Gel Chromatographic Fractionation of Flavor Components for Their Individual Sensory Evaluation

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Components of two tomato juices which had significantly different flavors were separated by gel chromatography on Sephadex G-15 of sera prepared by centrifugation of the juices. The eluate was monitored by uv absorbance, tests for reducing sugars, and by sensory evaluation. In sensory evaluation of the isolated fractions, 12 to 15 flavor notes were reported. Minor differences between the flavor notes detected in unconcentrated tomato juice vs. those in a reconstituted tomato juice were found. Preliminary gas chromatography of hexane extracts of sensorially significant gel chromatographic fractions showed up to five components present in each fraction.

In any flavor investigation, determination of the flavor significance of isolated fractions or components by sensory analysis increases the value of the analytical data. The sensory assessment made by sniffing at the exit port of a gas chromatograph (GC–sniff) (Guadagni, 1968; Tassan and Russell, 1974; von Sydow et al., 1970) can be helpful in describing the aromas of compounds or in screening fractions to establish those upon which further attention should be focused.

Gel permeation chromatography (GPC) has been used extensively to separate proteins and polymers, primarily on the basis of molecular weight. More recently, it has been used successfully in the separation of smaller nonvolatile compounds such as tea phenols (Wilkins, 1973), wine ultraviolet light absorbing compounds (Somers and Ziemelis, 1972, 1973), monosaccharides (Bertoniere et al., 1971), and low molecular weight urinary constituents (Jagenburg et al., 1968). Walradt and Shu (1973) used GPC to evaluate the extent of polymeric formation in volatile compounds such as 2,4-pentadienal and dodecalactone. Following solvent removal, phenolic components of cider, separated on Sephadex LH20, were evaluated by a sensory panel in a phenol stripped cider base to determine which fractions produced bitterness and/or astringency (Lea and Timberlake, 1974).

As the logical complement to GC–sniff procedures, in this study the use of GPC to separate flavor components of unconcentrated aqueous systems for sensory evaluation of the isolated fractions or components (GPC–taste) has been investigated.

EXPERIMENTAL SECTION

Materials. To investigate the use of the GPC–taste technique, two tomato juices which had been demonstrated to be different at the 1% level of significance by triangle tests (Gill, 1973) were selected for evaluation and comparison. Unconcentrated tomato juice (I) of 5% soluble solids (s.s.) and tomato juice (II) reconstituted to 5% s.s. from a paste of 20% s.s. were processed as described previously (Gill, 1973; Gill and Noble, 1974). These two juices were further evaluated by six panelists, who were members of a laboratory sensory evaluation panel which had been evaluating tomato juices daily for several months. Round table discussions were held in which brief flavor descriptions were generated.

The juices were prepared for GPC by centrifuging them at 10000 rpm for 30 min at 0 °C. In informal bench top evaluations, it was established that the sera obtained by centrifugation were characterized by the same odor and taste as those present in the parent juices. Further, suspension of the centrifuged residue in distilled water, followed by recentrifugation, yielded sera of less than 1% s.s. with a slightly sour taste, but with no characteristic tomato flavor.

Gel Chromatographic Analysis. A 110 × 5 cm column was prepared from Sephadex G-15 (Pharmacia Fine Chemicals' AB, Uppsala, Sweden) which had been swollen in 0.05 M sodium phosphate overnight. The column was jacketed and maintained at 4 °C. The flow rate of the eluting solvent was maintained at 30 ml/h by gravity flow. The void volume of the column was 540 ml, determined with Blue Dextran 2000 (mol wt 2.0×10^6; Pharmacia AB). For determination of elution volumes of standard components, the column was loaded with 30 ml of a standard solution of 2% (w/v) maltose, 2% fructose, 0.5% caffeine, and 0.75% monosodium glutamate, in the eluting solvent. For juice fractionation the column was loaded with 30 ml of the sera. Twenty-milliliter fractions of the eluate were collected in a refrigerated fraction collector (4 °C). Eluate absorbance at 280 nm was recorded continuously using a double beam uv analyzer.

The solvent used for eluting the components, 0.005 M monosodium phosphate (pH 4.9), was selected after preliminary runs were made using higher concentrations of sodium phosphate buffer (0.01–1.0 M). The more concentrated buffers interfered with the tasting of the fractions. The dilute solvent chosen, however, had minimal taste interference and was above the manufacturer's suggested minimum ionic strength.

Informal bench top comparison of the taste of the freshly prepared 0.005 M monosodium phosphate with that of the column eluate, both before and after runs, indicated no detectable taste or flavor contribution was made by the packing material.

