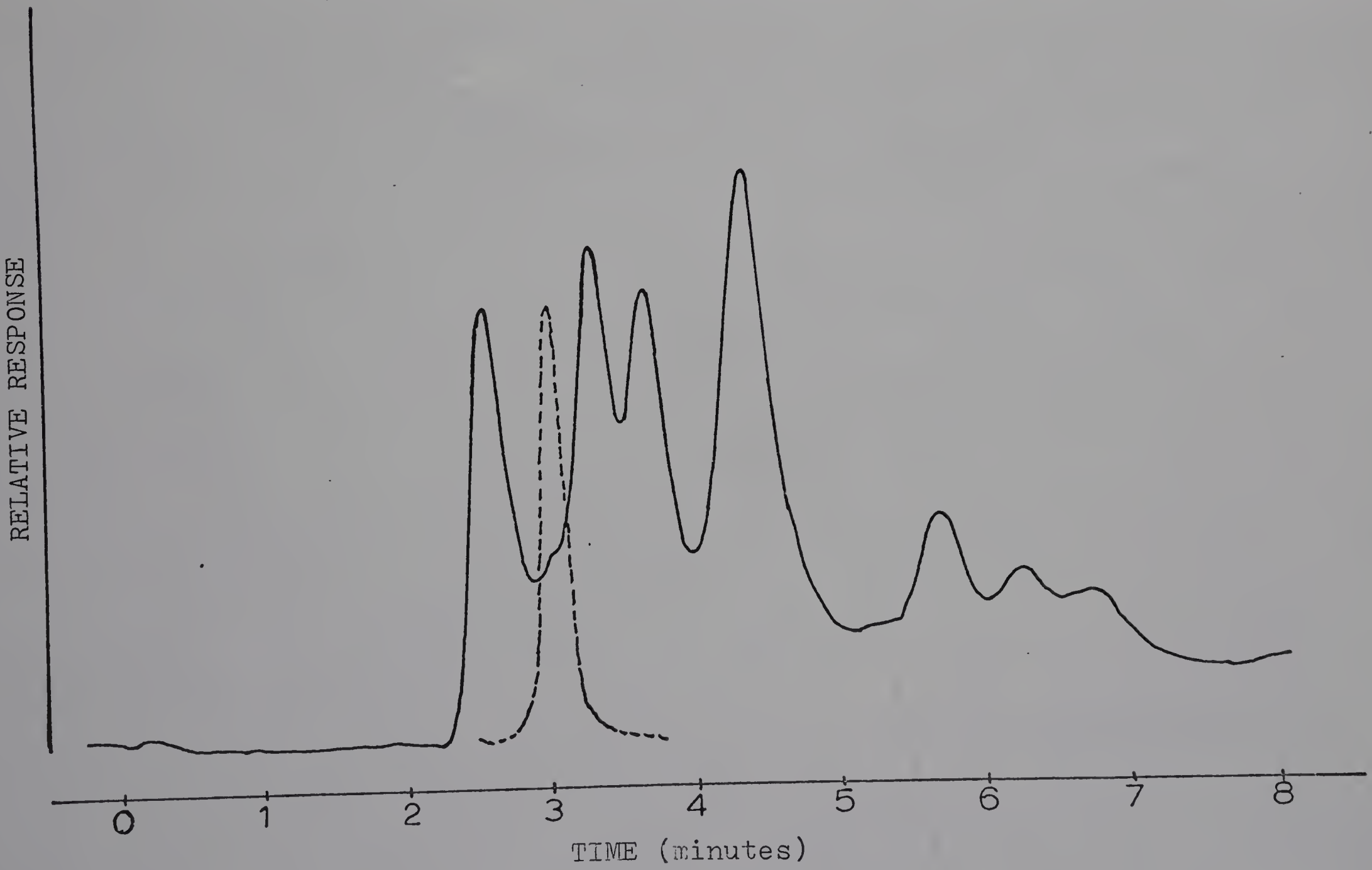

Figure 2. Typical chromatogram of a 2 ml sample of apple juice headspace. The peak representing acetaldehyde is shown by dotted lines.

more, the trapped substances must be quantitatively transferable from the system to the gas chromatograph used for measurement. To assure that comparable extraction and transfer has occurred among different runs, a suitable "internal standard" substance is also needed.

Attempts were made to completely remove the acetaldehyde from the apple slurry by extracting for various periods up to 5 hours. Complete removal was tested by GLC analysis of the air over the slurry. It was impractical to maintain the liquid N_2 traps for the period of time that might be necessary to obtain complete removal of acetaldehyde. In addition, during evaporation the outer portion of slurry froze, which impeded the removal of the acetaldehyde. This was overcome by immersing the flask containing the slurry in a 40°C water bath. When the traps were removed from the liquid N_2 and their contents thawed, a positive pressure built up inside the traps, which was released slowly using balloons. Following thawing, the contents of the 2 traps were combined and transferred to sample vials. However, this combination and transfer involved exposure to the atmosphere with the concomitant possibility of vapor loss.

An extensive search was made to find a suitable "internal standard". A suitable internal standard should be as volatile as acetaldehyde, not originally present in apple tissue, be separable by gas chromatography, and should not interfere with the separation of acetaldehyde. None of the many com-

pounds examined possessed these attributes. Those with boiling points near that of acetaldehyde either interfered with acetaldehyde separation by GLC, or appeared on the chromatogram at or near the point of a naturally occurring substance in the volatile mixture.

For the above reasons, it was concluded that this cryogenic trapping system could not be used for quantitatively measuring acetaldehyde present in apple tissue.

III. Reaction with 2,4-dinitrophenylhydrazine (2,4-DNPH)

A system that was developed which allowed maceration of frozen tissue in an enclosed system is shown in Figure 3. Any acetaldehyde released during the maceration would be withdrawn by continuous evacuation and collected in 2 traps containing 200 ml of 2,4-DNPH in 2N HCl. After maceration for 10 seconds, the system was opened to 100 ml of cold 3.8×10^{-3}M $HgCl_2$ or 0.15N H_3PO_4 solution which was quickly drawn into the macerate by the vacuum. Further blending resulted in gradual melting of the frozen macerate. A continuous flow of air or N_2 freed of carbonyls by a prewash in acidified DNPH was flushed through the system for varying periods of time and the carbonyl compounds were collected in the 2 hydrazine traps. The 2,4-DNP-hydrazones formed in the 2 traps during a 5-hour extraction period were combined and collected by vacuum filtration on glass fiber filter paper. The precipitate was washed first with 2N HCl and then with distilled water, and then was dried in a desiccator

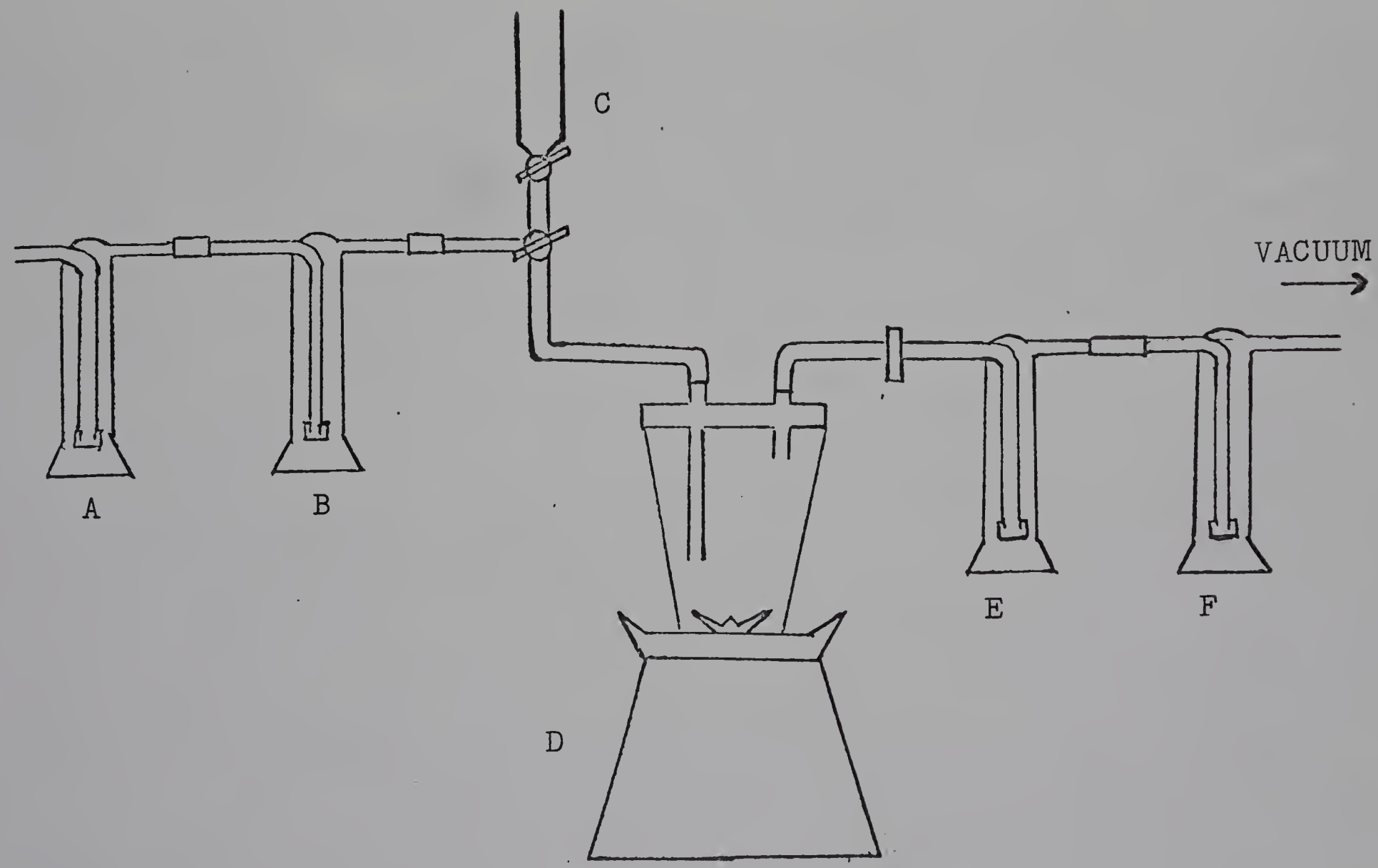

Figure 3. Illustration of extraction and trapping apparatus. (A) - 200 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2N H_2SO_4; (B) - Trap containing glass wool; (C) - Reservoir for enzyme-inactivation solution; (D) - Blendor with screw cap (fitted with inlet and outlet tubing); E and F - 200 ml each of 2,4-dinitrophenylhydrazine (2 g/liter) in 2N HCl.

over $CaCl_2$. The dried sample was dissolved in benzene, spotted on glass plates coated with aluminum oxide G (Type E), and developed for ½ hour with ethyl acetate/ Skelly B (15:85 v/v, saturated with distilled water) (36). A yellow spot corresponding to acetaldehyde was clearly visible.

