

ditions of the chick feeding study, choline chloride at all three levels was significantly effective in both increasing growth and preventing perosis on the semipurified basal diet and in decreasing perosis at the 400 mg/kg level in the practical diet. Although stachydrine did not effect a growth response, it did produce a significant decrease in the incidence of perosis at the 1158 mg/kg level in both diets. Betaine and methionine did not prevent perosis to a statistically significant degree.

In studying the various responses of methyl donors in animals, Moyer and du Vigneaud, (1942) fed to rats an amino acid diet devoid of methionine and cystine but containing homocystine and vitamin supplements. On this diet, choline chloride increased growth. Betaine, when added to a similar diet (Chandler and du Vigneaud, 1940; du Vigneaud *et al.*, 1939), increased growth, but choline was more effective than betaine. Jukes and Stokstad (1952) obtained similar results on low vitamin B₁₂ diets with and without vitamin B₁₂ additions.

In looking at the problem of perosis, Jukes (1940) found that choline, but not betaine, prevented perosis in turkeys. The diet fed was not deficient in methionine or cystine.

In studying compounds acting as methyl donors in man, Ciusa and Nebbia (1948) identified stachydrine, choline, betaine, and several other compounds as effective methyl donors.

The diets used in this present study meet NRC requirements of methionine and do not show great differences in growth response among the supplements. In fact, in the practical type basal diet, the average weight in each supplemented group did not differ statistically from the control. However, choline and stachydrine both effected a decrease in the incidence of perosis. Jukes (1971) has discussed the question of why betaine and methionine are ineffective as substitutes for choline in preventing perosis in chicks and turkeys. He suggests that choline may function

directly in bone formation as opposed to functioning as a precursor of betaine and methionine in the prevention of perosis. The results of our study indicate that stachydrine decreases the incidence of perosis, but no mechanism can be postulated at this time.

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Flavor Compounds: Volatilities in Vegetable Oil and Oil-Water Mixtures. Estimation of Odor Thresholds

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Air to vegetable oil partition coefficients have been determined experimentally for a number of organic flavor compounds. These are shown to be, in general, of the same order as values calculated from solution-vapor theory, assuming that their activity coefficients in vegetable oil are equal to 1. A simple method of calculating the volatilities (air to mixture partition coefficients) of com-

pounds in vegetable oil-water mixtures is derived and shown to compare reasonably well with experimental results for such mixtures. A method is also developed for calculating odor thresholds for compounds in vegetable oil solutions from their known thresholds in water solutions. This is shown to give values which are of the same order as experimentally determined values.

The authors are carrying out a continuing study of the aroma and flavor of fried foods (*cf.* Buttery and Ling, 1972; Guadagni *et al.*, 1972). An important factor in the effectiveness of various aroma compounds in foods is their volatility in the food medium. This is controlled to a considerable extent by the affinity of the compounds for the particular medium(s) in the food.

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With complex systems such as we have in foods, it is useful to have some model systems to relate to. Studies of these model systems can at least give us an approximation of the behavior we might expect in the actual practical system. The authors have previously studied the volatilities of a number of flavor compounds in a pure water medium (Buttery *et al.*, 1969, 1971). This could be considered as one type of model system. A second important model system with foods could be the volatilities of various flavor compounds in vegetable oil. A third model system could be that for water-vegetable oil mixtures. Some

properties of the latter two systems are reported in the present paper.

EXPERIMENTAL SECTION

Materials. Organic compounds were obtained from reliable commercial sources. They were purified by gas-liquid chromatography before use.

The vegetable oil used throughout the work was high-oleic safflower oil "Oleinate 181" obtained from Pacific Vegetable Oil Corporation, Richmond, Calif. It was stored under nitrogen, in the dark, and at refrigerator temperatures.

Gas Chromatography. The method used was essentially that described previously for water solutions (Buttery *et al.*, 1969, 1971). However, in the case of water solutions, it was possible to inject some of the solution for comparison with the vapor peak. With oil solutions, a standard solution of the material in water, hexane, or ether was used for comparison with the vapor peak.