Evaluation of Fractions. Within 24 h of their collection, the fractions were evaluated. Four runs each of juices I and II and two runs of the standard solution were made. On these runs reducing sugars were determined using the Fehling test (Triebold and Aurand, 1963) and the fractions were tasted informally by 2–4 people with the free exchange of comments encouraged. Once the reproducibility of the elution pattern was established by these tests, the fractions from 5 runs for each juice were evaluated by inspection of the uv recording and by sensory analysis. The fractions were evaluated one at a time independently by four panelists who had been introduced to the same descriptive terminology in evaluations of the preliminary runs. Five milliliters of each fraction was presented to the judge in a 50-ml glass beaker. Each

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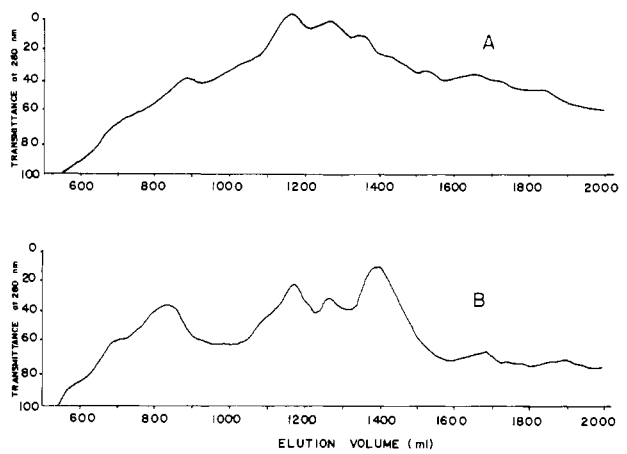

Figure 1. Typical gel chromatographic elution profiles at 280 nm of sera of (A) unconcentrated tomato juice and (B) reconstituted tomato juice.

panelist recorded his description of the perceived aroma and/or taste immediately after tasting each fraction. At any one sitting up to 20 fractions were evaluated.

RESULTS AND DISCUSSION

By descriptive analysis of the tomato juice flavors, the unconcentrated tomato pulp was characterized sequentially by the following descriptions: "fresh ripe tomato", "sweet", and "sour". The flavor, which left no aftertaste, was described overall as "fresh" and "rich tomato". Flavor notes reported in the reconstituted tomato juice were "green tomato", "cooked tomato", and "sour". The overall flavor was termed "flat" with a sour aftertaste.

Typical uv absorbance curves for the eluates are shown in Figure 1. The concentrations of the components in the separated fractions were high enough for their direct sensory assessment although, in all but a few cases, no aroma could be perceived by sniffing the fractions. The flavor notes described were readily detected, however, when the sample was taken into the mouth. Despite the lack of resolution as indicated by the broad uv absorbance bands, there was adequate separation of components for perception of distinctly different flavor notes upon sensory analysis of the fractions. Band broadening did occur, however, for example in the five consecutive fractions (48–53), which were reported as sweet. The highest intensity of sweetness was reported in fractions 50 and 51 with the rest being described as slightly sweet or faint, sweet.

In Table I the flavor descriptions of the fractions of both juices are listed. The descriptors reported are those used by at least 3 of the 4 panelists for 3 out of 5 runs. Where no flavor was detected or there was a lack of agreement on descriptors, no flavor notes are indicated. The elution volume of the fraction in which the flavor note was most intense is reported as the elution volume (V_e) for that descriptor. The results of sensory evaluation of the fractions of the standard solution of authentic compounds and of the Fehling test on these fractions are shown in Table II.

Despite the large difference in flavors between the two tomato juices, only a few flavor notes differed between the two samples in the GPC–taste evaluations. Of particular interest, in examining the flavor descriptors used for the fractions of two juices, are the fresh tomato note with a V_e of 1160 ml which was reported only in the unconcentrated tomato pulp and the bitter note, at an elution volume of 1750 ml, detected only in the reconstituted juice. In both samples, other fractions contained notes described as tomato-like. None were characteristic of the aroma or

Table I. Flavor Notes Present in GPC Fractions

Unconcentrated tomato juice		Reconstituted tomato juice	
V_e,[a] ml	Descriptor	V_e,[a] ml	Descriptor
765	Green meaty	765	Green meaty
880	Meaty, like MSG	880	Meaty, like MSG
925	Astringent		
940	Sour, tomato-like	940	Sour, tomato-like
1010	Sweet	1010	Sweet
1070	Sour	1071	Sour
1095	Green tomato, tomato-like		
1160	Ripe, fresh tomato		
		1200	Green tomato
1240	Sulfide	1240	Sulfide
1285	Rotten, strong sulfide	1290	Rich mouthfeel
1320	Tomato-like	1330	Meaty, tomato-like
1380	Canned corn	1375	Tomato-like aftertaste
		1420	Floral
		1490	Canned green bean
		1530	Like squash leaves
		1750	Bitter

[a] Elution volume for the descriptor is that of the fraction in which the maximum intensity of the descriptor was perceived.

Table II. Evaluation of GPC Fractions of Standards

	V_e, ml	Fehling test for reducing sugars	Flavor notes perceived on sensory evaln of fractions
Maltose	860	+	Faint
MSG	880	–	Meaty, like MSG
Fructose	1010	+	Sweet
Caffeine	1230	–	Bitter

flavor of fresh, ripe tomatoes, but were described as green tomato, green, meaty, or merely tomato-like. Several notes were reported that could not be detected in the parent tomato juices such as floral, canned corn, canned green bean, or rotten.