To determine if acetaldehyde removal was complete, headspace samples were taken from the Waring blendor after 2, 4, 7, and 13 hours of extraction. Two ml of vapor were injected into the F & M Scientific gas chromatograph. Even after 13 hours of extraction, a peak corresponding to acetaldehyde appeared on the chromatogram. To confirm the continued presence of acetaldehyde in the slurry, 100 ml of acidified DNPH were added to 25 ml of the slurry after 5 hours of extraction. Precipitate was extracted and spotted as above and a clearly discernible spot corresponding to acetaldehyde was separated. It was apparent that excessively long periods of extraction would be necessary for exhaustive extraction of the macerate.

IV. *in situ* Formation of acetaldehyde derivative

Since a quantitative procedure was desired, a more direct method of trapping acetaldehyde as its 2,4-DNP-hydrazone was developed. Approximately 30 g of apple tissue were sliced into and frozen in liquid N_2. The frozen slices were transferred to a pint Mason jar containing 200 ml of 2,4-DNPH in 2N HCl, and blended for 5 minutes using an

Osterizer. After this time, the tissue was completely macerated and the solution had warmed to room temperature. The 2,4-DNPH-containing macerate remained at room temperature for at least 45 minutes. To determine if the hydrazone formation was complete by this time, comparable samples were extracted immediately after blending, and after intervals up to 12 hours. No increase in acetaldehyde-2,4-DNP-hydrazone recovery occurred with increased reaction time.

The carbonyl-free 2,4-DNP-hydrazones were removed from the macerate by extracting for 15 minutes with 50 ml of benzene in a slowly rotating (3 rpm) separatory funnel. The extraction was repeated with a second 50 ml portion of benzene. A third 50-ml wash with benzene was found to remove little of any additional acetaldehyde, and in fact, most was removed by the first 50-ml portion. The benzene extracts were combined and concentrated to approximately 20 ml using a rotary evaporator and quantitatively transferred to graduated test tubes. The extract was then concentrated to 10 ml using an Evapo Mix[3] apparatus.

When Delicious apple tissue was used, 300-800 λ of the concentrated extract were spotted as a band on a Silica Gel G - coated glass plate (0.25 mm thickness) and developed 3 times with cyclohexane/acetone (7:3 v/v) mixture.

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[3] Buchler Instruments, Fort Lee, New Jersey.

The separated band corresponding to acetaldehyde-2,4-DNP-hydrazone was scraped off into a test tube, eluted with 2 ml of chloroform, and read in a Beckman DU-2 Spectrophotometer[4] at 354 nm, the absorption maximum of 2,4-DNP-hydrazone (14, 31). The amount of acetaldehyde-2,4-DNP-hydrazone in chloroform was read directly from a standard curve prepared by plotting OD readings against concentrations of authentic acetaldehyde-2,4-DNP-hydrazone (Figure 4). This standard curve was linear between 0.5 and 10.0 µg/ml acetaldehyde-2,4-DNP-hydrazone.

When McIntosh apple tissue was used, a modification of the analytical method was necessary. Complete separation of the acetaldehyde derivative from other carbonyl derivatives was not achieved using Silica Gel G and cyclohexane/acetone (7:3 v/v). Other adsorbents were tried, and numerous other solvent systems were tested before a satisfactory separation could be obtained. The procedure ultimately found to be satisfactory required that the sample first be developed on Silica Gel G using benzene and Skelly B (3:1); this produced a clear band containing the acetaldehyde derivative, but also other interfering carbonyl derivatives (Figure 5a). This band was scraped from the plate, eluted with about 15 ml of chloroform and evaporated to dryness. The entire residue

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[4] Beckman Instruments, Incorporated, Scientific Instruments Division, Fullerton, California.

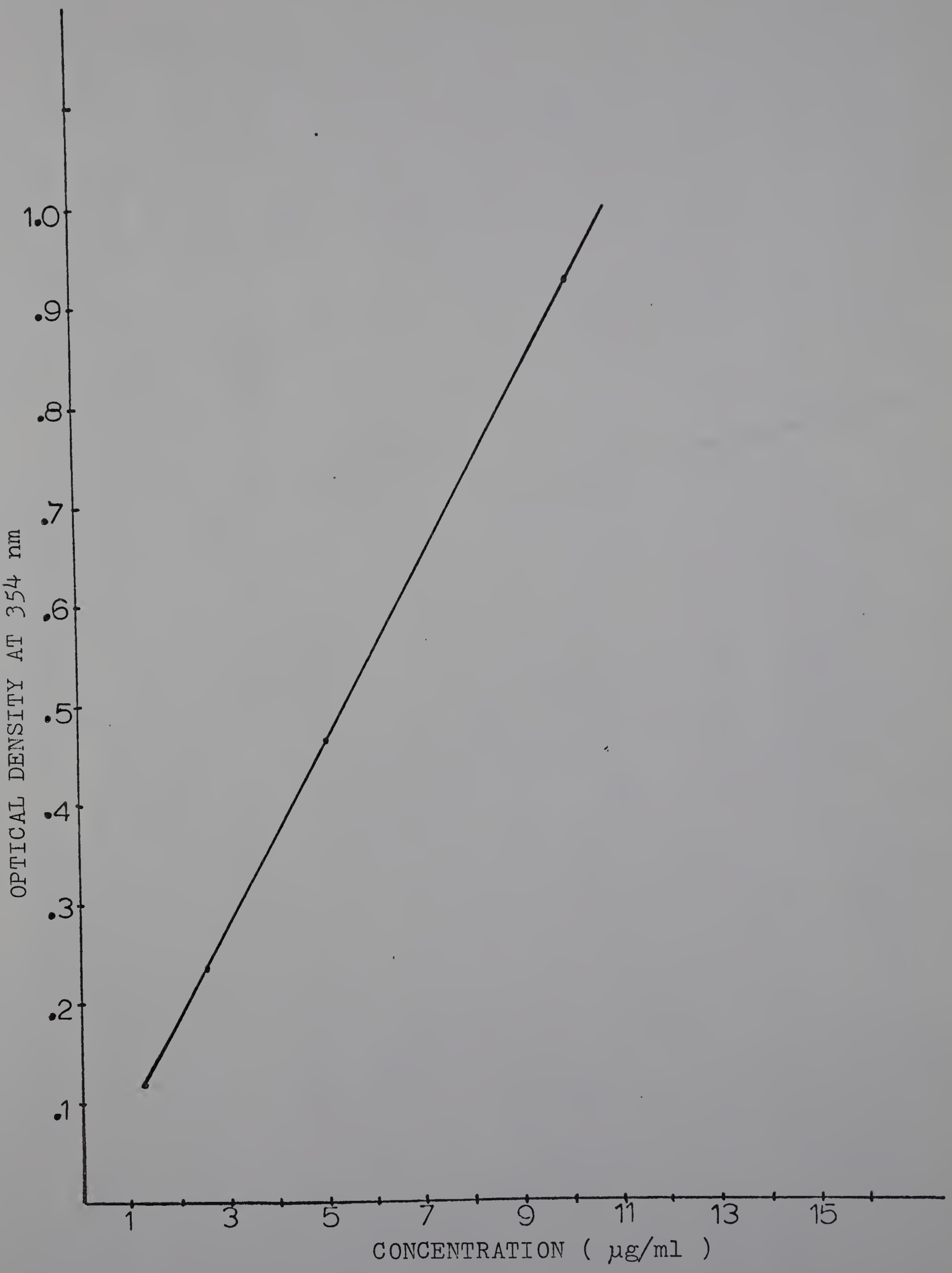

Figure 4. Standard curve plotting concentration of acetaldehyde-2,4-DNP-hydrazone against absorbance at 354 nm (OD).

Figure 5. TLC plates showing separation achieved by each step in purification procedure.

(a) - 800λ of 10 ml concentrated benzene extract of McIntosh apples on Silica Gel G and developed once with benzene and Skelly B (3:1, v/v). Acetaldehyde-2,4-DNP-hydrazone standard was spotted on both sides of the band.

(b) - Band corresponding to acetaldehyde eluted with chloroform and spotted on aluminum oxide G-coated glass plate. Developed twice using ethyl acetate/Skelly B (15:85 v/v, saturated with distilled water). Acetaldehyde-2,4-DNP-hydrazone standard was spotted on both sides of the band.

(a)

(b)

was taken up in benzene and spotted on an aluminum oxide G-coated glass plate, and this was developed with ethyl acetate/Skelly B (15:85 v/v, saturated with distilled water). Using this procedure, good separation was attained as illustrated in Figure 5b.

