Partition Coefficients for Acetates in Food Systems

Theo G. Kieckbusch[1] and C. Judson King*

Flame-ionization gas chromatography was used to determine equilibrium partition coefficients for C_1–C_5 acetates at high dilution between air and water, aqueous solutions of various carbohydrates, vegetable oils, and mineral oil. A modified sampling and injection technique was used to overcome sorption effects for vapor samples. Measurements were made over a range of temperatures from 25–50 °C. Partition coefficients between air and solutions of sucrose, maltose, and dextran (M_r 90 000) increased sharply with increasing dissolved-solids content. For the disaccharide solutions this could be attributed qualitatively to a loss of free water due to hydration of sugar molecules. For solutions of maltodextrin, dextrin, and coffee solids, the acetates were held into solution more at the higher dissolved-solids contents, and the partition coefficient for pentyl acetate actually decreased with increasing concentration of dissolved solids. Partition coefficients between air and the oils were much lower and indicated an activity coefficient of about 0.7 for the acetates in coffee and peanut oils.

The natural volatile components (aroma) of most foods are present in such dilute concentrations that they tend not to interact with one another. This corresponds to the ideal state of infinite dilution, and Henry's law is then valid; the partial pressure of the solute in the equilibrium vapor above the solution is directly proportional to the solute concentration in the liquid. Under such conditions, a convenient partition coefficient (K_{AL}) can be defined simply as the ratio of concentration of the solute in the vapor phase (C_{VA}) to its concentration in the liquid (aqueous) phase (C_{VL}):

$$K_{AL} = C_{VA}/C_{VL} \quad (1)$$

K_{AL} will be denoted as K_{AW} for partitioning of a solute between air and otherwise pure water.

Due to the complex nature of food products, most of the published equilibrium data for volatile components are limited to simplified food models. A systematic study of partition coefficients for various volatile organic compounds between air and water was made by Buttery et al. (1969, 1971). Later they extended this work to include a vegetable oil phase (Buttery et al., 1973). The effect of dissolved solids on the partition coefficient has also received some attention (Massaldi and King, 1973; Maier, 1970; Chandrasekaran and King, 1972). Other data on the effects of a second liquid phase have also been published (Radford et al., 1974; Massaldi and King, 1974).

The work reported here concerns partition coefficients of acetates in water, food, and nonfood systems at various temperatures.

EXPERIMENTAL TECHNIQUE AND WORKING EQUATIONS

A Perkin-Elmer Model 3920 flame-ionization gas chromatograph was used, with Porapak Q as column packing material.

The equilibrium cells consisted of 120-mL glass bottles equipped with a magnetic bar. The acetate solutions were prepared individually, weighed directly into the bottles. The bottles were sealed with a thick stopper made of Teflon with a small perforation necessary for sampling. They were placed in a water bath with the temperature kept within ±0.01 °C. Solutions typically contained 400 ppm of the acetate.

Department of Chemical Engineering, University of California, Berkeley, California 94720.

[1]Present address: Universidade Estadual de Campinas, Brazil.

In principle, the air–water partition coefficients, K_{AW}, can be readily determined using gas–liquid chromatography with consecutive injections of gas and liquid samples. However, the measurements are strongly affected by sorption phenomena occurring during sampling and injection of the gas phase. These effects are particularly intense with higher-molecular-weight solutes, as pointed out by Buttery et al. (1969). To minimize this interference a modified chromatographic sampling and injection procedure was set up, and the air–water partition coefficients, K_{AW}, of the acetates were carefully measured. Details of equipment alterations and measurement technique are given by Kieckbusch and King (1979).

For the remaining systems, with liquids other than pure water, the partition coefficients can be determined as a function of K_{AW}, and injections of only the gas phase are required. This is advantageous since the presence of nonvolatile solutes may foul the GC equipment. Furthermore, when a clean syringe is used, the sorption phenomena for vapor samples appear to be reproducible, and a linear response can be obtained for solute concentrations up to half of the saturation level (Kieckbusch, 1978; Kieckbusch and King, 1979). This facilitated the measurements, and, once the air–water partition coefficients had been determined using the modified technique, a simple syringe (Precision Scientific, Series 1000, 1 mL) was used for subsequent vapor samples. For safer handling, the syringe was locked inside a mantle made of Plexiglas. Water, at a temperature slightly higher than the equilibrium-bath temperature, was circulated through this mantle, but the gas circulation described elsewhere (Kieckbusch and King, 1979) was not used.

