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The Formation of Carbonyl Compounds in Cucumbers

SUMMARY—The carbonyl contents of benzene extracts of aqueous cucumber homogenates were estimated spectrophotometrically as the 2,4-dinitrophenylhydrazones. A large increase in the formation of carbonyl compounds occurred when cucumbers were blended with water in the presence of oxygen. This formation of carbonyl compounds was prevented by three methods: blending the cucumbers at pH 1.0; blending in an oxygen-free atmosphere; and heating whole cucumbers to an internal temperature of 77°C before blending.

Chromatographic assays indicated that negligible amounts of the 2-enals, 2,6-nonadienal, 2-nonenal, and 2-hexenal are present in intact cucumbers; but a rapid synthesis of this class of carbonyl compounds occurred when fresh cucumbers were blended in the presence of oxygen. The most significant increase occurred in the formation of 2,6-nonadienal, the aldehyde largely responsible for the flavor of fresh cucumbers. There were indications that ethanal and propanal were present in appreciable levels in intact cucumbers.

These observations suggest that the characteristic flavor components of fresh cucumbers are generated enzymatically as a consequence of cutting or mechanically rupturing the fruit.

INTRODUCTION

IT IS WELL KNOWN that the metabolic activity of plants is altered when the tissue is cut or crushed, sometimes resulting in the formation of new products (Virtanen, 1962). This phenomenon, which may be considered an artifact by the plant physiologist, is of practical as well as theoretical interest to those concerned with the flavor and other organoleptic properties of fruits and vegetables.

Evidence indicates that certain compounds important to the flavor of metabolically active fruits and vegetables may be formed when the tissue is physically damaged (Weurman, 1963). A classic example of this phenomenon is the formation of garlic and onion flavor compounds when the bulbs are crushed (Stoll *et al.*, 1951; Schwimmer *et al.*, 1961).

More closely associated with the work reported here is the evidence that 2-hexenal is not present in several plant materials including certain leaves (Nye *et al.*, 1943), strawberries (Winter *et al.*, 1964), bananas (Hultin *et al.*, 1961), and apples (Drawert *et al.*, 1965); but this aldehyde is formed when the above products are crushed.

The flavor of fresh cucumbers has been attributed largely to aldehydes and to a lesser extent certain corresponding alcohols (Forss *et al.*, 1962). The pleasant element was attributed to 2,6-nonadienal, while two other unsaturated aldehydes, 2-hexenal and 2-nonenal, and three saturated aldehydes, ethanal, propanal, and hexanal were considered to contribute secondarily to the overall flavor. The objectives of the present study were to learn if the above carbonyl compounds are present in intact cucumbers and to study the effect of grinding cucumbers in a blender on the formation of these compounds.

MATERIALS AND METHODS

FRESH MODEL variety pickling-type cucumbers, approximately 1¼ in. in diameter, were used in this study.

The carbonyl contents of cucumbers were quantitatively estimated by extracting aqueous homogenates of cucumbers with benzene, conversion of the carbonyls in the extracts to 2,4-dinitrophenylhydrazones and subsequent spectrophotometric assay. Cucumbers were peeled (1 to 2 mm thickness of the skin, stainless steel knife) and ground with an equal weight of distilled water in either a Waring Blendor or a Sorvall Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.). The slurry was then saturated with sodium chloride and shaken in a separatory funnel with 1/5 volume of benzene.

After centrifugation to break the emulsion, a portion of the benzene layer was removed and assayed for carbonyl content by the Henick *et al.* (1954) procedure. Reagent grade benzene (Fisher Scientific Co., ACS Certified) was found to be sufficiently carbonyl free for these extractions without further purification. One to five ml of benzene extract and sufficient benzene to make a total of 5 ml were added to 50 ml volumetric flasks containing 3 ml of 4.3% trichloroacetic acid in benzene and 5 ml of benzene saturated with 2,4-dinitrophenylhydrazine (Matheson Coleman and Bell). The flasks were glass-stoppered and heated for 30 min at 60°C and then cooled. Ten ml of potassium hydroxide in ethanol (4%, w/v) was added slowly while mixing and absorbances at 430 and 460 mμ were determined 10 min later.