To determine if freezing in liquid N_2 was necessary in the sampling procedure, 2 portions of the same apple were sampled with and without freezing prior to maceration with the reagent. The results, shown in Table 6, indicated that considerably larger amounts of acetaldehyde-2,4-DNP-hydrazone were usually recovered when the tissue was sliced directly into the reagent. It was also observed that without freezing the solution became considerably warmer after blending. The effect of temperature on the results was investigated by allowing frozen apple tissue to remain submerged until the temperature of the reagent rose to 19°C. Blending for 3 minutes caused the temperature to rise to 39°C. When apple tissue was directly macerated without freezing, the temperature of the reagent rose from 26°C to 43°C during 3 minutes of blending. Samples of the same tissue extracted by both methods gave comparable results (Table 7). Because of these results McIntosh apple tissue was analyzed without the use of liquid N_2. The full procedure employed for both varieties is schematically illustrated in Figure 6. From sampling of the fruit through reading the concentration in the spectrophotometer, the procedure required approximately 4 hours for

Table 6. Comparison of sampling procedure: direct maceration vs. freezing in liquid N_2 prior to maceration.

Delicious apple[a]	Acetaldehyde concentration (ug/g FW)	
	Direct	Liquid N_2 freezing
Apple 1	17.7	10.0
Apple 2	22.6	14.7
Apple 3	35.1	34.7
Apple 4	27.0	13.5

[a] One-half of an apple was directly macerated and the other half was frozen in liquid N_2 prior to maceration.

Table 7. Comparison of sampling procedure: direct maceration vs. freezing in liquid N_2 followed by warming prior to maceration.

Delicious apple[b]	Acetaldehyde concentration (ug/g FW)	
	19°C	26°C
Apple 1	13.36	13.55
Apple 2	15.24	14.44

[b] One-half of an apple was frozen in liquid N_2 (-196°C) and warmed to 19°C; the other half was macerated at 26°C.

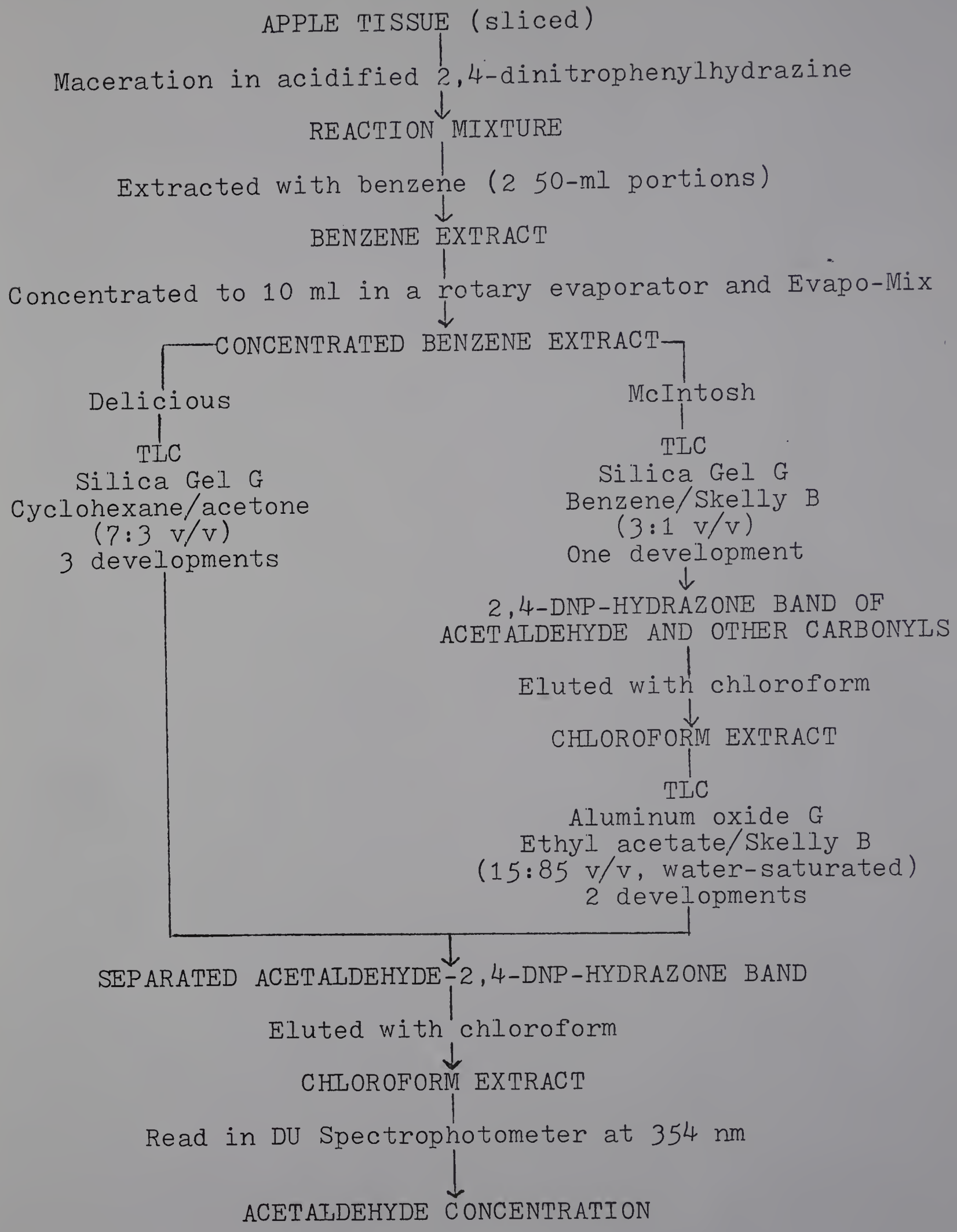

Figure 6. Scheme of procedure for in situ measurement of acetaldehyde content of Delicious and McIntosh apple tissue.

a single sample.

B. Identification of Acetaldehyde

To ascertain that acetaldehyde was being trapped, and that it was being separated as pure acetaldehyde-2,4-DNP-hydrazone, the flash-exchange regeneration procedure of Jones and Monroe (37) was attempted. Six mg of the derivative were mixed with 2 mg of Celite and then 15 mg of a regeneration agent (1:1 (w/w) p-dimethylaminobenzaldehyde and oxalic acid dihydrate). A capillary tube bent at a right angle was filled to a height of 1.5 cm with the mixture and the open end was fitted into a hypodermic needle through a silicone rubber septum. The needle was inserted into the injection port of the chromatograph and the reaction carried out by heating the contents of the capillary tube to 250°C for 10-15 seconds. Heating was carried out by immersion in a heated mercury bath or by wrapping a 90-volt nichrome wire around the capillary tube and applying 9.5 volts for 14 seconds.

This procedure was not successful. Erratic results were obtained using authentic compounds and an acetaldehyde peak was obtained when only the reaction mixture was heated. Although this method of regenerating carbonyl compounds from their 2,4-DNP-hydrazones reportedly has been used successfully (45,55), it has been necessary to employ exacting conditions, such as thorough mixing, use of pure reagents and uniform heating, to obtain reproducible results.

We abandoned this approach in favor of identification by mass spectrophotometry.

A sample was prepared for mass spectrophotometric analysis by eluting the band corresponding to acetaldehyde from 3 separate plates. A Hitachi-Perkin Elmer Model RMU-61[5], equipped with a direct probe, was used for the analysis. A graphic representation of the results appear in Figure 7, which showed definitively that the band being separated by TLC contained acetaldehyde-2,4-DNP-hydrazone.

Since a different procedure was involved when McIntosh apple tissue was sampled, it was essential to verify the identity of the band corresponding to authentic acetaldehyde-2,4-DNP-hydrazone. Identification was done by gas chromatography since the mass spectrophotometer previously employed was not available at this time. A Varian Aerograph Moduline Series 2700 equipped with a H^3 electron capture detector was used for the analysis. The presence of acetaldehyde was confirmed by retention times and peak characteristics (38), using two different columns (Figures 8 and 9). Columns used were a 6ft x $\frac{1}{4}$ in. OD, glass column packed with 2% SE 30 on 80/100 mesh Chromosorb W A/W DMCS, and a 5ft x 1/8 in. OD, stainless steel column packed with 1.5% OV 17 on 100-

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Hitachi Limited, Tokyo, Japan.