Odor Thresholds. Odor thresholds were determined essentially as described previously for water solutions (Guadagni *et al.*, 1963, 1972) using teflon bottles and tubing. In this case Oleinate 181 vegetable oil was used as solvent.

RESULTS AND DISCUSSION

Many authors have studied some aspect of the volatility of flavor compounds in foods. This work has been previously reviewed by the authors (Buttery *et al.*, 1969).

For our model systems we can consider a closed teflon bottle approximately half full of the liquid medium under consideration. We will consider only dilute solutions less than about 1% of single organic compounds dissolved in the medium, and at a temperature of 25° and at atmospheric pressure. We will consider also that the bottle is swirled gently (horizontally) to facilitate the attainment of an equilibrium. Our main interest is in being able to relate the concentration of a compound in the headspace to its concentration in solution. Much can be understood from established theory on solutions and volatility. An experimental approach is also desirable, partly because of the lack of fundamental data, but also to verify the validity of theoretical calculations.

The most practical factor for food aroma work is the air-to-solution partition coefficient. This is, of course, the ratio of solute concentration in the atmosphere above the solution to that in solution, at equilibrium, as indicated in eq 1. This can be related to the Henry's law constant C , as shown in eq 2.

air to solution partition coefficient

$$K_{as} = \frac{\text{(solute concentration in air)}}{\text{(solute concentration in solution)}} \quad (1)$$

$$K_{as} = C \times \text{(solvent conversion factor)} \quad (2)$$

The solvent conversion factor is a simple number which involves conversion of pressure units to mass units using gas laws and conversion of solvent volume to mole fraction terms. This factor is dependent on the molecular weight of the solvent but is independent of the solute molecular weight which cancels out in the calculation of this factor. For water this factor is 0.97×10^{-6} and for vegetable oil it is 5.2×10^{-5} . As outlined previously (Buttery *et al.*, 1971) from solution-volatility theory

$$C = p_0 \times \gamma \quad (3)$$

$$C = p_0 \times 1/N_s \text{ (if solubility is low)} \quad (4)$$

where p_0 is the vapor pressure of the pure compound, γ is the activity coefficient in that solvent, and N_s is the solubility of the compound in that solvent in mole fraction terms.

In previous work we have shown that air-to-water partition coefficients calculated using these types of equations compared fairly well to experimental values (Buttery *et al.*, 1971). Activity coefficients for aliphatic aldehydes in water solutions can vary from a value of 4.2 for acetaldehyde to 71,000 for nonanal (Bomben and Merson, 1969). These activity coefficients were calculated from literature data (Pierotti *et al.*, 1959). Unfortunately very few such values are available for vegetable oil solutions. Some are available for paraffin oil, but this has very different solubility properties than vegetable oil. Experiments by the authors showed, however, that vegetable oil was a remarkably good solvent for a wide variety of food aroma compounds. In fact many such compounds seemed completely miscible with vegetable oil. In this case N_s , the solubility in mole fraction units, is equal to 1. The activity coefficient γ generally also approaches 1. We should point out, however, that eq 4 is only meant for cases where the solubility is low. From our experimental results it seems that for many common flavor compounds, the activity coefficient in vegetable oil solutions is at least of the order of 1, which is quite different from the very large variation found in water solutions.

Table I lists experimental air to vegetable oil partition coefficients as determined by methods previously described by the authors (Buttery *et al.*, 1971). For comparison, the values calculated assuming that the activity coefficient γ is equal to 1 are also listed. Except for pentanol, the calculated and experimental values are of the same order. With hydrophilic compounds such as the aliphatic alcohols and probably also free organic acids, etc., we might expect γ to be considerably greater than 1, especially for the lower homologs. In the case of pentanol, γ appears to be roughly 18. It is interesting to compare experimental air-to-water partition coefficients with air-to-vegetable oil partition coefficients for homologous aliphatic aldehydes, as is shown by the solid lines in Figure 1. The relations show quite a contrast. For a compound such as butanal, there is not very much difference between the values for oil and water, but with octanal the difference is of the order of 1000.