The partition coefficients for the solutions were calculated by comparing the head-space response with the corresponding response for an air–water system. The equations to be used can be developed from a mass balance. If a mass, m_{VL}, of a volatile component is added to a bottle containing volumes L of solution and A_L of air, it will partition at equilibrium between the phases according to the following:

$$m_{VL} = C_{VA_L}[A_L + (L/K_{AL})] \quad (2)$$

where C_{VA_L} is the air-phase concentration of volatile component and K_{AL} is the partition coefficient between air and the liquid solution in mass/volume (eq 1). By the same token, a mass m_{VW} will partition between a volume of water, W, and a volume of air, A_W:

$$m_{VW} = C_{VA_W}[A_W + (W/K_{AW})] \quad (3)$$

where C_{VA_W} is the equilibrium concentration of the volatile

0021-8561/79/1427-0504$01.00/0

Table I. Partition Coefficients of Acetates in Sucrose Solutions at Different Temperatures and Concentrations, $K_{AL} \times 10^3$

% sucrose	25 °C	30 °C	35 °C	40 °C
		Methyl Acetate		
0	5.27	6.72	8.72	
20	6.96	8.76	11.0	
40	10.6	12.7	16.5	
60	20.6	25.3	30.5	
		Ethyl Acetate		
0	6.94	9.2		15.2
20	9.24	12.0		19.2
30	11.3			
40	13.8	17.7		27.7
50	18.0			
60	23.6	30.3		45.3
		Propyl Acetate		
0	8.91	11.9		20.6
20	11.9	16.1		26.7
30	13.9			
40	17.4	22.8		37.2
50	21.0			
60	28.6	31.4		55.9
		Butyl Acetate		
0	11.5	16.1		28.5
20	15.1	20.9		35.8
30	17.7			
40	21.5	28.4		47.1
50	25.6			
60	34.5	45.4		72.2
		Pentyl Acetate		
0	14.5	20.9		39.4
20	19.0	26.8		49.3
30	22.1			
40	26.3	36.5		61.5
50	31.5			
60	39.2	52.5		89.7

component in the air phase and K_{AW} is the air–water partition coefficient. Taking the ratio between eq 2 and 3 and noticing that the gas-phase concentration is proportional to the chromatograph response, one arrives at:

$$K_{AL} = \frac{L}{(R_W/R_L)(m_{VL}/m_{VW})[A_W + (W/K_{AW})] - A_L} \quad (4)$$

where R_S/R_L is the ratio of the chromatograph responses of gas samples taken from vapor phases in equilibrium with the water phase and with the solution.

If the liquid-phase depletion of the volatile component necessary for equilibration with the air phase is neglected, a simplified equation results:

$$K_{AL} = L\left(\frac{R_L}{R_W}\right)\left(\frac{m_{VW}}{m_{VL}}\right)\left(\frac{K_{AW}}{W}\right) \quad (5)$$

Equation 5 was used in the present investigation since the error introduced can be neglected. This is a result of the low partition coefficients found for the acetates and the comparative procedure used.

For runs at different temperatures, the responses were compared to the response of the same bottle at 25 °C. The expression for K_{AL_t}, i.e., the partition coefficient at a temperature t, can also be derived by mass balance. The final expression is:

$$K_{AL_t} = \frac{L}{\left[\frac{R_{25}}{R_t}A + \frac{R_{25}}{R_t}\frac{L}{K_{AL_{25}}} - A\left(\frac{273 + t}{298}\right)\right]} \quad (6)$$

The measurements were made with a cyclic variation of the temperature levels. The ratio R_{25}/R_t is the average between values for the same temperature measured during the rising and the falling periods. Typically, these differed by no more than 2%.

Table II. Partition Coefficients for Ethyl and Pentyl Acetates in Sucrose Solution at 25 °C, Converted to Free-Water Basis

		$K_{AWf} \times 10^3$	
% sucrose	hydration no.	ethyl acetate	pentyl acetate
0	6.72	6.94	14.5
20	5.80	7.38	15.2
40	4.96	8.05	15.3
60	4.02	8.28	13.8