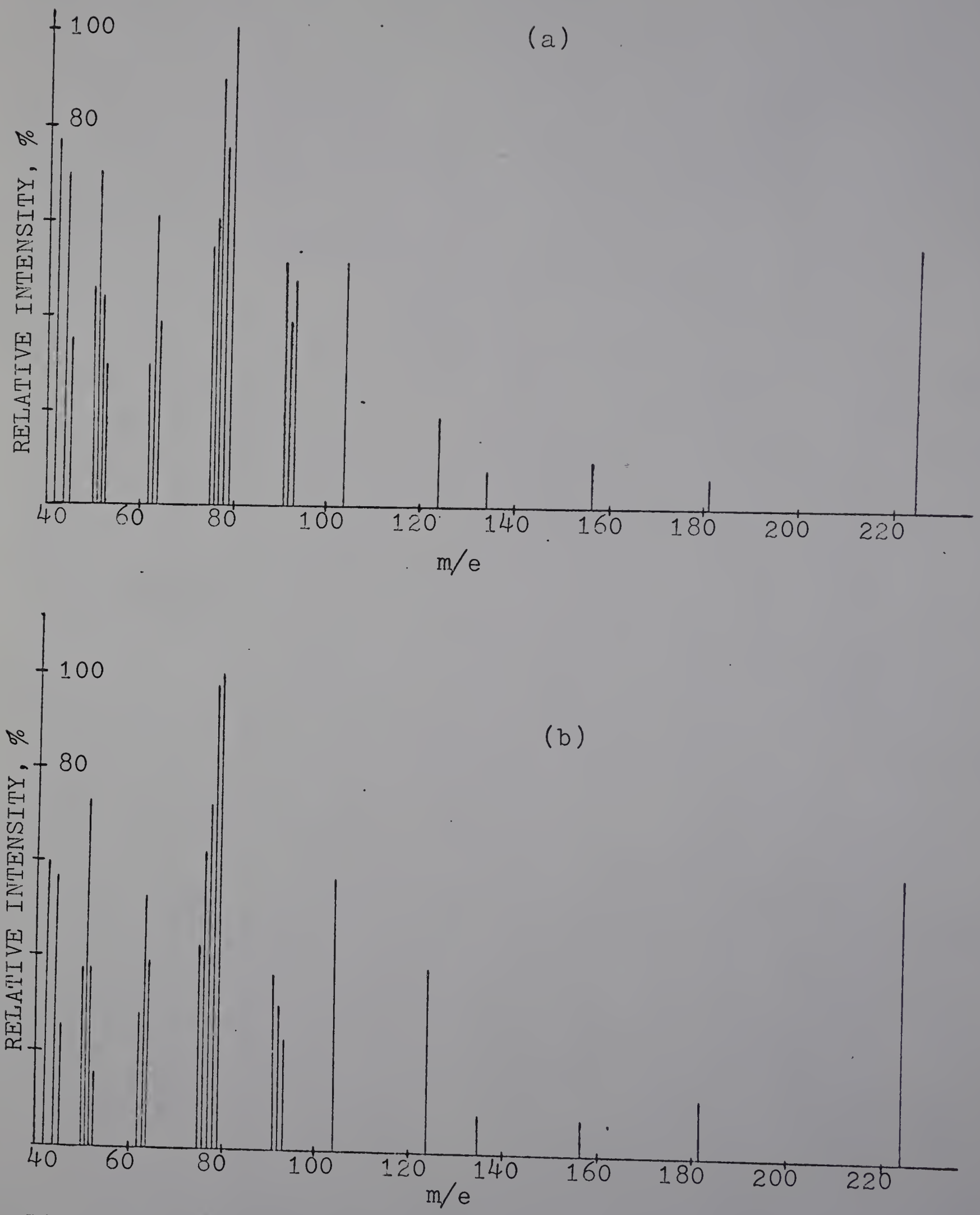
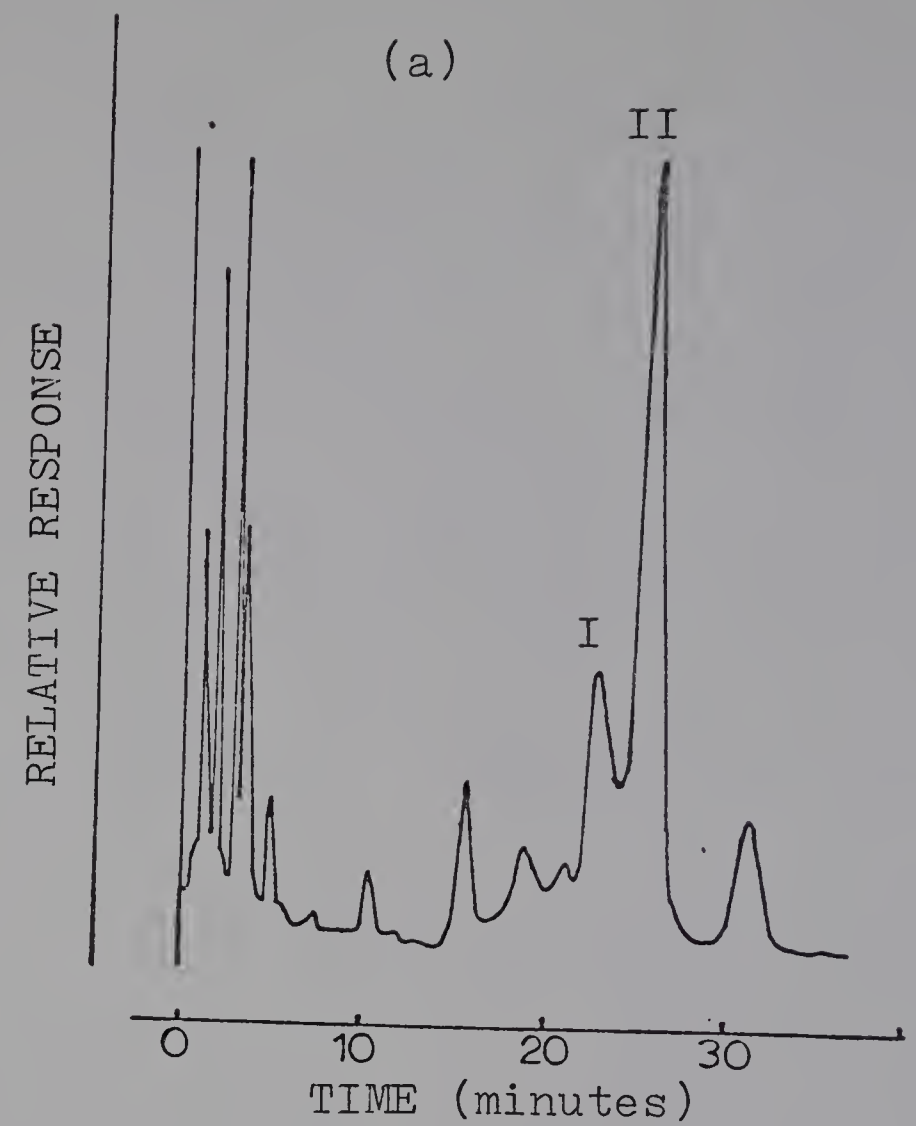


Figure 7. Mass spectra of (a) acetaldehyde-2,4-DNP-hydrazone from Delicious apple tissue and (b) authentic acetaldehyde-2,4-DNP-hydrazone.

Figure 8. Gas-liquid chromatograms of 2,4-DNP-hydrazones resolved on a 2% SE 30 column at 190°C using a H^3 electron capture detector. Peaks I and II represent acetaldehyde-2,4-DNP-hydrazone.

(a) - Analysis of 2.0 ul of TLC-purified acetaldehyde-2,4-DNP-hydrazone in benzene from McIntosh apple tissue. Attenuation: 16 x 10^{-10}A.

(b) - 1.0 ul (2.5 ug/ml) authentic acetaldehyde-2,4-DNP-hydrazone in benzene. Attenuation: 16 x 10^{-10}A.


(a)
II
I
RELATIVE RESPONSE
0
10
20
30
TIME (minutes)

