Interactions of Volatile Flavor Compounds with Propyl Gallate and Other Phenols As Compared with Caffeine

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In order to understand the partitioning of flavors that occurs during both food processing and food consumption, we determined the air–water partition coefficients for several volatile flavor compounds by means of GC. In this way it was shown that the aqueous solubility of anisole, 2,3-diethylpyrazine, and ethyl benzoate increased in solutions containing propyl gallate or other phenols such as chlorogenic acid or naringin. The phenol-induced solubilization of flavor compounds was compared to a similar effect induced by caffeine in systems where the formation of a molecular complex between caffeine and the flavor compound is possible. Partition coefficients are also given for systems containing both a phenol and caffeine.

Interactions that result in increased aqueous solubility of flavor volatiles are especially interesting in consideration of the complex partitioning that occurs among air, water, and lipid phases not only during food processing but also during food consumption.

Phenols are widely distributed in the plant kingdom. They serve such diverse and necessary functions as growth regulators, antioxidants, and vitamins. They can be responsible for color as well as flavor. Phenols often create problems, however, when plants are processed for food. Many phenols are substrates for enzymatic oxidation or browning. The presence of phenols also may contribute to bitterness or too much astringency. For these reasons it might be desirable to modify the phenolic content in foods.

Most polyphenols are nonvolatile and only sparingly soluble in water. Distillation and extraction processes, such as those commonly used to prepare aroma concentrates from fruit juices, may effectively reduce the phenolic content. In light of this treatment it is interesting to consider the possibility of phenol–volatile flavor interaction.

Propyl gallate is commonly used as an antioxidant in a number of different foods ranging from ghee to fish and also in various oils, chewing gum, and packaging material (Furia, 1968). Propyl gallate is sufficiently soluble in water so that measurements of its influence on the solubility of volatile flavor compounds could be obtained at different concentrations.

The interactions between propyl gallate and volatile flavor compounds such as anisole, ethyl benzoate, and diethylpyrazine have been investigated. Data from these and other interactions between the same flavor compounds and phenols such as chlorogenic acid and naringin, or purines such as caffeine, are compared.

EXPERIMENTAL SECTION

Materials. Anisole, (+)-limonene, L-tyrosine, (+)-catechin hydrate, ethyl benzoate, anhydrous powdered caffeine, and chlorogenic acid were purchased pure from Fluka AG (CH-Buchs). Naringin and caffeine–potassium–chlorogenate (CKC) were obtained pure from Senn Chemicals (CH-Dielsdorf). 2,3-Diethylpyrazine (DEP) was donated by Firmenich SA (CH-Geneva).

Gas Chromatography. A Carlo Erba Fractovap 2200 gas chromatograph with an FID was used. It was equipped with a Spectra-Physics SP 4000 data system. The 2 m × 2 mm i.d. glass column contained 2.5% OV-17 on 80–100-mesh Chromosorb G. It was operated isothermally at 130 °C with a nitrogen flow rate of 25 mL/min. The injector temperature was maintained at 250 °C. Headspace samples (1 or 2 mL, depending on volatility) were injected by means of a 2.5-mL Hamilton gas-tight syringe. The syringe was stored at 50 °C between injections to prevent condensation during sampling. (For experiments at 60 °C, the syringe was also maintained at 60 °C.) Injections of the liquid phase for flavors dissolved in water were 0.1–0.3 μL. A calibration curve was made for aqueous solutions of each volatile flavor compound (VFC) at known concentrations. In this way it was possible to convert GC response to molar concentration. (The same factor was assumed for both liquid and gaseous injections of the flavor compounds.)

Method. Glass serum flasks of equal volume (320 mL) were fitted with blue silicone septums (Hauri & Kempf AG, CH-Zurich) in metal screw caps. For prevention of septum adsorption of the flavor compounds, disposable Teflon liners (Angst & Pfister, CH-Zurich) were used.