Vegetable Oil-Water Mixtures. In many foods we have reasonable amounts of both oil (or fat) and water as the two main liquid phases. Milk is a good example of this. With an equilibrium of a solute between an oil-water mixture and air, we have a three-phase system. This is more complicated than a two-phase system, but still can be handled fairly easily. Using thermodynamic quantities, Bomben and Merson (1969) have developed equations for handling such mixtures. Following a similar line of reasoning to these authors, a somewhat simpler relation can

Table I. Air to Vegetable Oil Partition Coefficient (25°) Determined Experimentally and Calculated Assuming the Activity Coefficient (γ) is Equal to 1

Compound	Air to vegetable oil partition coefficient	
	Experimental	Calculated assuming $\gamma = 1$
Butanal	2.3×10^{-3}	4.7×10^{-3}
Pentanal	1.0×10^{-3}	2.0×10^{-3}
Hexanal	3.5×10^{-4}	6.2×10^{-4}
Heptanal	1.0×10^{-4}	2.4×10^{-4}
Octanal	4.0×10^{-5}	8.8×10^{-5}
Butan-2-one	1.9×10^{-3}	5.2×10^{-3}
Heptan-2-one	1.03×10^{-4}	0.78×10^{-4}
But-2-enal	1.4×10^{-3}	1.8×10^{-3}
Hex-2-enal	1.6×10^{-4}	2.4×10^{-4}
Pentanol	2.3×10^{-3}	1.3×10^{-4}
2-Methylpyrazine	2.0×10^{-4}	5.2×10^{-4}

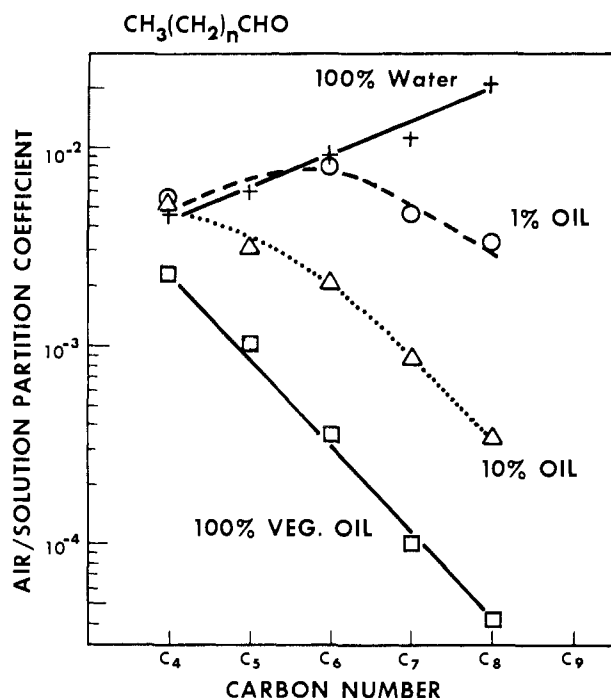


Figure 1. Comparison of air to solution partition coefficients (25°) for aliphatic aldehydes in water (+) and vegetable oil (□) (both solid lines) and for 1% (O) and 10% (Δ) mixtures of oil in water (broken and dotted lines).

be obtained in the following way. The air-to-mixture partition coefficient is given by:

air to vegetable oil-water mixture partition coefficient

$$K_{am} = \frac{\text{(solute concentration in the air)}}{\text{(solute concentration in the mixture)}} \quad (5)$$

If C_a , C_w , and C_{ol} are the concentrations of the solute in the air, water, and oil, respectively, and F_{ol} and F_w are the fraction of oil and water in the mixture, respectively, then the upper part of eq 5 can be replaced with C_a . The lower half of eq 5 is the total weight of solute in the mixture (which equals that in the water phase $C_w \times F_w$ plus that in the oil phase $C_{ol} \times F_{ol}$) divided by the total volume, $F_w + F_{ol}$, which is equal to 1. Equation 5 then leads to

$$K_{am} = C_a / (C_w \times F_w + C_{ol} \times F_{ol}) \quad (6)$$

Table II. Air to Mixture Partition Coefficients (25°) Determined Experimentally and Calculated for 1% and 10% Mixtures of Vegetable Oil in Water