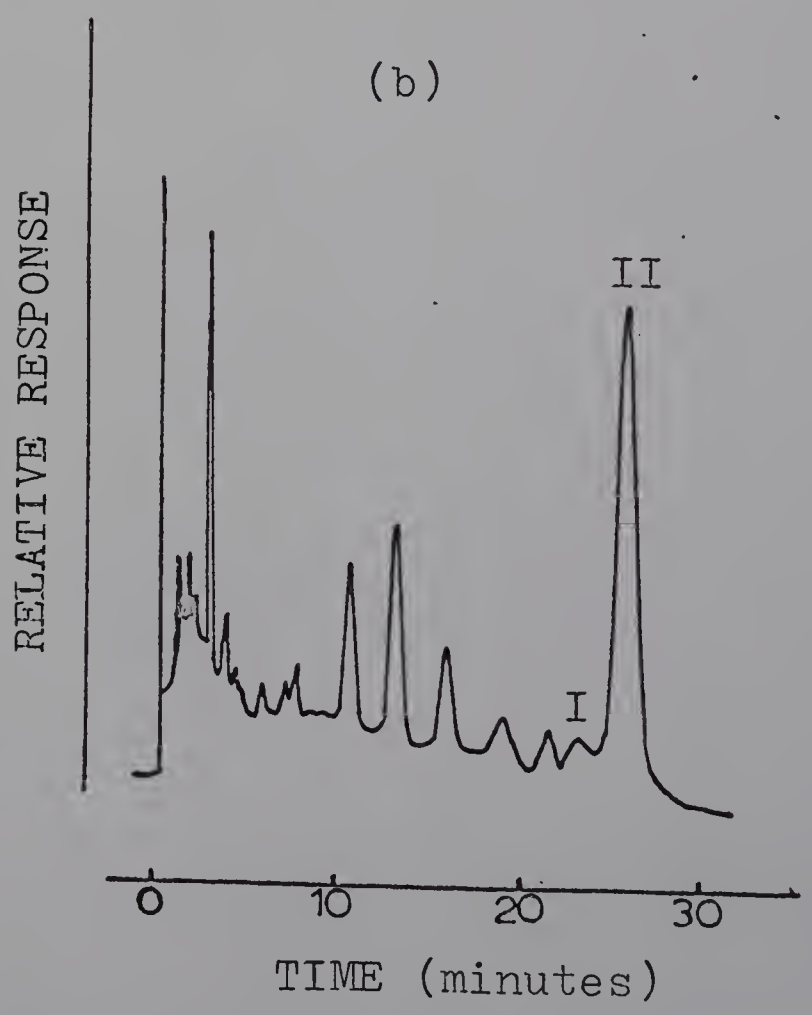
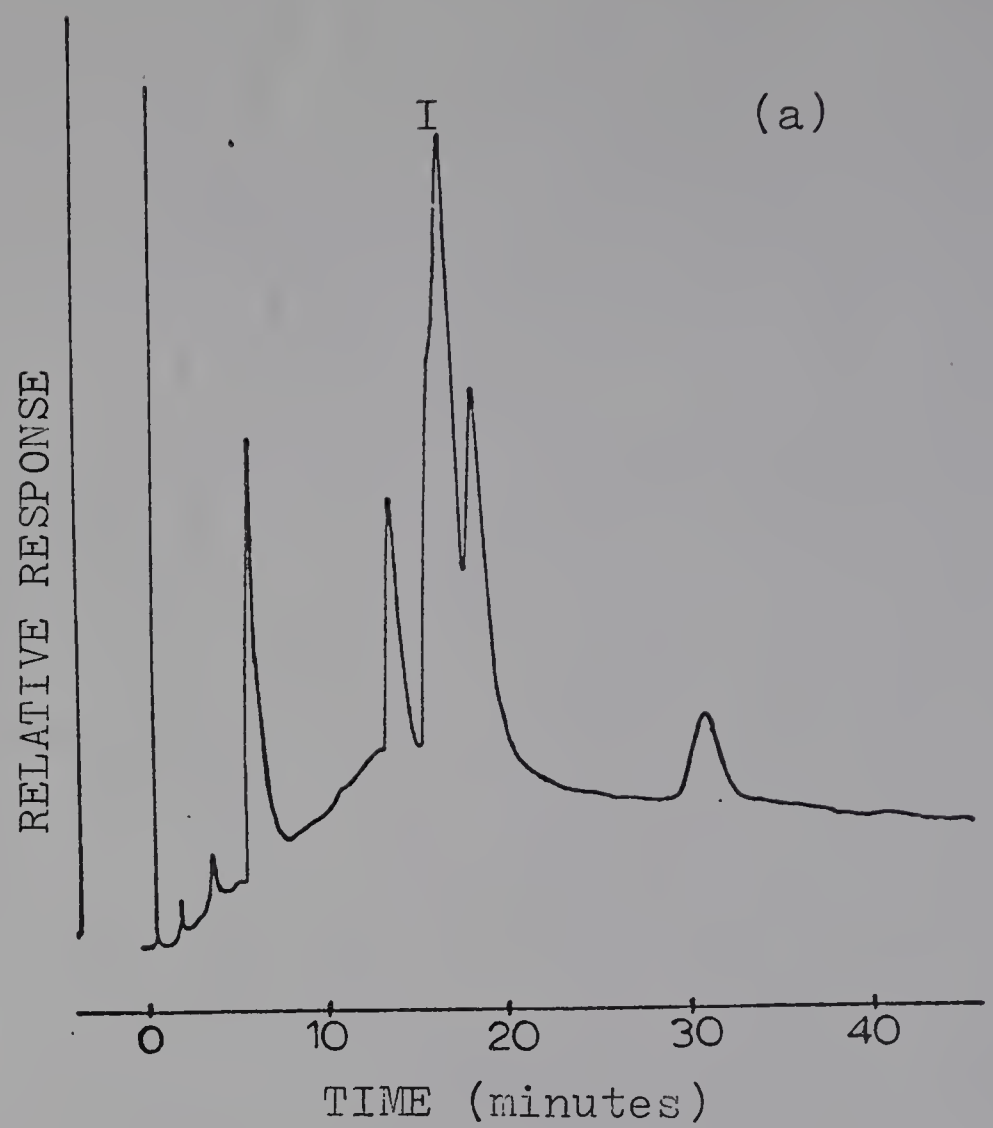
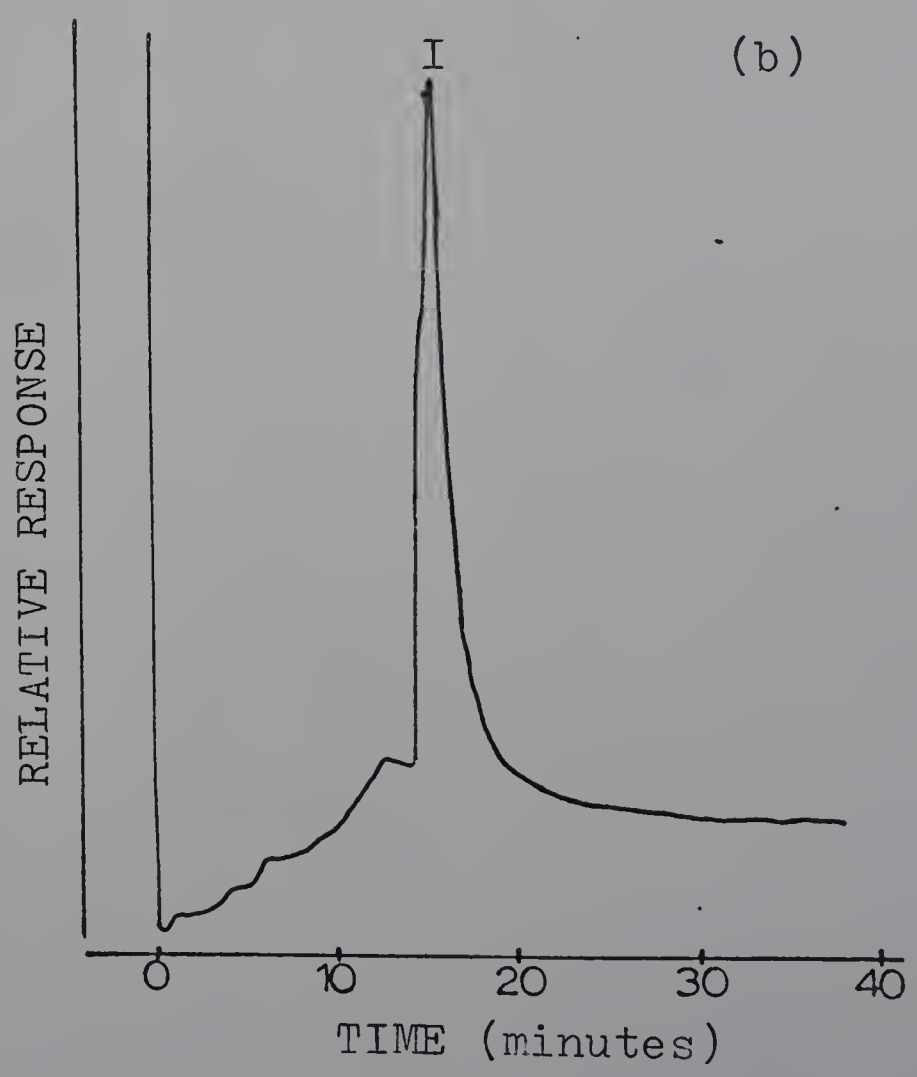

(b)
II
I
RELATIVE RESPONSE
0
10
20
30
TIME (minutes)

Figure 9. Gas-liquid chromatograms of 2,4-DNP-hydrazones resolved on a 1.5% OV 17 column using a H^3 electron capture detector. Temperature programmed 150-200°C at 4°C/minute. Peak I represents acetaldehyde-2,4-DNP-hydrazone.

(a) - Analysis of 1.0 ul of TLC-purified acetaldehyde-2,4-DNP-hydrazone in benzene from McIntsoh apple tissue. Attenuation: 128×10^{-10}A.

(b) - 1.0 ul (5.0 ug/ml) of authentic acetaldehyde-2,4-DNP-hydrazone in benzene. Attenuation: 128×10^{-10}A.


(a)
I
RELATIVE RESPONSE
0
10
20
30
40
TIME (minutes)


(b)
I
RELATIVE RESPONSE
0
10
20
30
40
TIME (minutes)

120 mesh Chromosorb G, H/P. Authentic acetaldehyde-2,4-DNP-hydrazone and samples were dissolved in benzene and 1-2 ul samples were analyzed. To increase the ability of the columns to resolve the DNP-hydrazones, it proved advantageous to silylate the columns with 10-15 ul of Silyl 8[6]. The temperature of the 2% SE 30 column was held at 190°C. When the 1.5% OV 17 column was used, it was necessary to increase the temperature from 150° to 200°C at a rate of 4°/minute to achieve good separation. Acetaldehyde-2,4-DNP-hydrazone consistently exhibited a double peak (Figure 8) suggesting the presence of two isomeric forms (38).

The chromatograms showed that in addition to acetaldehyde, other substances were present. To determine the extent to which these other substances interfered in the quantitation by absorbance at 354 nm, the acetaldehyde concentration in two samples was determined by both UV absorbance and by quantitative GLC. A standard curve was constructed plotting concentration of authentic acetaldehyde-2,4-DNP-hydrazone against peak areas as measured by a disc integrator (Figure 10). Quantitation by GLC produced slightly lower values than quantitation by UV absorption indicating that there was some

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6 Pierce Chemical Company, Rockford, Illinois.