Calculations for concentrations of saturated and unsaturated carbonyl compounds in these flasks were based on the extinction coefficients and equations reported by Henick *et al.* (1954) for the analysis of hexaldehyde and crotonaldehyde mixtures. From these calculations and correction for dilution, the total contents of carbonyls in the extracts were expressed as μ moles per 100 g fresh cucumber. The proportion of unsaturated carbonyls of this total was expressed as mole %.

Spectrophotometry was performed with a Bausch and Lomb 505 spectrophotometer.

Gas chromatographic analyses were performed with a Barber-Colman Model 10 gas chromatograph equipped with a hydrogen flame detector. A stream splitter permitted 50% of the column effluent to exist through the collector port for odor monitoring. Separations were ac-

complished with Apiezon L, 5%, on Chromosorb G (60 to 80 mesh, AW-DMCS, Applied Science Laboratories, Inc.) contained in a 6-ft, ¼-in. internal diameter glass column. Conditions for the analyses were: column, 120°C; cell, 200°C; nitrogen carrier gas, 17 psi inlet, 10 ml/min through collector; hydrogen, 15 psi; oxygen 25 psi.

Column chromatographic separations of 2,4-dinitrophenylhydrazones were carried out by adsorption on magnesia as a modification of the Schwartz *et al.* (1963) procedure. Fifteen g each of Seasorb 43 and Celite 545 (Fisher Scientific Co.) were slurried in 150 ml of chloroform and poured through a funnel into a glass column, 2.5 × 51 cm, containing a small wad of glass wool at the base. The column was packed under 2 to 3 psi nitrogen, allowing the solvent to reach a level of about 5 mm above the packed portion. Benzene extracts of cucumbers were diluted to the same carbonyl concentration, 2.5×10^{-4}M. These solutions were saturated with 2,4-dinitrophenylhydrazine and combined with an amount of 4.3% trichloroacetic acid proportionate to that defined in the Henick *et al.* (1954) assay procedure. The solutions were heated for 30 min at 60°C, cooled, and then washed four times with two volumes of distilled water to remove the acid.

After drying the solutions over sodium sulfate, the benzene was removed by evaporation *in vacuo* at 40°C. A 2-ml sample containing 10 micromoles of the 2,4-DNPH derivatives contained in chloroform was pipetted onto the column, allowed to be adsorbed, and then eluted with a sequence of solvents consisting of 200 ml chloroform, 100 ml each of 1, 3, 6, 15 and 50% methanol in chloroform, respectively, and finally 200 ml methanol. Five-ml fractions were collected with an LKB type 3402B automatic fraction collector and absorbances determined at 360 mμ.

Thin-layer chromatography (TLC) of 2,4-dinitrophenylhydrazones was performed with kieselguhr G plates (20 × 20 mm) coated with 2-phenoxyethanol as described by Urbach (1963). The plates were spotted 3 cm from the origin and developed in heptane which had been saturated with 2-phenoxyethanol. Four ascents of the solvent to about 3 cm from the top of the plate were allowed, the plate being removed and dried a few minutes before return to the developing tank for a subsequent ascent. This procedure was used by Urbach for the separation of hydrazones of an homologous series and was a valuable aid in identifying the components in eluates from class separations by column chromatography.

RESULTS AND DISCUSSION

Total carbonyl content of cucumber extracts

Over 80% of the unsaturated carbonyl compounds of fresh cucumbers was extracted into benzene in the first extraction when one part benzene to 5 parts of a sodium chloride-saturated cucumber slurry was shaken in a separatory funnel and the emulsion centrifuged. The saturated carbonyl compounds were less completely extracted into benzene and were present in the aqueous phase in appreciable quantities after three successive extractions. It was assumed that the more hydrophilic carbonyl compounds, e.g. ethanal and acetone, were responsible for the less complete extraction of the saturated carbonyls into benzene. Since the longer chain unsaturated carbonyls were of primary interest, a single benzene extraction was adopted for use in quantitative estimations. Benzene extracts of peeled cucumbers absorbed negligibly when appropriately diluted and read at the wavelengths specified in the assay. With unpeeled cucumbers the assay was influenced slightly due to the presence of pigments in or near the skin, hence the reason for removing the skin prior to extraction.

