gas chromatograms of tocopherols from almond and apricot oil are shown in Figure 2, a and b. Also here, as in the previous tlc separations (see above), the pattern of the tocopherols was entirely different for the two investigated oils. The gas chromatograms of the tocopherols from pure almond and apricot oils reveal that the tocopherol fractions did not include interfering components from unsaponifiables with the same retention time as either α- or γ-tocopherol. In the gas chromatogram of tocopherols from a 95:5 mixture of almond and apricot oils (Figure 2c) the peak height of γ-tocopherol increased distinctly as compared with the corresponding peak in the gas chromatogram of almond oil (Figure 2a). The ratio of α- to γ-tocopherol in pure almond oil was 94:6 (Figure 2a), while for the adulterated oil it was decreased to 83:17. It was concluded also that gas chromatographic analysis may be used as a means for detection of adulteration of almond oil with apricot oil.

To summarize, three different techniques suitable for detecting adulteration of almond oil with apricot oil were described. The first one (tlc of the unsaponifiables) may be used in a primary investigation of a possibility of adulteration. The other two methods (whose results were in good agreement between themselves) were combinations of tlc of the unsaponifiables, followed by either colorimetric or gas chromatographic determinations of tocopherols. Both of these methods were found sensitive and are recommended for evaluation of the extent of the adulterations.

A survey of the level of γ-tocopherol in different varieties and crops of the investigated almond and apricot oils may help in establishing the usefulness of the γ-tocopherol content as an indication of the purity of almond oil.

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A Method for Obtaining Reproducible Quantitative Gas Chromatograms of Volatiles Isolated from Foods

Statistical interpretation of the gas chromatographic data obtained from volatile flavor compounds isolated from foods requires reproducible gas chromatograms. Injection of the volatiles isolated from frying oils into a high sensitivity gas chromatograph yielded similar but quantitatively different gas chromatograms in consecutive injections. It was found that after the gas chromatographic column has been saturated with the volatiles by repeated injections, qualitatively as well as quantitatively reproducible gas chromatograms can be obtained.

Recent publications in the field of volatile flavor analysis by gas chromatography employ serious efforts in statistical interpretation of data (Dravnieks *et al.*, 1973; Pattee and Singleton, 1972). Since the volatiles isolated from the food samples are usually minute in quantity, high sensitivity gas chromatography is generally used to produce profile chromatograms. The most significant prerequisite for the statistical interpretation of such chromatograms is therefore the assurance of reproducibility of quantitation of each peak in the chromatograms.

McGugan and Howsam (1972) reported that the analysis of volatiles by gas chromatography is not always quantitative. It is generally thought that thermal degradation of labile compounds and "irreversible" sorption of compounds account for the nonquantitative passage of samples through the gas chromatograph.

In our recent study of the volatile flavor components in deep fat fried foods, we simulated commercial and restaurant practices, according to the method of Krishnamurthy *et al.* (1965), by frying moist cotton balls containing 75% by weight of water in six different oils, namely, cottonseed oil, corn oil, peanut oil, and soybean oil hydrogenated to iodine values of 70, 89, and 115, respectively. The volatile flavor compounds were then isolated from each of the fried oils by subjecting them to 90° under 0.05 mm for 6 hr.

A sample of the isolated volatiles from cottonseed oil was chromatographed with a Beckman GC-55 gas chromatograph using a hydrogen flame ionization detector set at high sensitivity. Two profile chromatograms, obtained by injecting the same sample into the instrument twice under exactly identical conditions, were qualitatively similar, but quantitatively different (Figure 1). Similar results were obtained with 1.8-mm i.d. stainless steel columns packed with either 10% methyl silicone SE-30 on 60–70 mesh Anakrom ABS, Porapak Q, 60–80 mesh, or 5% OV-101 on 80–100 mesh Chromosorb W-HP (AW-DMCS).