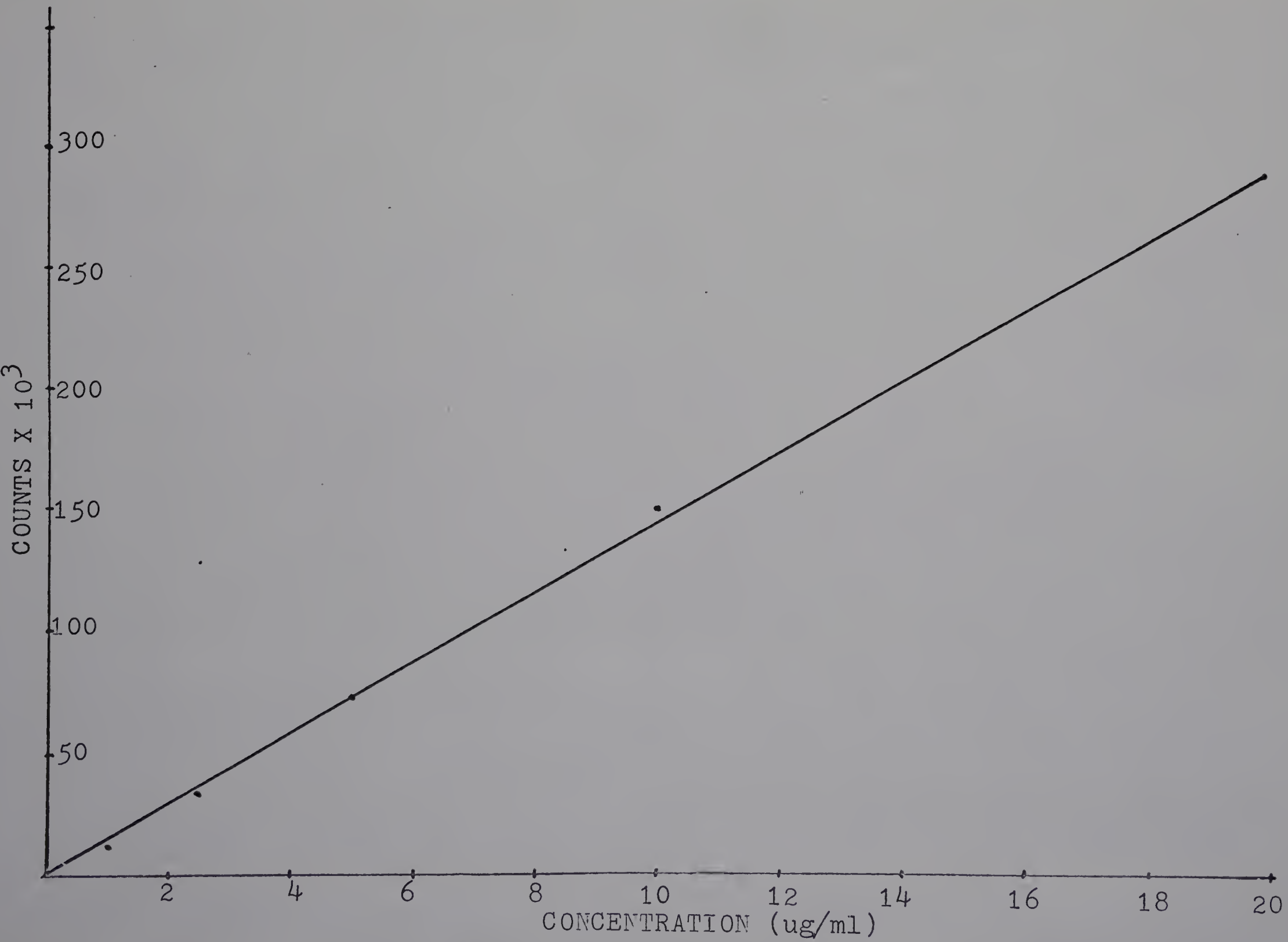

Figure 10. Standard curve plotting concentration against peak area measured by a disc integrator.

absorbance at 354 nm by contaminating substances. However, differences were small (1.98 vs. 1.75 ug/g FW) and did not warrant GLC analysis as the standard procedure.

C. Recovery Studies

To determine if acetaldehyde was being quantitatively recovered from the apple tissue, recovery stuides were performed in two ways. A known weighed amount of acetaldehyde in a sealed capillary tube was added to a 200 ml Mason jar containing tissue slices and reagent. The contents were macerated and the normal extraction procedure was followed. A portion of the same sample to which acetaldehyde had not been added was also analyzed. A second approach involved adding increments of an aqueous solution of a known amount of acetaldehyde to the apple slices and reagent and carrying it through the extraction procedure.

When 4.4 and 8.0 mg of acetaldehyde in sealed capillary tubes were added to apple tissue and the normal extraction procedure followed, recovery was 10.0% and 9.1%, respectively.

Addition of smaller amounts of acetaldehyde in aqueous solution to apple tissue in the reagent resulted in higher but inconsistent recoveries. When 10 ml aliquots containing 77 ug and 56 ug of acetaldehyde were added, recoveries of 90% and 48%, respectively, were obtained. Using 20 ml aliquots, addition of 154 ug and 112 ug of acetaldehyde resulted in a 50% and 42% recoveries, respectively. Higher recoveries were obtained when the amount of acetaldehyde

added was closer to the amount of acetaldehyde found in the apple but the values were inconsistent (42-90%). The manner in which the acetaldehyde was introduced, by pipetting aliquots of an aqueous solution of acetaldehyde, may have been inefficient and involved losses. Also, the acetaldehyde in the tissue is expected to be rather uniformly distributed and the introduction of a more concentrated aliquot of acetaldehyde may have resulted in larger losses.

D. Accumulation of Acetaldehyde in Apples

I. Delicious apples

An experiment was conducted to determine the accumulation of acetaldehyde in apples affected by watercore, which has been suggested to predispose the fruit to internal breakdown (3,6,41,42). Delicious apples grown at the Horticulture Research Center in Belchertown, Massachusetts were harvested on October 3, 1972, at which time they were free from watercore. On October 30, a second harvest was made from these same trees; at this time watercore was abundant in the fruit. Following harvest, all fruits were stored in air at 0°C. A Biospect Spectrophotometer[7] was used to detect watercore and internal breakdown within the intact fruit. Light transmittance was measured at 740 nm, 805 nm, and

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7 Agricultural Specialties Company, 11313 Frederick Avenue, Beltsville, Maryland.

840 nm. The optical density difference $\Delta OD_{740-805}$ was used as an index of watercore intensity in the fruit, and $\Delta OD_{840-740}$ was used as an index of internal breakdown (3,22). All apples were held at room temperature before and during determination of internal characteristics and measured while in vertical position with the light beam passing parallel to the core.

About 300 fruits harvested on October 30 were analyzed for watercore and internal breakdown at harvest time and at two-week intervals until March 6. In this manner, a record of the watercore history was compiled for each fruit noting the disappearance of watercore as well as the occurrence of incipient internal breakdown.

To determine the concentration of acetaldehyde in watercored fruits during storage, fruits were sampled at biweekly intervals during November and thereafter to January 16, by which time no more severe watercore was detected. Fruits previously having severe watercore but which had lost it were sampled on February 8. At each sampling date 4 severely watercored apples and 4 apples that never possessed the disorder were individually analyzed. Each apple constituted a replication of the condition and time. For sampling, 20 to 40 g of a single fruit were sliced and extracted as shown in Figure 6. Peel and core were avoided in sampling, so that only pulp tissue was extracted. A representative chromatogram is shown in Figure 11.

Figure 11. A representative TLC plate after 800 λ of concentrated benzene extract of Delicious apple tissue was spotted as a band on a Silica Gel G plate and developed 3 times with cyclohexane/acetone (7:3, v/v).

Changes in acetaldehyde content during storage of watercored and nonwatercored fruits are represented in Figure 12. After three months of storage in air at 0°C, larger amounts of acetaldehyde accumulated in watercored apples than in controls. Both groups contained a low concentration of acetaldehyde at the beginning of the experiment and this subsequently increased during ripening. The longer the tissue remained watersoaked, the more acetaldehyde accumulated. After an initial increase in acetaldehyde content during the first month of storage, control fruits maintained a relatively constant level of acetaldehyde, about 3.5 ug/g FW, which was significantly lower than the acetaldehyde content of watercored fruit after mid-December. Fruits which had contained severe watercore but which had subsequently lost their watersoaked condition also contained higher levels of acetaldehyde than controls. There was no significant change in acetaldehyde level with loss of watercore.

Although a distinct relationship between watercore and internal breakdown of Delicious apples has been previously described (3,6,41,42), during the 1972-73 storage season watercored apples developed virtually no internal breakdown. (This was also observed throughout New England for commercially stored Delicious apples.) Thus, the acetaldehyde levels measured (Figure 12) were apparently below a threshold level for inducing internal breakdown in these Delicious apples if

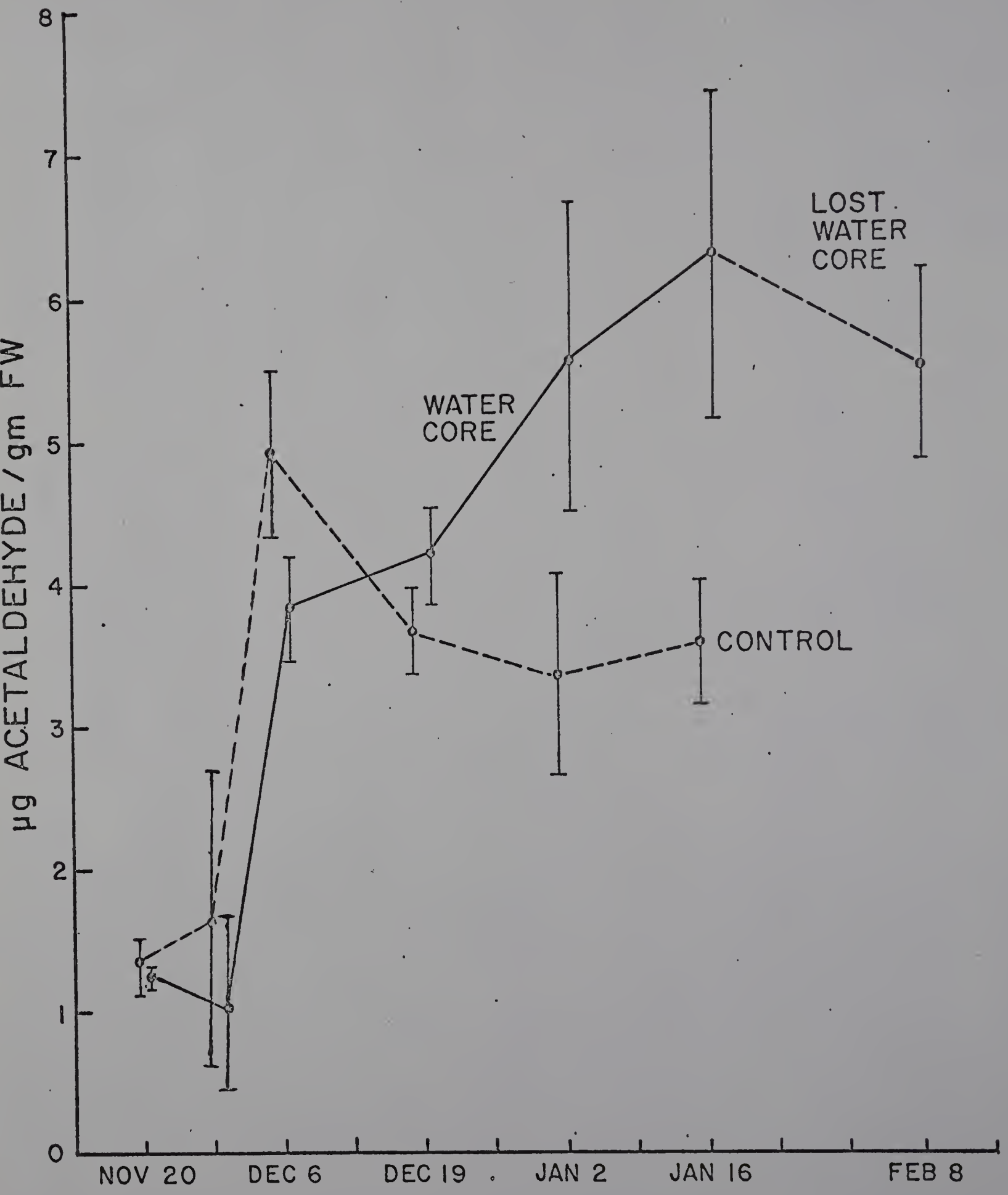

Figure 12. Acetaldehyde content (µg/g FW) of the pulp of severely watercored Delicious apples and controls stored in air at 0°C. Each point represents an average of 4 replications.

acetaldehyde is the causal agent of internal breakdown.