Earlier studies in this laboratory indicated that the carbonyl content of intact fresh cucumbers is comparatively low. This conclusion was made after a comparison of the carbonyl content of fresh cucumbers blended in distilled water with that of cucumbers blended in acidified water (final pH about 1.0). It was assumed, therefore, that the major portion of carbonyl compounds are formed enzymatically as a consequence of crushing the cucumbers. Several prior reports have shown that 2-hexenal is formed enzymatically upon crushing plant leaves and certain fruits. Nye *et al.* (1943) found that 2-hexenal is formed by leaves of *Ailanthus glandulosa* upon grinding in the presence of air, but is not present in the intact leaves. A similar observation was reported by Major *et al.* (1963) for leaves of the tree, *Ginkgo biloba.* In both instances, oxygen was essential for the formation of 2-hexenal and an enzymatic mechanism was assumed to be involved.

An experiment was designed to learn if oxygen is essential for the formation of carbonyl compounds in ground cucumbers. Cucumbers were blended under controlled aeration with a modified Sorvall Omni-Mixer. The stainless steel cover of the apparatus was fitted with two 1-in. ports (3 mm I. D.) for inlet and outlet of gases. A 100-g sample of peeled and coarsely diced (about 1-in. cubes) cucumbers and 100 ml of distilled water were placed in a pint Mason jar and then the jar was coupled to the cover and blade assembly by means of an adapter ring. One sample was purged with nitrogen for 5 min and then blended. The slurry was acidified to approximately pH 1.0 by slowly adding 1 *N* HCl with a syringe through the outlet port; nitrogen gassing continued. The sample was then removed from the jar, saturated with sodium chloride and immediately extracted with benzene. The data in Table 1 show that the total carbonyl content of this extract was only about 20% of that when the cucumbers were blended in the presence of oxygen before acidifying. The cucumber slurry was still capable of forming carbonyls after being blended under a nitrogen atmosphere.

After gassing with nitrogen and blending as described above, a sample was then gassed with oxygen for 5 min before acidifying. It was concluded from these results that the activity of an enzymatic, oxygen-requiring, carbonyl-forming system in cucumbers is greatly increased when the tissue is mechanically ruptured; this system may be incapable of operating in the intact tissue. The system operates rapidly at room temperature as no appreciable increase in carbonyl content occurred beyond 5 min after blending the cucumbers.

The enzymatic formation of carbonyl compounds in cucumbers was prevented by another means, heating prior to blending. Whole cucumbers were packed into ½-gal jars, 1 kg per jar. The jars were then filled with distilled

Table 1. Control of carbonyl formation in blended cucumbers.

Treatments[1]	Total carbonyl content (μmoles/ 100 g)[2]	Unsaturated carbonyl content (mole %)
Aeration control during blending		
Blended under N_2, then acidified	8.5	31.2
Blended under N_2, gassed with oxygen, then acidified	45.9	39.7
Blended under oxygen, then acidified	42.2	39.5
Heat inactivation of enzymatic activity		
Heated whole cucumbers, then blended	10.3	19.4
Blended to allow carbonyl formation, then heated	39.5	13.1
Blended, not heated	44.1	42.7

[1] See text for detailed description of procedure.
[2] Expressed on a fresh weight basis of the cucumbers.

water, capped ("Twist-Off" cap, White Cap Co., Chicago, Ill.) and immersed and heated in an 82°C water bath to an internal temperature of 77°C. A control jar containing a thermometer placed in the center of a cucumber served to indicate the internal temperature. The jars were cooled to room temperature and the cucumbers were removed, peeled and then ground in a Waring Blendor and extracted as described earlier. The total carbonyl content of the heated whole cucumbers was less than 25% that of the fresh, unheated cucumbers (Table 1). Also, the proportion of unsaturated carbonyls was lower in the heated cucumbers.

A third sample served to assess the effects of heat on destruction of carbonyls. Peeled cucumbers were blended with an equal weight of distilled water to allow carbonyl formation. This homogenate was transferred to ½-gal. jars, and then the jars were capped and heated to an internal temperature of 77°C as were the whole cucumbers. It was found that the total carbonyl content of this sample was slightly reduced when compared to the fresh, unheated cucumbers. The proportion of unsaturated carbonyl compounds was appreciably lower, indicating partial destruction of this class of carbonyl compounds by heat (Table 1).