Since the injected volatile flavor compounds were from the same sample, any losses of heat-sensitive compounds should have been the same and should not have affected

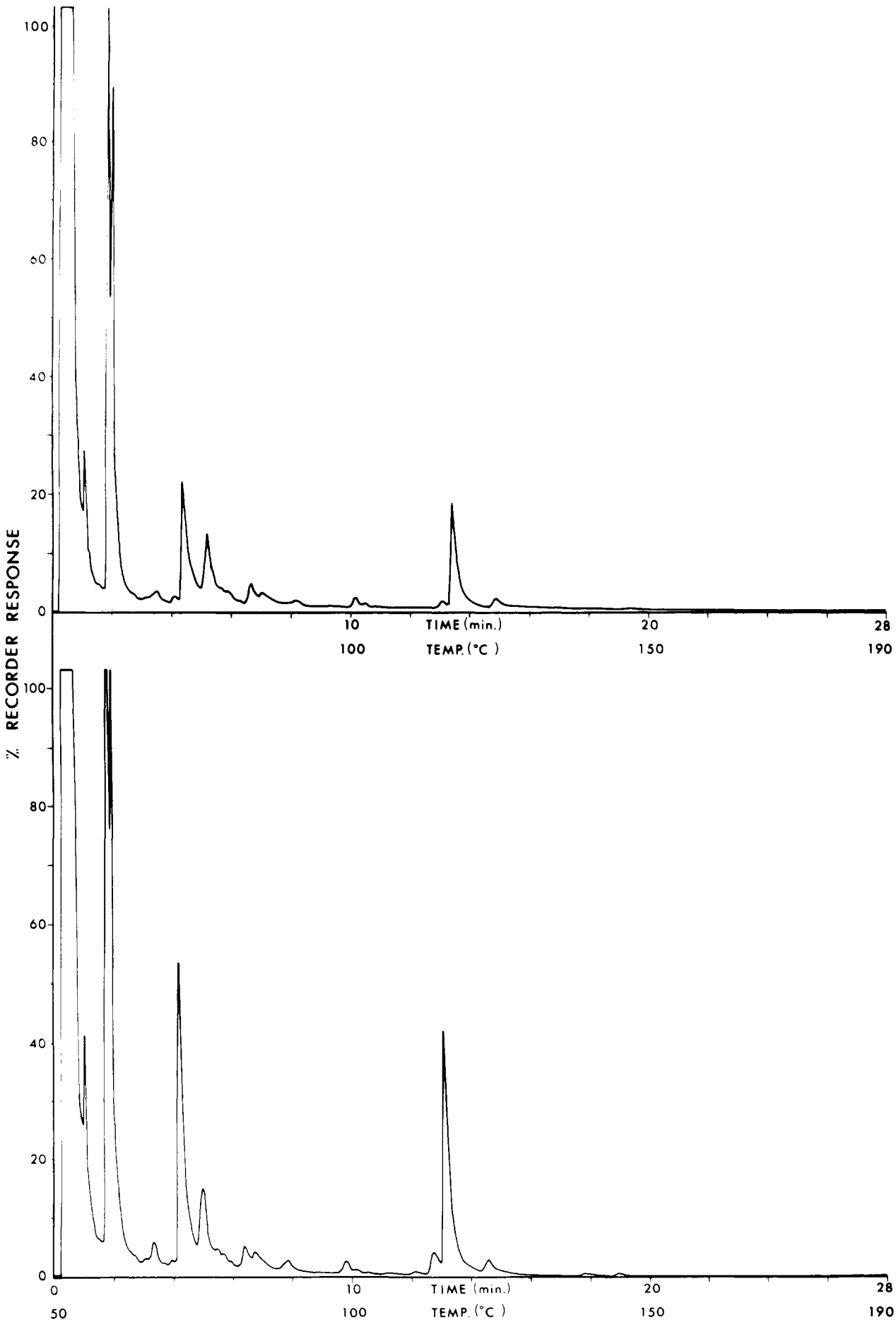

Figure 1. Nonreproducible gas chromatograms obtained by consecutive injection of the volatiles isolated from a cottonseed oil used for simulated deep fat frying.