It was of interest to measure acetaldehyde accumulation during fruit ripening. For this, 28 Delicious apples harvested on October 3 were removed from 0°C cold storage on November 28 and kept at room temperature (approximately 21°C). Four apples were analyzed 24 hours after removal from cold storage and subsequently after 1, 2, 3, 4, 12, and 14 weeks at room temperature. Analytical procedures were as in Figure 6.

The acetaldehyde content of Delicious apples kept at room temperature changed little during the first four weeks but by the 12th and 14th week they contained greatly increased amounts of acetaldehyde, about 2-3 times as much during the first four weeks (Figure 13). By the end of the experiment the apple tissue was mealy but no internal breakdown was observed. Therefore, it again appeared that insufficient concentrations of acetaldehyde accumulated in these fruits to induce internal breakdown.

II. McIntosh apples

McIntosh apples grown at the Horticulture Research Center in Belchertown, Massachusetts were also used to study changes in acetaldehyde concentration during ripening. Fruit from an experiment in which ethephon ((2-chloroethyl)phosphonic acid) had been applied to trees to accelerate ripening (15) were employed. Ethephon is a potent ripening agent on apples, and it was observed in Maine in 1972-73 (W. G. Stiles, personal communication) that McIntosh treated with ethephon had greatly

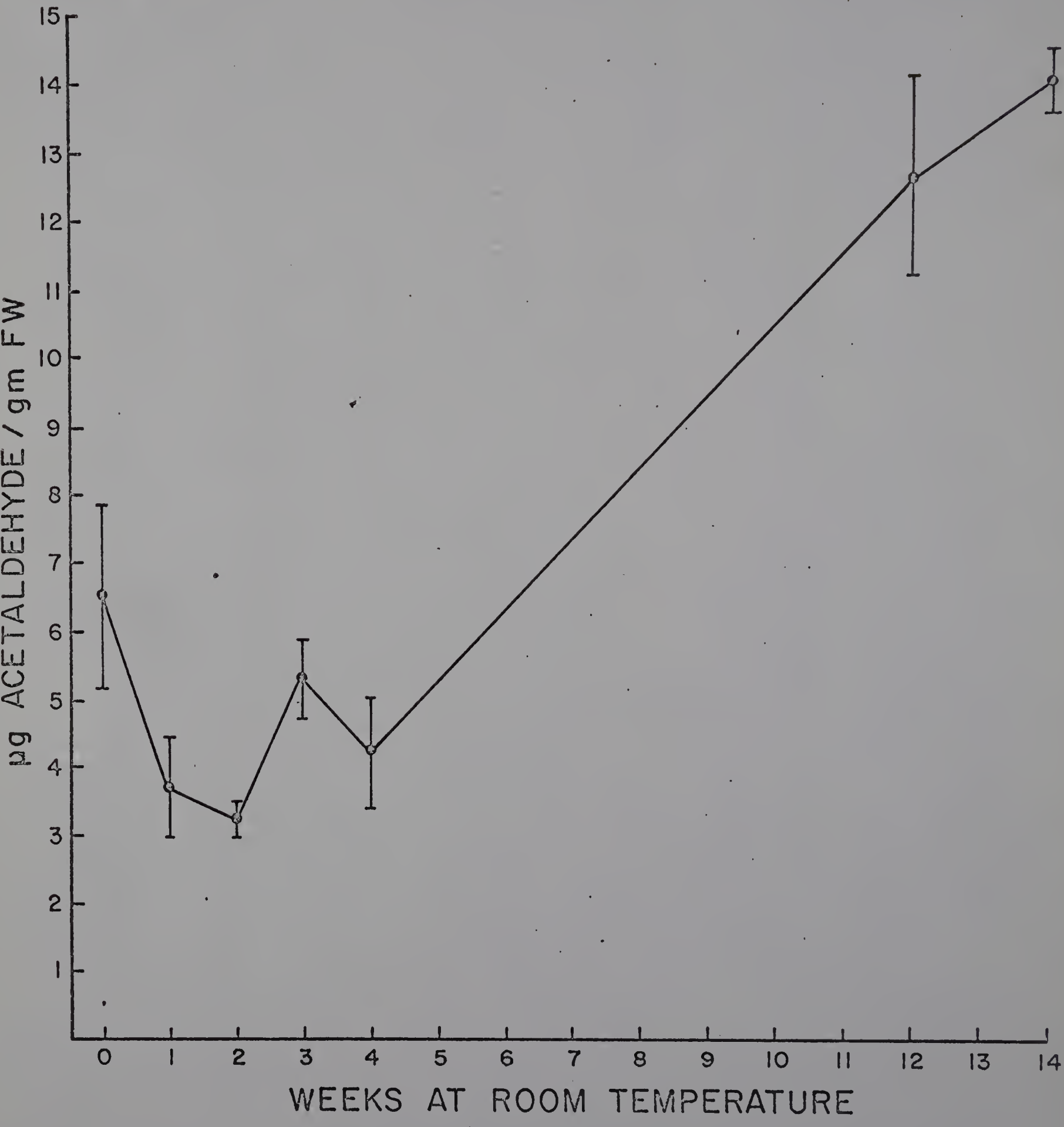

Figure 13. Acetaldehyde content (ug/g FW) of pulp from Delicious apples kept at room temperature (21°C) for 14 weeks. Each point represents an average of 4 replications.

increased susceptibility to internal breakdown.

Trees sprayed with 150 ppm ethephon on September 6 were harvested two weeks later on September 20 and the fruits were kept at 3°C in controlled atmosphere storage (3% O_2 + 5% CO_2) until March. On March 20 and April 11, 20 fruits of each group, ethephon-treated and control, were removed from storage and placed at room temperature. Fruits were sampled after 1, 3, 6, and 15 days at room temperature. Fruits were selected at random on March 20, but on April 11 they were carefully chosen for uniform red color in the case of ethephon-treated apples, and green color in the case of the controls. Individual fruits were sampled using 20-40 g of pulp tissue from each as shown in Figure 6, and each condition was replicated four times.

The McIntosh apples removed from storage on March 19 had been selected at random from bulk lots. The analytical results obtained for these fruits were very erratic and are not presented. This was attributed to lack of uniformity among the fruit, and greater selectivity was considered necessary.

The apples removed from storage on April 20 were carefully selected with two things in mind, firstly, to obtain uniform samples and secondly, to compare groups of apples at two distinctly different stages of ripeness. The results obtained are shown in Figure 14. After an initially low level of acetaldehyde, slightly less than 1 ug/g FW, the acetaldehyde

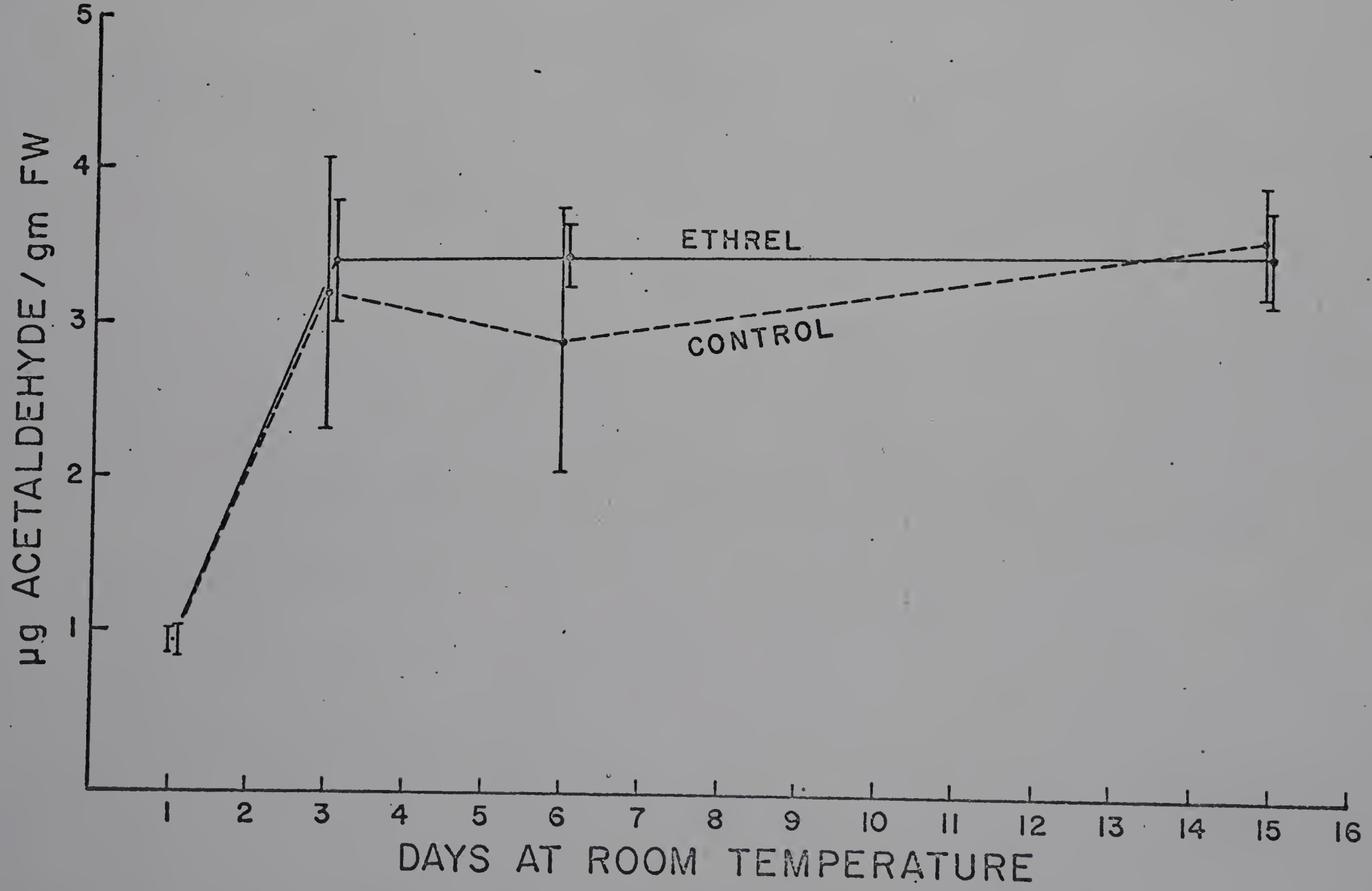

Figure 14. Acetaldehyde content of McIntosh apple pulp during ripening at 21°C.

content increased to about 3.4 ug/g FW where it remained for the length of the experiment. There was no significant difference between acetaldehyde levels of the two lots of fruit.