EXPERIMENTAL RESULTS

Sucrose Solutions. The partition coefficients measured for the five lightest acetates at different sucrose concentrations and different temperatures are displayed in Table I. A sharp increase of K_{AL} with increasing sucrose concentration can be observed. Similar trends for other esters have been found by Chandrasekaran and King (1972) and Massaldi and King (1973). Some investigators (e.g., Nawar, 1971) have found a milder variation in head-space concentration of volatile species with the addition of sugars. Their result, however, is a consequence of a different approach used. Nawar compared the head-space contents of bottles with the same water and volatile contents. That procedure gives, in fact, a closer estimate of the effective interaction between the molecules of sugar and of volatile species. The apparent effect of sucrose can be better appreciated if K_{AL} is converted to K_{AWf}, a fictitious partition coefficient which assumes that only the free water acts as solvent for the acetate. This is a very simplistic approach to the theory of solutions, suggested by Glasstone and Pound (1925). Data on degree of hydration were taken from Schliephake (1963) since he included corrections due to additional structural water retained by the clustering of sucrose molecules at concentrations above 40%. The volume of free water, W_f, to be used instead of L for determining K_{AWf} from eq 5 is:

$$W_f = (L\rho_L/\rho_W)(1 - w) \quad (7)$$

where w is the mass fraction of the solution that corresponds to the sucrose plus the hydration water.

Table II gives the hydration number and K_{AWf} calculated for several sucrose concentrations at 25 °C. These corrected partition coefficients remain much more nearly constant, suggesting that sucrose hydrates exclude the acetates. Low affinity of simple sugars for acetates in solution can be expected since binding can occur only through loose hydrogen bonds, in which case the acetate has to compete with water.

Maltodextrin Solutions. The partition coefficients obtained with Mor-rex 1917 (CPC International) solutions are given in Table III. Mor-rex 1917 is a partially hydrolyzed corn starch (maltodextrin) with D.E. of 9–12. The effect of the maltodextrin content on the partition coefficient is rather complex, exhibiting different trends for different acetates. For ethyl acetate, the increase in K_{AL} with increasing maltodextrin concentration is slightly less than with the equivalent sucrose concentration. As the molecular weight of the acetate increases, the stronger is its affinity to the liquid phase at higher maltodextrin contents. For pentyl acetate the trend reverses, and the presence of maltodextrin solids depresses the partition coefficient.

Coffee Extract Solutions. Roasted ground Brazil-Santos coffee (purchased at a local supermarket) was

Table III. Partition Coefficient of Acetates in Maltodextrin Solutions at Different Temperatures and Concentrations, $K_{AL} \times 10^3$

weight %	25 °C	30 °C	45 °C
	Ethyl Acetate		
0	6.94		
15.7	7.23	12.0	19.5
19.0	7.98		
23.0	9.46	15.9	24.8
38.0	10.9		
47.8	13.2	21.1	32.3
	Propyl Acetate		
0	8.91		
15.7	10.2	17.5	29.0
19.0	10.2		
23.9	11.2	19.1	30.6
38.0	12.6		
47.8	13.8	22.6	35.1
	Butyl Acetate		
0	11.5		
15.7	12.3	21.2	35.4
19.0	12.5		
23.9	12.6	21.2	34.4
38.0	13.0		
47.8	13.0	21.4	34.0
	Pentyl Acetate		
0	14.5		
15.7	14.1	23.5	37.3
19.0	13.4		
23.9	14.0	21.5	34.5
38.0	13.0		
47.8	11.5	18.2	29.0

Table IV. Partition Coefficient of Acetates in Brazil-Santos Coffee Extract at Different Temperatures and Concentrations, $K_{AL} \times 10^3$

weight %	25 °C	30 °C	40 °C
	Propyl Acetate		
0	8.9		
17.0	11.4	15.5	26.3
40.7	16.3	21.5	35.4
	Butyl Acetate		
0	11.5		
17.0	13.4	18.6	32.8
40.7	16.1	21.2	37.2
	Pentyl Acetate		
0	14.5		
17.0	13.8	19.3	35.6
40.7	12.7	17.0	31.1

submitted to countercurrent three-stage, batch extraction with boiling water at ambient pressure. The extract was filtered through glass wool and evaporated to almost complete dryness in a vacuum oven at 90 °C. Solutions were prepared, and the pH was increased from about 4.5 to 6.5 using concentrated sodium hydroxide solution. The solutions were then centrifuged.

An instant spray-dried coffee was treated with petroleum ether in a Soxhlet extractor for over 12 h in order to extract any added oil phase. The solids were dried in a vacuum oven at 60 °C. Solutions were prepared and the pH increased to 6.5 with concentrated sodium hydroxide solution.