The flasks contained a liquid volume of 50 mL. Flavor compounds were injected into the liquid phase of each flask by means of a Hamilton syringe held so that the needle tip was below the surface of the liquid. The flasks were fixed in a specially constructed water bath equipped with magnetic stirrers driven by compressed air. For most experiments the temperature of the water bath was either 20 or 45 °C. Naringin experiments, however, were conducted at 60 °C because of the greater solubility of naringin at this temperature. GC analysis was begun 2 h after the flasks were placed in the bath. Care was taken to flush the syringes repeatedly before injection and to clean them properly afterward. Only one headspace injection was made per flask. For flavors dissolved in water, injections of the aqueous phase were made immediately after the respective headspace injection.

Experiments were conducted so that flasks containing dissolved purines and/or phenols were equilibrated simultaneously with flasks in which the liquid phase was water. Each experiment included 10 different initial concentrations of the flavor compound for each aqueous system. Experiments were repeated at least 5 times, and the data were pooled.

Partition coefficients were determined from the linear regression analyses of the pooled data for each system. Excellent reproducibility was shown by the fact that the worst correlation coefficient was 0.98. In the case of flavors dissolved in water, the air–water partition coefficient (K_{AW}) was determined directly by comparison of the concentra-

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tions measured in both the air and the water phases. From this value and the measured value of flavor concentration in the headspace over any other liquid phase, it is possible to calculate air–liquid partition coefficients (K_{AL}) (Kieckbusch and King, 1979a).

RESULTS AND DISCUSSION

The sorption phenomena that can occur during sampling and/or injection of both vapor and liquid phases into the chromatograph have been discussed by Kieckbusch and King (1979b). In order to obtain accurate partition coefficients, they proposed a specially constructed thermostated syringe for sampling, followed by a needle-less injection into the chromatograph. They admit, however, only a very small benefit in using their system as opposed to an unmodified one.

The intention of the present paper is to show changes in partition coefficients caused by the addition of purines and/or phenols rather than absolute values of K_{AW} for any specific flavor compound. For this reason the problem of sorption as such was not investigated. Errors due to sampling or injection are undoubtedly consistent for all experiments using a given flavor compound. It should be stated, however, that there was minimal if any measurable GC response when the gas-tight syringes used were injected consecutively without prior cleaning.

The accuracy of the entire experiment was controlled by comparing the sum of the amounts of VFC measured in each phase to the amount of VFC originally introduced into the closed system. Differences calculated in this way amounted to at most 10%. The sum of the measured amounts was lower than the amount originally present in the system for all flavor compounds except DEP. The liquid-phase concentration measured for DEP was slightly but consistently higher than the amount of DEP originally introduced into the system. It is possible that the original concentration of DEP in the system was actually higher than assumed due to syringe inaccuracies. As the least volatile compound examined, DEP was used in the highest aqueous concentrations. The amount of DEP injected into the liquid phases at the beginning of an experiment required use of a 100-μL syringe whereas a 10-μL syringe was employed for all other flavor compounds.

In practice, it is usually easier to obtain information regarding the aqueous solubility of volatile flavor compounds by measuring concentration in the gas phase (headspace) as opposed to the liquid phase. The VFC headspace concentration increases in direct proportion to the concentration of that compound in the aqueous system until saturation of the liquid phase is achieved. By use of the headspace and GC setup described, it was shown that equilibrium in the headspace flasks was established on the order of 30–60 min. No significant variations in the VFC headspace concentration could be measured over the time span needed to complete the GC analyses of all flasks in an experimental series.

If VFC aqueous solubility can be increased in some way, this effect will be indicated by a corresponding decrease in the headspace concentration. Thus it may be concluded from Figure 1 that adding propyl gallate to aqueous solutions of ethyl benzoate increased the aqueous solubility of ethyl benzoate.