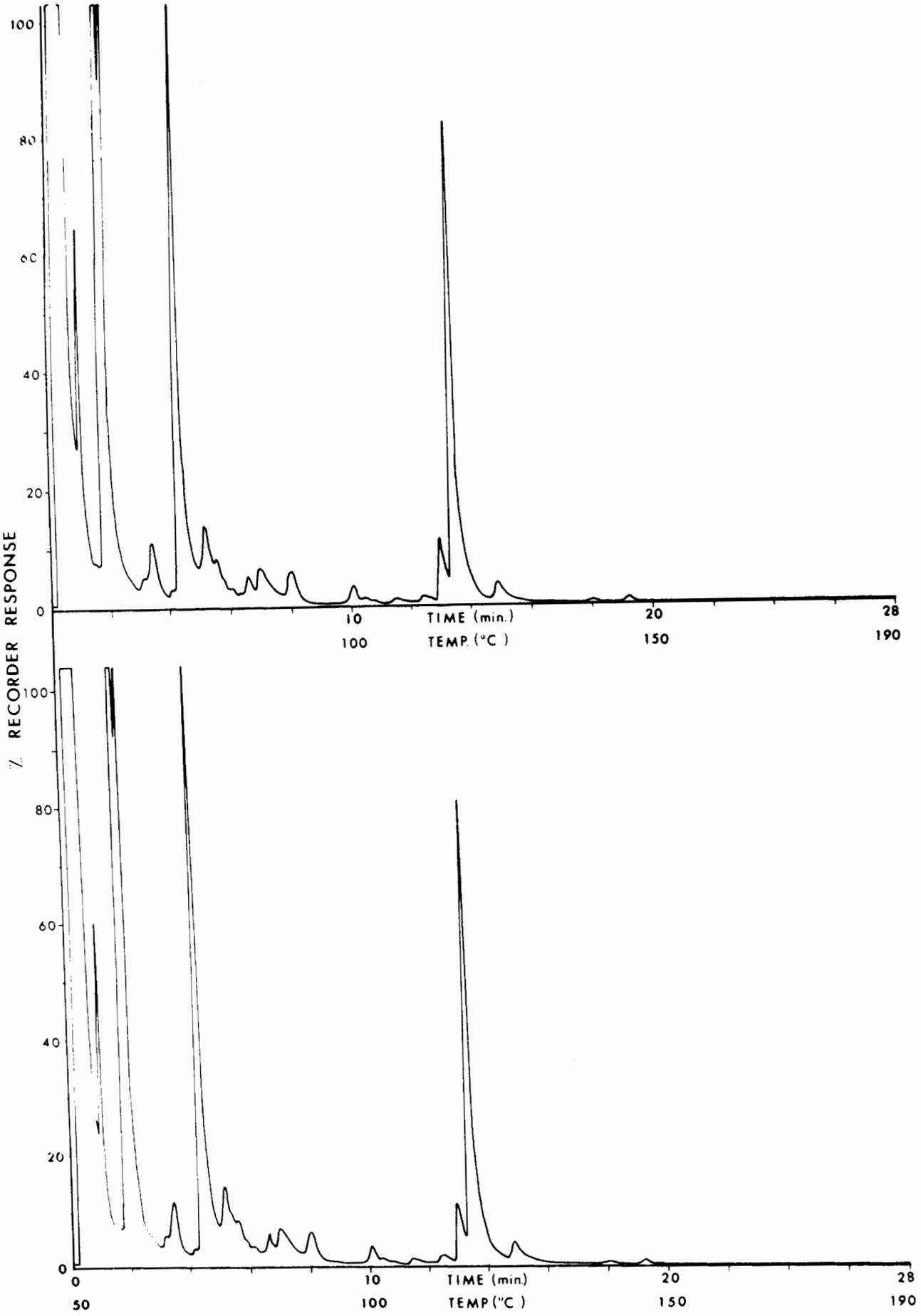

Figure 2. Reproducible gas chromatograms obtained after the saturation of the column by consecutive injections of the volatiles isolated from a cottonseed oil used for simulated deep fat frying.

quantitation of the chromatograms. It was hypothesized that the quantitative differences were due to the retention of materials at numerous active sites on the column. This being true, the quantitative differences should be eliminated after the column active sites are saturated with the sample.

We therefore repeatedly injected the volatiles isolated from the fried cottonseed oil into the OV-101 column. Following each injection at 50°, the column was programmed from 50° to 190° at 5°/min and automatically cooled to 50°. After a minimum of seven consecutive injections of 5-μl samples of the ethyl ether solution of the isolated volatiles, excellent reproducible gas chromatograms were obtained, as shown in Figure 2. Equally reproducible gas chromatograms were obtained with the volatiles isolated from all the oil samples after 6–12 injections on either Porapak Q, OV-101, or methyl silicone SE-30 columns. Furthermore, closely reproducible gas chromatograms were obtained, with the use of the saturation method, from volatile flavor compounds isolated from canned beef stew.