Compound	Air to mixture partition coefficients	
	Experimental	Calculated
10% vegetable oil		
Butanal	5.0×10^{-3}	4.3×10^{-3}
Hexanal	2.1×10^{-3}	2.6×10^{-3}
Heptanal	8.4×10^{-4}	9.3×10^{-4}
Octanal	3.4×10^{-4}	3.9×10^{-4}
1% vegetable oil		
Butanal	5.3×10^{-3}	4.7×10^{-3}
Hexanal	7.1×10^{-3}	7.0×10^{-3}
Heptanal	4.6×10^{-3}	3.9×10^{-3}
Octanal	3.2×10^{-3}	3.4×10^{-3}

Dividing both top and bottom lines by C_a gives us eq 7.

$$K_{am} = \frac{1}{\frac{C_w \times F_w}{C_a} + \frac{C_{ol} \times F_{ol}}{C_a}} \quad (7)$$

We can call the air-to-water partition coefficient (C_a/C_w) K_w , and we can call the air-to-oil partition coefficient (C_a/C_{ol}) K_{ol} . Equation 7 is then simplified to

$$K_{am} = \frac{1}{F_w/K_w + F_{ol}/K_{ol}} \quad (8)$$

Using eq 8, if we know the air-to-water partition coefficient, the air-to-oil partition coefficient, and the fraction or percentage of oil, we can calculate the air-to-mixture partition coefficient quite readily.

Table II lists some calculated air to vegetable oil-water mixture partition coefficients for 1 and 10% mixtures of oil in water. Also listed in Table II are the experimentally determined values for such mixtures. It can be seen that the experimental and calculated figures agree quite closely. It is interesting to compare the values obtained for 1 and 10% vegetable oil-water mixtures for the homologous aliphatic aldehydes with the values for pure water and pure vegetable oil. This is shown in Figure 1. It can be seen that even 1% vegetable oil in water can affect the equilibrium quite markedly. The effect is more and more noticeable as we go to the higher homologs.

A practical system that might be compared to our model system is the effect of various odorants in coffee. A cup of black coffee would be expected to be more favorable for detection of the aroma of the higher aliphatic aldehydes (and related compounds) than coffee that has had cream added to it. The effect on pyrazines would be expected to be less than that for aliphatic aldehydes because the difference between the air to solution partition coefficients for pyrazine compounds in vegetable oil and water is not nearly as great as it is with aliphatic aldehydes.

Odor Thresholds. We have determined odor thresholds for many aroma compounds in both water and vegetable oil solutions. The solution is placed in a teflon container similar to that shown in Figure 2. An equilibrium is established in the teflon bottle between the solution and the atmosphere above the solution. By gently squeezing the bottle, the vapor above the solution is delivered to the ol-

Table III. Threshold Values Calculated for Vegetable Oil Compared with Experimental Values

Compound	Odor threshold in vegetable oil in parts per 10 ⁹	
	Calculated	Experimental
Hexanal	75 ^a	120
Heptanal	350 ^a	250
Nonanal	1500 ^a	1000
Hex-2-enal	210 ^a	850
Hept-2-enal	220 ^b	1500
Oct-2-enal	300 ^b	500
Non-2-enal	47 ^b	150
Dec-2-enal	540 ^b	2100
Deca-2,4-dienal	340 ^b	135
Pent-1-en-3-one	1.3 ^b	5.5
2,5-Dimethylpyrazine	3000 ^b	2600
2-Ethyl-5-methylpyrazine	600 ^b	320
2,5-Diethylpyrazine	800 ^b	270
2-Ethyl-3,6-dimethylpyrazine	16 ^b	24

^a Calculated using experimental K_{ol} and K_w values. ^b Calculated from experimental K_w values and calculated K_{ol} values assuming $\gamma = 1$.

factory senses with minimum dilution from outside air. For reliable threshold determinations, background odors must be consistently kept to a minimum. Extraneous odors are more easily removed from water than from most other liquids. Vegetable oil is more difficult to purify because of the oxidative deterioration which is always going on in the presence of air. We have used refined high-oleic safflower oil (*e.g.*, Pacific Vegetable Oil Corp., Oleinate 181) for odor thresholds because it is relatively free from background odors. Threshold measurements are designed to determine the concentration of the odorous compound in the solution at a specified level ($p < 0.01$). Obviously the human subjects are detecting the compound at its equilibrium concentration in the atmosphere above the solution. The air threshold (T_a) can be readily calculated from the threshold concentration in water solution as follows.