Results of partition coefficient determinations at different temperatures are given in Table IV and Table V. The behaviors of the different acetates follow the same trend as found with maltodextrin solutions, although in a more pronounced way. A small quantity of coffee oil (about 0.3%, dry basis) could exert a similar influence. It is doubtful, however, that such an amount could still be present in the solids, especially for the instant coffee, which exhibited the strongest volatility depression. It is reasonable to speculate that long-chain carbohydrates are

Table V. Partition Coefficient of Acetates in Commercial Instant Coffee Solutions at Different Temperatures and Concentrations, $K_{AL} \times 10^3$

wt %	25%	30 °C	40 °C
	Propyl Acetate		
0	8.9		
20	11.1	14.9	25.0
40	13.8	18.1	26.9
50	14.2	17.8	26.6
	Butyl Acetate		
0	11.5		
20	12.6	16.5	25.8
40	13.7	17.7	32.0
50	12.7	16.1	25.8
	Pentyl Acetate		
0	14.5		
20	13.7	18.6	23.7
40	11.6	15.8	34.0
50	9.8	12.5	20.6

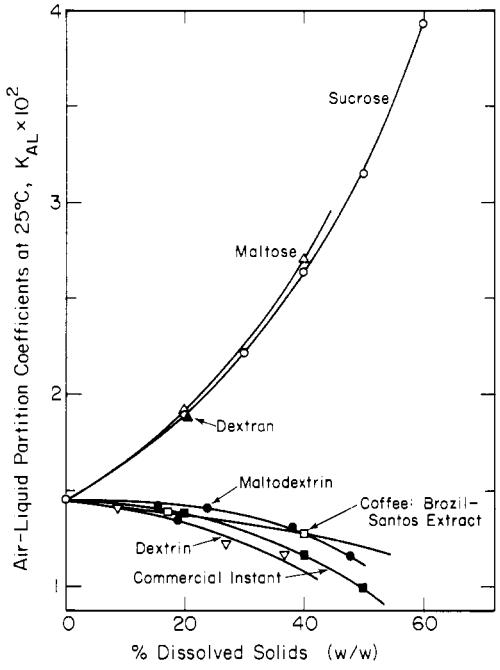

Figure 1. Partition coefficients of pentyl acetate as influenced by content of different solutes.

responsible for the observed behavior, in line with the results for maltodextrin. The difference in values of K_{AL} for the two coffees may come from more severe extraction conditions, and consequent higher solubilization of cellulosic material, for the commercial instant product.

Other Polysaccharides. Partition coefficients for acetates between air and solutions of dextran (nominal average M_r = 90 000), maltose, and dextrin were also determined at 25 °C. The effects of maltose and dextran were nearly identical with that of sucrose. The dextrin solutions exhibit a behavior similar to the maltodextrin solutions. Results for K_{AL} of pentyl acetate are plotted in Figure 1. Tabulated values for the other acetates are given elsewhere (Kieckbusch, 1978). The behavior with dextran solutions differs markedly from that for solutions of other high-molecular-weight carbohydrates.

Oils. Partition coefficients for acetates between air and oil, K_{AO}, were determined following a similar procedure to that used with aqueous solutions. Coffee oil (obtained from Folger Coffee Co., South San Francisco, CA), peanut oil (purchased at a local supermarker), and commercial mineral oil were used. The coffee oil was deodorized by stripping it with nitrogen, under vacuum, for 48 h; it was

Table VI. Partition Coefficients of Acetates in Oil, at Different Temperatures, $K_{AO} \times 10^4$

acetates	25 °C	30 °C	35 °C	50 °C
		Coffee Oil		
butyl	3.11		5.30	10.68
pentyl	1.10		2.20	5.22
hexyl	0.39	0.59	0.89	
isopentyl	1.98	2.70	3.59	8.09
		Peanut Oil		
isopentyl	2.01		3.71	8.42
		Mineral Oil		
isopentyl	3.50		6.33	13.2

also kept refrigerated. Results are given in Table VI.

The activity coefficients of the acetates in coffee oil, assuming a molecular weight of 885.4 which corresponds to triolein, are about 0.7. This indicates a strong affinity between the host and solute molecules. Similar behavior was also found for aldehydes by Buttery et al. (1973). Mineral oil shows less affinity for the ester since it is a mixture of hydrocarbons.

Partition coefficients between oil and an aqueous phase for the acetates can be computed as $K_{OL} = K_{AL}/K_{AO}$.