Gas liquid chromatography of cucumber benzene extracts

Portions of benzene extracts from the heat inactivity study reported in Table 1 were assayed by gas chromatography (GLC). The benzene from 50 ml of extract, representing the carbonyl content of 125 g of cucumbers, was removed by evaporating slowly at 40°C *in vacuo* in a flash evaporator. The concentrate from the "heated whole" cucumbers had a hay-like, cooked vegetable odor; no odor of fresh cucumbers was evident. The concentrate from the fresh unheated cucumbers had a pleasant and strong odor of fresh cucumbers. The concentrate from cucumbers blended prior to heating had a hay-like, cooked vegetable odor but in addition an odor of fresh cucumbers.

The oily residues were taken up in 0.2 ml of re-distilled carbon disulfide. Five microliters of these samples were gas chromatographed on Apiezon L (isothermally, 120°C). There is a striking contrast in the GLC profiles of Fig. 1.

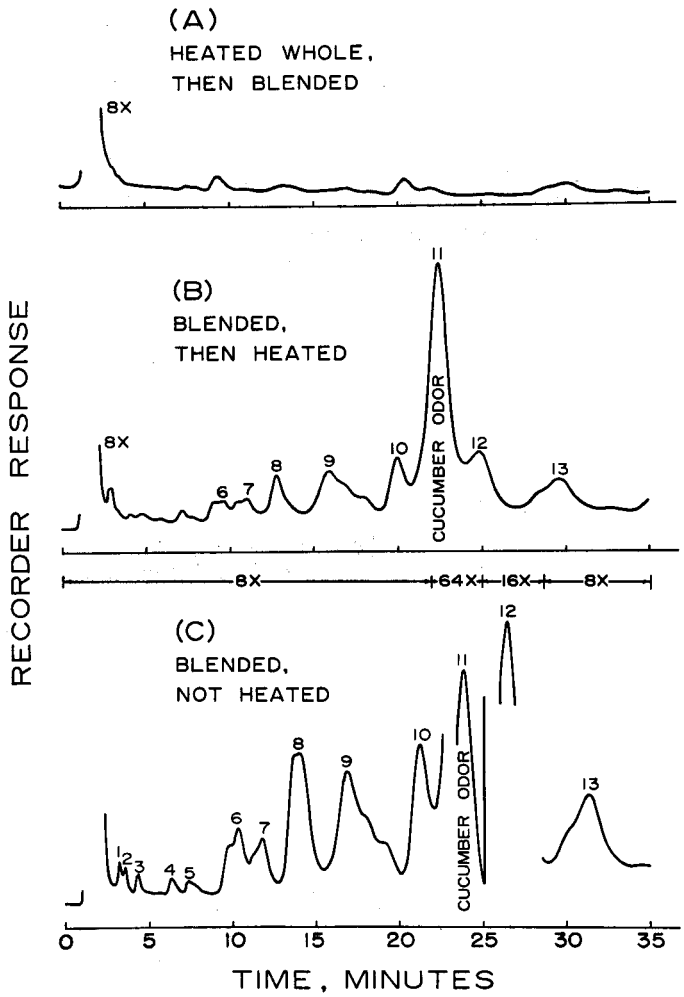

Fig. 1. Gas chromatograms of benzene extracts of cucumbers. The extracts were prepared from the samples included in the heat inactivation study of Table 1. All three samples represented extracts from equal quantities of cucumbers.

The profile of the "heated whole" cucumbers is devoid of most of the peaks showing in the fresh cucumber concentrate, and most of those appearing are greatly reduced. The concentrate from cucumbers heated after blending indicates the presence of most of the compounds of the fresh sample, but at lower levels. Most of the lower boiling compounds were undoubtedly lost during concentrating; however, some of the higher boiling carbonyls, such as the 9-carbon aldehydes, should have been retained.