Partition coefficients for ethyl benzoate in both 20 and 40 mM solutions of propyl gallate, as well as in a number of other aqueous systems, are given in Table I. The solubility of ethyl benzoate was shown to increase in proportion to the amount of propyl gallate in the system. The percent increase in aqueous ethyl benzoate solubility $[(K_{AW} - K_{AL})/K_{AW}]$ can be calculated as 9.2% for 20 mM

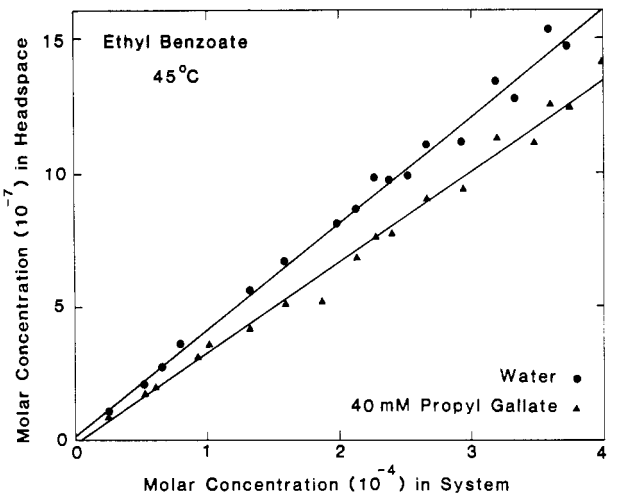

Figure 1. Propyl gallate induced solubilization of ethyl benzoate.

Table I. Partition Coefficients for Ethyl Benzoate in the Concentration Range 0.027-0.373 mM

system	$10^{-3}K$	temp, °C
air-water	0.902	20
air-water	5.99	45
air-water	11.7	60
air-40 mM chlorogenic acid	4.46	45
air-20 mM propyl gallate	5.44	45
air-40 mM propyl gallate	4.89	45
air-20 mM naringin	9.53	60
air-2.48 mM L-tyrosine	6.17	45
air-20 mM (+)-catechin hydrate	6.09	45
air-20 mM caffeine	0.548	20
air-20 mM caffeine	4.40	45
air-40 mM caffeine	3.56	45
air-40 mM CKC	3.68	45

and 18.3% for 40 mM solutions of propyl gallate.

Additional partition coefficients are given in Table I for ethyl benzoate in the presence of several other phenols. It can be seen that a 20 mM solution of catechin was not effective as a solubilizer. Similarly, no increase in solubility was observed for solutions containing tyrosin. It should be noted, however, that the tyrosin concentration is quite low due to the sparingly soluble nature of tyrosin itself.

At higher temperatures the flavanone glycoside naringin, an important constituent of grapefruit, is quite soluble in water (Pulley, 1936). Experiments conducted at 60 °C showed that naringin was an effective solubilizer for ethyl benzoate. Used as a 20 mM solution, naringin increased the solubility of ethyl benzoate by 18.7%.

Air-water partition coefficients are obviously dependent on temperature. We have recently reported data from headspace experiments using aqueous solutions of ethyl benzoate and DEP that suggest that there is formation of a caffeine–VFC complex with these compounds (King and Solms, 1981). The values obtained for both K_{AW} and K_{AL} (20 mM solutions of caffeine) increased with increasing temperature. It was suggested that the formation of a caffeine–VFC complex with these compounds was not temperature dependent because parallel curves were obtained by plotting K_{AW} and K_{AL} as a function of temperature.