LITERATURE CITED

Buttery, R. G., Ling, L. C., Guadagni, D. G., *J. Agric. Food Chem.* **17**, 385 (1969).
Buttery, R. G., Bomben, J. L., Guadagni, D. G., Ling, L. C., *J. Agric. Food Chem.* **19**, 1045 (1971).
Buttery, R. G., Guadagni, D. G., Ling, L. C., *J. Agric. Food Chem.* **21**, 198 (1973).
Chandrasekaran, S. K., King, C. J., *AIChE J.* **18**, 513 (1972).
Glasstone, S., Pound, A., *J. Chem. Soc.* **127**, 2660 (1925).
Kieckbusch, T. G., PhD Dissertation, University of California, Berkeley, 1978.
Kieckbusch, T. G., King, C. J., *J. Chromatogr. Sci.*, in press (1979).
Maier, H. G., *Angew. Chem. Int. Ed. Engl.* **9**, 917 (1970).
Massaldi, H. A., King, C. J., *J. Chem. Eng. Data* **18**, 393 (1973).
Massaldi, H. A. King, C. J., *J. Food Sci.* **39**, 434 (1974).
Nawar, W. W., *J. Agric. Food Chem.* **19**, 1057 (1971).
Radford, T., Kawashima, K., Friedel, P. K., Pope, L. E., Gianturco, M. A., *J. Agric. Food Chem.* **22**, 1066 (1974).
Schliephake, D., *Zucker* **16**, 523 (1963).

Received for review August 10, 1978. Accepted January 2, 1979. This work was supported, in part, by Grant No. ENG76-17270 from the National Science Foundation, as well as by a grant from General Foods Corporation. One of the authors (T.G.K.) received financial aid from the Conselho Nacional de Desenvolvimento Cientifico e Technologico, Proc. 3671/73, Brasil.

Racemization of Amino Acids in Alkali-Treated Food Proteins

Patricia M. Masters and Mendel Friedman*

Casein, lactalbumin, Promine-D (soy protein), and wheat gluten exhibit significant racemization of aspartic and glutamic acids (or their amides), phenylalanine, and alanine when subjected to 0.1 N NaOH at 65 °C for 3 h. In order of lability to racemization, the proteins are Promine-D > casein > wheat gluten > lactalbumin. While racemization rates of individual amino acids vary among proteins, in any given protein, the relative rates are similar. The pH kinetics of aspartic acid racemization in casein indicate that k_{asp} is first order with respect to hydroxide concentration above pH 10. Racemization can impair nutritional values of food proteins by decreasing the amounts of the essential amino acid L enantiomers present, by decreasing digestibility, and as a result of specific toxicity of certain D enantiomers.

Commercial processing of food proteins often entails heating in alkaline solutions. Such treatments are intended to alter flavor and texture, destroy microorganisms, enzymes, toxins, or proteolytic inhibitors, and prepare protein concentrates. Undesirable changes also occur in the amino acid composition of proteins under such processing conditions. For instance, amino acid cross-linking (Provansal et al., 1975; Friedman, 1977, 1978; Hurrell and Carpenter, 1977), degradation (Asquith and Otterburn, 1977), and racemization (Provansal et al., 1975; Tannenbaum et al., 1970) have been reported.

The ability of strong alkali to racemize amino acids has been demonstrated in early chemical studies on proteins (Dakin, 1912–1913; Levene and Bass, 1927, 1928, 1929). The reaction is thought to proceed by abstraction of the α proton from an amino acid or amino acid residue in a peptide or protein to give a negatively charged planar carbanion (Neuberger, 1948). A proton can then be added back to either side of this optically inactive intermediate, thus regenerating the L form or producing the D enantiomer. The reaction can be written as

$$\text{L-amino acid} \underset{k'}{\overset{k}{\rightleftharpoons}} \text{D-amino acid} \qquad (1)$$

where k and k' are the first-order rate constants for interconversion of the L and D enantiomers. For the amino acids discussed in this paper, $k = k'$. (Amino acids with two asymmetric centers exhibit k' slightly different from k.) Only L-amino acids are initially present in most proteins due to the stereospecificity of biosynthesis.

Provansal et al. (1975) have studied lysine racemization in sunflower protein under varying conditions of heat and hydroxide concentration. Enzymatic enantiomeric analysis disclosed measurable amounts of D-lysine in solutions in NaOH more concentrated than 0.2 N NaOH heated at 80 °C. Using microbiological assays, Tannenbaum et al. (1970) found methionine to be nearly completely racemized in fish protein concentrate heated 20 min at 95 °C in 0.2 N NaOH.

The present study uses quantitative chromatographic methods to measure the extent of racemization of seven amino acid residues from proteins subjected to relatively mild alkaline treatments. We have determined D/L en-

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093 (P.M.M.), and the Western Regional Research Center, Science Education Administration, U.S. Department of Agriculture, Berkeley, California 94710 (M.F.).

0021-8561/79/1427-0507$01.00/0