The odors of the components emerging from the stream splitter of the gas chromatograph were monitored. Peak no. 11 of the fresh cucumber concentrate had a strong, definite fresh cucumber odor. Peak nos. 1, 2, 9, 10 and 12 were described as either aldehyde or cucumber-like. These peaks, although smaller, also were present in the sample from cucumbers heated after blending. Synthetic 2,6-nonadienal (Haarmann and Reimer Corp., Union, New Jersey) had the same retention time as did peak no. 11. The "heated whole" cucumber sample gave no peak or odor in the region where cucumber and aldehyde-like odors emerged with the other two samples.

Chromatography of 2,4-DNPH derivatives

It was desired to learn the relative amounts of carbonyl classes, as well as individual carbonyl compounds, in the fresh as compared to the heated cucumber samples (heat inactivation study, Table 1). Ten micromoles of 2,4-dinitrophenylhydrazones, prepared from the benzene extracts of these three samples, were pipetted onto Seasorb 43-Celite 545 columns and eluted with chloroform containing increasing concentrations of methanol (Materials and Methods). The carbonyl compounds of interest were eluted by the time the 15% methanol in chloroform solution had passed through the column. Only a relatively small quantity of carbonyls was eluted with higher concentrations of methanol and the components of these eluates were not studied.

Elution profiles for the three samples are shown in Fig. 2. Peak tubes were evaporated to dryness and the hydrazones dissolved in chloroform. Absorption spectra of these fractions were determined and the wavelength maximum for each of the peaks is indicated in Table 2. The assignment of peak fractions to carbonyl classes was made on the basis of the absorption maximum and verification by TLC analysis of the components. The slight deviation in the absorption maxima reported in Table 2 from the values reported for the pure 2,4-DNPH classes (Schwartz *et al.*, 1962) was expected since the chromatographic procedure did not render complete resolution. A blank containing the appropriate reagents and solvents was carried through the same procedure as the samples. From this blank it was ascertained that peak no. 1 of Fig. 2 was a reagent or solvent impurity; otherwise, only negligible amounts of carbonyl impurities were present.

After determining the absorption maxima, the peak tube samples were again evaporated to dryness, redissolved in 0.1 ml of chloroform and assayed by thin layer chromatography. Identification of the DNPH's (Table 2) was made by a comparison of R_f values with pure derivatives. There was a strikingly low level of 2-enal compounds in the "heated whole" cucumbers as revealed by column fractionation (Fig. 2) and the TLC data confirmed this observation. The "heated whole" cucumbers contained only a trace of 2,6-nonadienal and no detectable level of 2-nonenal and 2-hexenal whereas unheated cucumbers contained a significant amount of the former two aldehydes and a trace of 2-hexenal.

Heating caused a reduction in the level of 2-enals as evidenced by the cucumbers that were blended to allow carbonyl formation prior to heating (Fig. 2 and Table 2); but, TLC revealed a significant quantity of 2,6-nonadienal and 2-nonenal in the "blended, then heated" cucumbers. Appreciable quantities of ethanal and propanal, as well as a detectable amount of hexanal, were present in the "heated whole" cucumbers. These and foregoing observations (Table 1) indicate that the 2-enals, if present in intact cucumbers, are there in comparatively low amounts; a large increase in the formation of these aldehydes occurs when fresh cucumbers are blended in the presence of oxygen. The levels of alkanals also increased when fresh cucumbers were blended but they were also present in detectable levels in the intact fruit. Acetone was present