$$(\text{threshold in air}) T_a = T_w \times K_w \quad (9)$$

where T_w is the threshold concentration found for water and K_w is the air-to-water partition coefficient. A similar equation would also hold for oil solutions. For oil solutions we can call T_{oi} the threshold concentration in oil and K_{oi} the air-to-oil partition coefficient. Assuming no background odors, T_a should be the same for the same compound for both oil and water.

$$\text{i.e., } T_a = T_w \times K_w = T_{oi} \times K_{oi} \quad (10)$$

hence

$$T_{oi} = T_w \times K_w / K_{oi} \quad (11)$$

We can then use eq 11 to calculate odor thresholds for vegetable oil solutions if we already know the water thresholds and partition coefficients K_w and K_{oi} . It should be noted that the ratio K_w/K_{oi} is equal to the oil-to-water liquid-liquid partition coefficient which may be more available than either K_w or K_{oi} (Nelson and Hoff, 1968).

This calculation could be useful because many more thresholds are known for water solutions than are known for oil solutions. Thresholds are also difficult to measure in vegetable oil, partly because of the difficulty in getting a vegetable oil completely free of background odor. Although it is of less practical use, it may be noted that eq 11 could be used in the reverse way to calculate T_w values when T_{oi} values are known.

Table III compares calculated thresholds using eq 11 with those found by an established panel method (Guadagni *et al.*, 1963). Except for the aliphatic aldehydes, K_{oi} values were also calculated using eq 2 and 3, assuming that the activity coefficient γ was equal to 1 and obtaining vapor pressure values from the literature. The calculated and found threshold values for saturated aliphatic aldehydes are reasonably close. The somewhat higher figures for the found values in the case of the 2-enals may reflect the closeness of their odor characters to that of the natural background of the oil. Other differences may be due to some deviations from ideal behavior in the oil and

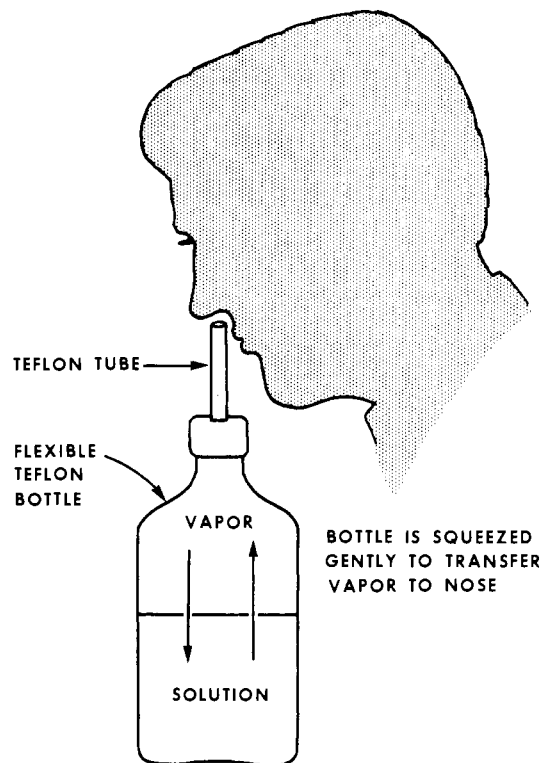


Figure 2. Container used to evaluate odor thresholds.

also to inaccuracies in vapor pressure values in the literature.

Most of the experimental values shown in Table III are significantly different from the calculated values ($p < 0.5$). However, all of the calculated figures are within a factor of 10 of the experimental values. Considering that odor thresholds for different compounds can range over values differing by a factor of 10^9 and that values for the same materials determined in different laboratories also frequently differ by a factor of 10 or greater, the calculated values represent a fairly reasonable estimate of threshold concentration in vegetable oil.

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