Table II indicates that a caffeine–anisole complex might well be temperature dependent, although the data do not permit calculation of such a dependence. From the partition coefficients given it can be seen that there is a larger percent increase in caffeine-induced anisole solubilization at 20 °C (18.7%) than at 45 °C (12.3%). Propyl gallate increased anisole solubility only at 20 °C. It should be

Table II. Partition Coefficients for Anisole in the Concentration Range 0.037–0.555 mM

system	$10^{-3}K$	temp, °C
air-water	8.95	20
air-water	24.0	45
air-20 mM chlorogenic acid	8.56	20
air-20 mM propyl gallate	8.55	20
air-20 mM propyl gallate	24.4	45
air-20 mM caffeine	7.28	20
air-20 mM caffeine	21.0	45
air-20 mM CKC	7.38	20

Table III. Partition Coefficients for 2,3-Diethylpyrazine in the Concentration Range 0.734–11.7 mM

system	$10^{-4}K$	temp, °C
air-water	1.33	20
air-water	13.8	45
air-water	30.8	60
air-20 mM chlorogenic acid	7.85	45
air-20 mM propyl gallate	11.5	45
air-20 mM naringin	27.8	60
air-20 mM caffeine	1.11	20
air-20 mM caffeine	9.67	45
air-20 mM caffeine	24.9	60
air-20 mM CKC	8.74	45

remembered that anisole is much more volatile than either ethyl benzoate or DEP.

Among the compounds examined, the greatest effect for propyl gallate induced VFC solubilization was shown to occur with DEP. Table III shows that a 20 mM solution of propyl gallate increased the aqueous solubility of DEP by 16.9%. The same table also shows that DEP experiences the greatest effect from chlorogenic acid induced solubilization (26.9%). Although the nature of phenol–VFC interactions has not been clarified, the possibility for acid–base interactions in these two systems may explain the extent of solubilization observed. Experiments conducted at 60 °C showed that naringin was an effective solubilizer for DEP. Used as a 20 mM solution, naringin increased the solubility of DEP by 9.6%.

Flavonoid constituents of *Citrus* are abundant and of special interest to the flavor chemist. Limonene is a major *Citrus* volatile. For this reason the solubilization effect of naringin on limonene was investigated. The data given in Table IV indicate that the aqueous solubility of limonene is not influenced by naringin. It should be noted that a 20 mM solution of caffeine also was ineffective as a limonene solubilizer. When the caffeine concentration was increased to 77 mM, an increase in solubility of only 7.6% was measured.

We have reported that many of the classes of volatile compounds that contribute to food flavor interact with purines (King and Solms, 1980). These interactions can probably be attributed to plane-to-plane stacking resulting from hydrophobic and π-electron interactions of a nature similar to that described by many authors for the self-association of purines (Chan et al., 1964; Lawaczeck and Wagner, 1974; Schimmack et al., 1975; Wagner et al., 1978; Cheng et al., 1980) and the interactions between purine and indole derivatives (Dimicoli and Hélène, 1973), between caffeine and chlorogenic acid (Horman and Viani, 1972), or between caffeine and benzo[*a*]pyrene (Nosaka et al., 1978). Limonene lacks the extended π-electron system that would enable it to form a complex with caffeine.

Table IV. Partition Coefficients for (+)-Limonene in the Concentration Range 0.025–0.148 mM

system	K	temp, °C
air-water	0.153	20
air-water	2.81	60
air-20 mM naringin	2.85	60
air-20 mM caffeine	2.92	60
air-77 mM caffeine	0.142	20

During the course of our work we have shown that the purine caffeine also forms complexes with such compounds as ethyl benzoate and DEP under conditions in which the caffeine is either self-associated or already in complexed form, for example, as the naturally occurring complex with chlorogenic acid (King and Solms, 1981). In each case where an interaction occurred, the aqueous solubility of the flavor compound was increased. As is seen from the data in Table II, the aqueous solubility of anisole also is increased in the presence of CKC.

From headspace experiments alone it is not possible to describe the nature of the CKC–VFC interactions. The data presented in this paper do show, however, that interactions could involve either the caffeine or the chlorogenate moiety or both parts of the molecule. Interactions between volatile flavor compounds and phenols are certainly different in character than the purine–VFC interactions that result in molecular complexes. In light of the wide distribution of phenols, purines, and flavor volatiles in foods, we feel that interactions among these components deserve further attention.

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