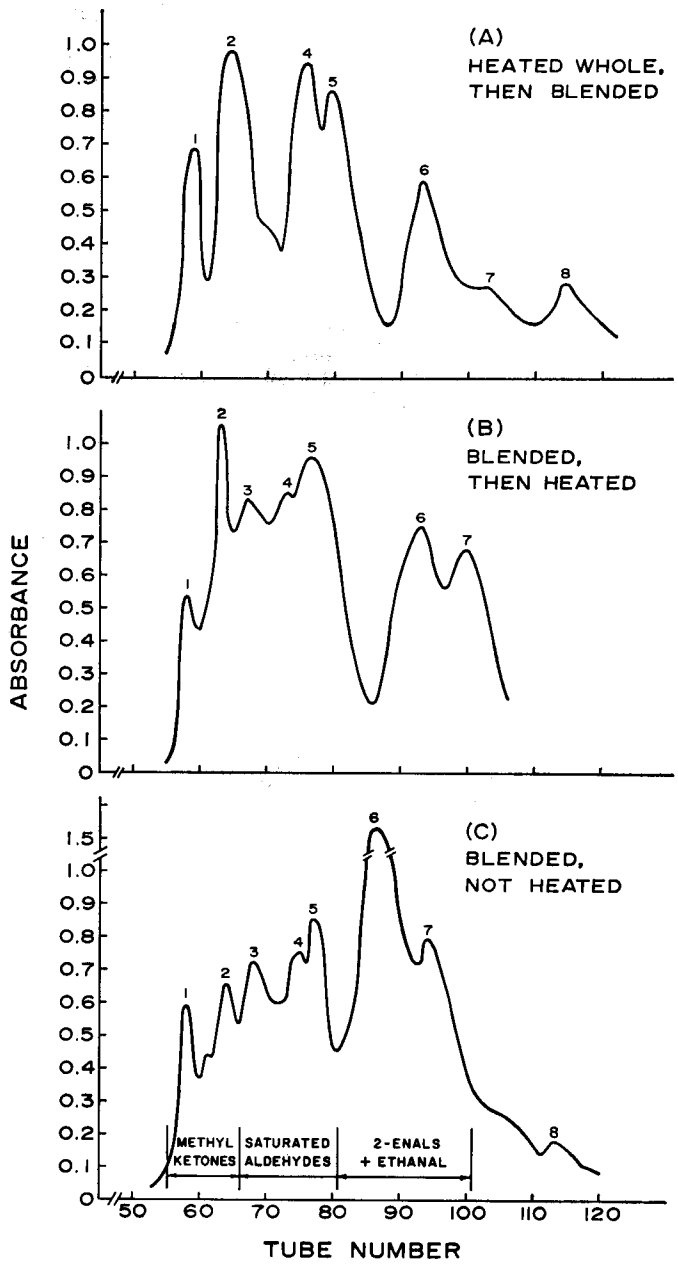

Fig. 2. Column chromatography of 2,4-dinitrophenylhydrazones from benzene extracts of cucumbers. The extracts were prepared from the samples included in the heat inactivation study of Table 1 and correspond to the samples that were analyzed gas chromatographically (Fig. 1). The profiles represent relative proportions of three carbonyl classes for each sample, when 10 μ moles of hydrazone were chromatographed. A quantitative comparison among samples may be made by considering the total carbonyl contents (Table 1).

in a relatively large amount in the "heated whole" as compared to the unheated cucumbers. Apparently acetone is present in intact cucumbers; but heating may have caused an additional amount of this compound. Several compounds were detected by TLC but were not identified. Three such compounds of possible significance were present in peak No. 3 of the "unheated" and the "blended, then heated" cucumbers. These compounds were not present in the "heated whole" cucumbers, and may be metabolic intermediates related to the formation of 2-enals.

Table 2. Identification of 2,4-dinitrophenylhydrazones in fractions from column chromatography.[1]

Peak no.	λ max	Carbonyl class	Tentative compounds	Detection of fractionated cucumber DNPH's by TCL[2]		
				Blended not heated	Blended then heated	Heated whole then blended
1	366	methyl ketone	unknown	+	+	+
2	362	methyl ketone	acetone	++	++	++
3	358	unknown	unknown	+	+	−
4	358	alkanal	hexanal	+	+	+
			nonanal	+	+	−
5	358	alkanal	propanal	++	++	++
6	372	2-enal	ethanal	+	++	++
		(+ ethanal)	2,6-nonadienal	++	−	−
			2-nonenal	+	−	−
			2-hexenal	trace	−	−
7	375	2-enal	2,6-nonadienal	++	++	trace
			2-nonenal	+	+	−
8	372	unknown	unknown	+		+

[1] These data were obtained from eluates of the column fractionations depicted in Fig. 2. Peak No. refers to the fraction so numbered in Fig. 2 and λ max values refer to the wavelength of maximum absorption of the fractions from the unheated cucumber sample (Fig. 2 C).

[2] The presence of a compound in the fraction, as indicated by TLC, is designated by a "+," compounds of highest concentration in the fraction (visual estimation) by a "++," and the absence by a "—."

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