The elution volume of the sweet tasting fraction (1010 ml) coincides with the V_e for the standard fructose. Fractions 48–53, which were described as sweet by the panelists, were the only ones to give a positive Fehling test. The meaty fraction, occurring at an elution volume of 880 ml, coincides with the V_e and with the taste of the standard monosodium glutamate. With the exception of speculations that a monovalent salt of glutamic acid and monosaccharides are present respectively in the meaty and sweet fractions, no attempts were made in this study to identify components in specific fractions. Estimation of molecular weight (mol wt) of any component on the basis of the GPC elution volume cannot be made. Although the elution volume is usually a linear function of the log of the molecular weight over a large range, aromatic components and highly polar compounds have an affinity for the gel phase and are eluted at a larger V_e than that predicted from the log of the molecular weight. This is shown by comparison of the elution volumes and molecular weights of the components in the standard solution (Table II) in which caffeine (mol wt 242) is eluted considerably later than monosodium glutamate or fructose (mol wt 170 and 180, respectively).

In preliminary work, gas chromatography of compounds extracted from several fractions with hexane showed up to five gas chromatographic peaks per fraction. However, no effort at identification or quantification of these components was attempted in this study. Further investigation using gas chromatographic–mass spectral

analysis of the sensorially significant GPC fractions is needed to interpret the significance of the minor differences in the flavor notes between the two juices.

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Ethylcarbamate in Fermented Beverages and Foods. I. Naturally Occurring Ethylcarbamate

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A method was developed for the determination of ethylcarbamate in food and beverages. The ethylcarbamate was extracted, purified on Florisil, and concentrated. Separation was done with a specially prepared liquid phase. Detection was with a Coulson nitrogen detector. Most fermented foods and beverages measured contained ethylcarbamate, ranging from a trace to 6.0 μg/l. A commercial sake was found to contain in excess of 150 μg/l. Naturally occurring ethylcarbamate in wines was confirmed by gas–liquid chromatography and mass spectral analysis. The source was postulated to be the ethanolysis of carbamyl phosphate. No ethylcarbamate was found in unfermented foods or beverages.

Boehm and Mehta (1938), who first synthesized diethyl dicarbonate (DEDC), commonly called diethyl pyrocarbonate (DEPC), suggested that reactions with the primary amines formed the carbamic esters:

$$RNH_2 + O(OCOR')_2 \rightarrow RNHCOOR' + R'OH$$

The specific reaction of DEDC with ammonia in wine was mentioned by Thoukis et al. (1962). A report by Lofroth and Gejvall (1971) was the first on the amount of ethylcarbamate produced by adding DEDC to beverages. They reported finding large amounts by a radioactive isotope dilution determination. In a white wine of pH 3.4 with an estimated ammonia content of 5 mg/l., the addition of DEDC at 500 mg/l. apparently produced ethylcarbamate at 2.6 mg/l. That work was immediately repeated by others: Fischer (1971–1972) and Industrial Bio-Test Laboratories, Inc. (Department of Health, Education and Welfare, 1972), who found a much lower level (by a factor of about 100-fold) of ethylcarbamate formed by their isotope dilution studies. The possibility of ambiguity in the Lofroth work was strongly suggested. The second report also covered work concerning gas chromatographic determination of ethylcarbamate by conventional methods. Flame ionization techniques and gas chromatography combined with mass spectrometry could not verify the extremely high results. Those studies indicated levels 100 to 200 times less than that reported by Lofroth and Gejvall (1971). Nevertheless, permission for the use of DEDC in beverages under the Federal Food, Drug and Cosmetic Act (*Fed. Regist.*, 1972) was rescinded. The reason given was that deficiencies in analytical methods did not permit an unequivocal conclusion as to the presence or absence of ethylcarbamate in either treated or untreated beverages. Walker et al. (1974) recently reported a method capable of detecting ethylcarbamate in fermented beverages at 100 μg/l. The Joint FAO/WHO Expert Committee on Food Additives (World Health Organization, 1972) concluded that ethylcarbamate at 10 μg/l. was a permissible level in soft drinks, that the compound should be used only in beverages at pH 4.0 or less, and that diethyl dicarbonate not be used in beverages such as milk with significant ammonia, protein, and amino acids. The Committee felt its use in other acid beverages is not technologically justified and questioned whether ethylcarbamate naturally existed in fermented beverages. A number of questions thus appeared unanswered as to the amounts of ethylcarbamate that might occur naturally in fermented foods and the levels that could actually result from added DEDC. A series of experiments was initiated to resolve those questions. The first group of experiments was designed to detect the presence of naturally occurring ethylcarbamate and to explain the mechanism of its formation.

MATERIALS AND METHODS

Apparatus. A 1-l. Kuderna-Danish evaporator with a

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