Some internal breakdown occurred among the fruit in these April 20 samples. Of these fruit that had been treated with ethephon and were bright red, 20% developed internal breakdown, whereas only 5% of the green, control fruit developed internal breakdown. It is clear in Figure 14 that the average ethephon-treated apple contained no more acetaldehyde, despite its higher susceptibility to internal breakdown, than the average control apple.

The acetaldehyde level in fruits already possessing internal breakdown was determined, and was found to be 4.08 ± 0.19 ug/g FW. Thus, these fruit contained about 20% more acetaldehyde than the corresponding sound fruit.

DISCUSSION

Acetaldehyde is a convenient substance for inclusion in proposed schemes for physiological deterioration of fruit tissue. It is endogenous, its toxicity is easily demonstrated, and there is ample data relating its accumulation to fruit deterioration. However, threshold levels for toxicity have not been determined and no mode of action has been proposed.

Despite its hypothetical convenience, acetaldehyde is a very difficult substance to carefully measure. Some of the difficulties involved are the following.

1. Acetaldehyde is a highly volatile substance, but in an intact apple the skin apparently serves as a barrier to its movement (19). Once this barrier is removed (e.g., in slicing), rapid loss from the tissue can be expected to occur. The longer cut tissue is exposed to the atmosphere, the greater will be the expected loss of acetaldehyde.

2. Despite its volatility, our experience has been that it is quite difficult to exhaustively remove acetaldehyde by evacuation. This may be due to its presence in combined forms within cell solutions and/or to continued formation.

3. Acetaldehyde is probably metabolized at very low rates within intact fruits under normal conditions, but during sampling (slicing) enzymatic changes may be greatly stimulated through disruption of cellular stucture (47).

4. Although acetaldehyde is a highly reactive carbonyl

compound, its measurement is complicated because its general reactions are shared by many other carbonyl compounds produced by fruit. In addition, its highly reactive carbonyl group and its small molecular size may make it subject to non-enzymatic changes at high temperature.

Past attempts to quantitatively extract acetaldehyde from fruit tissues have usually employed steam distillation. The high temepratures used have been presumed to inactivate enzymes and thus avoid artifact production after cell disruption. However, Wager (72) found that repeated distillations of the same sample of peas in water gave a slow but continuous recovery of volatile substances. Isherwood and Niavis (35) estimated α-keto acids in plant tissue through 2,4-DNP-hydrazone formation and compared methods of enzyme inactivation and α-keto acid extraction. They found that use of hot acid, strong alkali, or boiling methanol all led to both formation and destruction of individual α-keto acids by chemical reactions occurring during tissue disruption.

It would be desirable to avoid heating during extraction. Even if enzymes are heat inactivated and no nonenzymatic artifact-producing reactions occur, during the initial warming enzymes undergo a period of high activity prior to inactivation. An alternative approach developed by Isherwood and Niavis (35) was to freeze-inactivate plant tissue in a dry ice-methanol mixture (-70°C) and then dis-

integrate the frozen tissue in cold acid solution. Cooling is not only much more rapid than any feasible heating process, but also enzyme activity is progressively arrested during the cooling. We employed liquid N_2 freezing followed by acid inactivation, but found (Table 6) that acetaldehyde recovery was substantially reduced by the freezing. This was perhaps due to the volatility of acetaldehyde. Freezing may have disrupted cellular structure and released entrapped acetaldehyde, some of which escaped during transfer despite the low temperature. Since liquid N_2 freezing was used in the experiments with Delicious apples, the values shown in Figures 12 and 13 are likely underestimations of the endogenous acetaldehyde content of the fruit.

The extraction procedure that we have employed possesses a number of desirable features. High temperature is not used to inactivate enzymes. When tissue was frozen it was sliced directly into liquid N_2, where enzyme activity should have been arrested almost instantly. When tissue was not frozen, no more than 10 seconds elapsed between fruit slicing and contact with the tissue by the acidified 2,4-DNPH. Although temperature rose to 39-43°C during maceration, the low pH (2.0) should have protected against enzyme activation. Acetaldehyde reacts readily with 2,4-DNPH, and our observations are that this reaction is very rapid. Although the reaction with DNPH is not specific for acetaldehyde, the mixture of hydrazones collected may be separated by paper (1, 8, 21), column

(14,59), gas-liquid (38,63), and thin-layer (9,16,36) chromatography.

Although acetaldehyde reacts readily with 2,4-DNPH, recovery studies have failed to produce consistent results. The manner of introducing the highly volatile acetaldehyde as a concentrated solution into the system may be causing these inconsistencies by permitting escape before entrapment. Nevertheless, to verify that all the acetaldehyde in the apple tissue is being converted to acetaldehyde-2,4-DNP-hydrazone, further work is needed.

An important precaution in applying the in situ method was demonstrated by the necessity to modify the separation procedures when McIntosh rather than Delicious apple tissue was sampled. It is therefore necessary to verify that the acetaldehyde-24,-DNP-hydrazone has been adequately purified when applying this procedure to other plant tissues or even to the same tissue that has been subjected to different conditions.

It has been amply demonstrated (32,46,74) that carbonyls other than acetaldehyde are formed by apple tissue. Since the bisulfite reaction is not specific, procedures employing the reaction measure total aldehydes and ketones capable of reacting with bisulfite. Huelin (34) showed that other carbonyl compounds from apples were trapped by bisulfite. The extent to which the published "acetaldehyde" concentrations shown in Tables 1, 2, 4, and 5 are distorted by

measurement of other carbonyls is unknown.

A general comparison of data for acetaldehyde content of apples determined by the steam distillation, bisulfite addtion method (Table 1) and by the in situ method (Figures 12, 13,14) indicates that somewhat higher values were generally obtained using the former method. However, the only specific comparison that can be made is with Gerhardt's (23) measurement of acetaldehyde content of McIntosh and Delicious apples. Although the values for acetaldehyde content of Delicious apples at the beginning of storage are similar to our values (Figure 12), much higher values were reported by Gerhardt after 180 days and 225 days at -0.6°C. Our Delicious apples were not stored for this long period of time, but when the apples were kept at room temperature for 14 weeks they did contain amounts of acetaldehyde comparable to those reported by Gerhardt (23) for fruit stored for 225 days at -0.6°C.

The acetaldehyde content reported by Gerhardt (23) for McIntosh apples stored for 45 days at -0.6°C (Table 1) was also comparable to what we found in our study (Figure 14).

The much higher values for other varieties by Fidler (18) and Miller (47), leads one to question whether other carbonyl compounds are building up and interfering in the analysis. It is also possible that these values are real and indicate a large capacity for acetaldehyde accumulation in certain varieties or after longer periods of storage than examined in our studies.

The role that acetaldehyde plays in physiological deterioration of fruit tissue is still unclear. However, the association of high levels of acetaldehyde content with such a diverse group of physiological disorders and injuries (Tables 4 and 5) seems to support the viewpoint that its accumulation is a secondary phenomenon during physiological deterioration. Our studies reinforce this view by finding no significant difference in acetaldehyde content between 2 groups of McIntosh apples of different maturities. The more mature ethephon-treated fruits were more susceptible to internal breakdown but only the fruits exhibiting the breakdown contained significantly higher amounts of acetaldehyde. This may indicate that acetaldehyde build-up occurs after internal breakdown is initiated.

SUMMARY

A method was developed to measure the acetaldehyde content of apple tissue. Sampling involved maceration of tissue in acidified 2,4-DNPH followed by extraction of the carbonyl-2,4-DNP-hydrazones with benzene. Acetaldehyde-2,4-DNP-hydrazone was separated and purified by TLC and quantitatively determined by UV spectroscopy at 354 nm.

Studies of acetaldehyde accumulation during ripening support previous findings that initially low levels increase as the fruit ripens and that only during advanced senescence does acetaldehyde accumulation reach high levels.

No evidence was found that suggested a build-up of acetaldehyde prior to tissue disorganization. Watercored Delicious apple tissue accumulated significantly greater amounts of acetaldehyde than controls during storage but internal breakdown did not result.

Comparison of the acetaldehyde content between ethephon-treated and nontreated McIntosh apples, showed, on the average, no significant difference. More breakdown occurred in the ethephon-treated fruits even though the average acetaldehyde content was not significantly greater than the control. The acetaldehyde content of tissue exhibiting internal breakdown was about 20% higher than the corresponding sound fruit. This evidence supports the view that acetaldehyde accumulation may be secondary in nature and not the primary cause of internal breakdown.

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in situ MEASUREMENT OF ACETALDEHYDE IN SENESCING APPLE FRUITS

A Dissertation

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