It was further found that the active sorption sites would gradually become unsaturated if the column were left idle for some hours, even at room temperatures. Usually, a column would require saturation again when it was left idle overnight.

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Interaction of 5′-Nucleotides with Gelatin—a Model System for Flavor Potentiation

The association constants k (mM^{-1}) obtained with the equilibrium method in the system gelatin–5′-nucleotide–water (pH 6.5, 20°) are as follows: 5′-GMP 235, 5′-IMP 112, 5′-AMP 29, and 5′-CMP very small. They correspond in sequence and in relative magnitude exactly to the relative taste-potentiating activities of these compounds as reported by Yamaguchi *et al.* (1971). The described system, therefore, seems to be a valuable model for flavor potentiation. Possible mechanisms are stabilization of the helical structure and base stacking around the helices of proteins.

The theories of taste receptor stimulation are based upon the assumption that a chemical stimulus combines with some component of a receptor in such a way that the stimulus-receptor combination leads to neural activity and eventually to taste sensations. Several model systems are discussed in review papers (Beidler, 1961; Hofmann, 1969; Ohloff and Thomas, 1971; Unterhalt, 1970). For these interactions Beidler (1954) has proposed an equation which is derived from the mass action law. It is related to an equation described by Scatchard (1949), which is now widely used in binding studies with proteins (Steinhardt and Reynolds, 1969). Investigating the interactions of flavor compounds with food components (Solms *et al.*, 1973), we observed that 5′-nucleotides interact specifically with gelatin gel, and that the mode of action is a possible simulation of a taste-potentiating reaction. 5′-Nucleotides have no taste of their own, but have rather general well known taste-potentiating activities (Gutzeit-Walz and Solms, 1971; Kuninaka, 1967; Solms, 1967; Yamaguchi *et al.*, 1968). The relationships between taste-potentiating activity and structure have been extensively examined (Kasahara *et al.*, 1970; Mizuta *et al.*, 1972; Sato *et al.*, 1970). But nothing is known about the possible mechanism of taste potentiation; however, it can be assumed that it must be closely related to ordinary taste perception. In the present study, interactions in gelatin–nucleotide–water systems were investigated with the equilibrium method and the results were then compared with taste-potentiating activities reported in the literature.

EXPERIMENTAL SECTION

Materials. The nucleotides 5′-CMP, 5′-AMP, 5′-IMP and 5′-GMP were purchased from Zellstoff-Fabrik Waldhof, Mannheim, Germany. Gelatin R 220 was obtained from Ed. Geistlich Söhne, Wolhusen, Switzerland. In all calculations a value of 50,000 was used for the molecular weight of the gelatin.

Methods. A 10% gelatin gel was prepared by heating the dry gelatin in water to 50° with subsequent cooling. Samples of exactly 1 mg of gelatin gel were added to 25 ml of nucleotide solutions of different concentrations (5 to 90 mM) in 0.02 M sodium acetate buffer, pH 6.5. The systems were then equilibrated under shaking for 48 hr at 20°. The nucleotide concentrations in the supernatants were analyzed quantitatively by uv spectroscopy before (C_t) and after (C_f) equilibration. The bound nucleotides (C_b) were calculated as the difference (C_t) − (C_f). For the mathematical treatment of the data the Scatchard equation was used (Scatchard, 1949; Weder *et al.*, 1971)

$$\frac{\bar{r}}{n - \bar{r}} = kC_f$$

where $\bar{r}$ is the average number of moles of bound nucleotides per mole of gelatin, n is the maximum number of moles of nucleotides bound per mole of protein, k is an association constant (mM^{-1}), and C_f is the molar concentration of nonbound nucleotides at equilibrium. To determine the parameters k and n, one plots $\bar{r}/C_f$ against $\bar{r}$, as graphically presented in Figure 1. Each point shown in this figure averages six to eight measurements, with variations not exceeding ±6%. The drawing of the lines and the calculations of the binding parameters were done with a WANG 700 C computer, equipped with a writer plotter.