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Partitioning Behavior of Alkan-1-ols between Milkfat and Aqueous Phases As Influenced by Temperature

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Partitioning of volatile compounds between lipid and aqueous phases may influence flavor perception and availability to participate in flavor-generating reactions. The objective of this research was to characterize the partitioning of short-chain alkan-1-ols between milkfat and aqueous phases, as influenced by temperature, as compared to an octan-1-ol/water biphasic system (Log *P*). Temperature has a positive, but nonlinear, influence on Log *P* values for alkan-1-ols. There is an approximately 1 log decrease in Log *P* values of milkfat/water as compared to octan-1-ol/water systems; similar trends were observed across chain length. Temperature has a greater effect on alkan-1-ol partitioning in milkfat/water systems than octan-1-ol/water. The latter observation is primarily attributed to the solidification of milkfat at temperatures below 40 °C and the resulting reduction in liquid lipid solvent volume.

KEYWORDS: Volatile; cheese; Log *P*; temperature; aroma; partitioning

INTRODUCTION

Cheese flavor results from reactions and interactions of constituents within the cheese matrix during the manufacturing and aging process. The extent of these reactions is influenced by the presence of and accessibility to corresponding chemical substrate(s). The control of flavor-generating reactions may reside with an improved understanding of substrate availability. In multiphase foods, such as cheese, substrate availability is in part dependent on the partitioning properties between lipid and aqueous phases. In organic synthesis, Janssen et al. (*1*) demonstrated selective, predictable formation of mono-, di-, or triacylglyceride based on changes in phase polarity and relative volume. Understanding the factors that influence partitioning properties in a milkfat-containing system may aid in the control of important flavor-generating pathways for dairy foods such as cheese.

The fundamentals of phase affinity is often studied in vapor−liquid partitioning experiments. Lebert and Richon (*2*) extended this research to a food system, by determining activity coefficients of alkan-1-ols and alkanes in olive oil, over a range of temperatures. Distribution of compounds in biphasic liquid systems is commonly predicted by octan-1-ol/water partition coefficients (Log *P*). The topic of partitioning has been thoroughly reviewed by Leo et al. (*3*) and Sangster (*4*, *5*). Log *P* values can also be estimated by the addition of group activity coefficients with various corrective factors for certain molecular structures (*6*). A vast amount of standard Log *P* data is available; however, the conditions to determine Log *P* greatly deviate from those of an actual food matrix (*7*). Partitioning in milkfat-containing systems is further complicated by the presence of partially solid lipid phase at temperatures below 40 °C (*8*). Roberts et al. (*9*) suggest that the solid portion of milkfat may entrap volatiles, but does not absorb volatiles once in a solid state; larger proportions of solid fat resulted in greater flavor release, due to proportionately less liquid fat solvent.

Although octan-1-ol/water Log *P* values provide relative partitioning tendencies, thorough experimentation is needed to accurately determine partitioning behavior in more realistic food systems. There is a need to improve our understanding of factors that may influence cheese flavor development (*10*). Understanding the role of partitioning and substrate availability is a necessary component in manipulating cheese flavor development. Similar to the effects that relative phase volume has on flavor release and perceived flavor, partitioning properties are hypothesized to also affect the extent of flavor-generating reactions (e.g., oxidation, reduction, esterification, hydrolysis).

To better determine the partitioning properties of volatiles in a milkfat-containing system, a biphasic model system was developed using milkfat and water as the solvent phases. Partitioning properties were determined over a range of temperatures typical of cheese processing and storage. The objective of this research was to characterize alkan-1-ol partitioning between milkfat and aqueous phases, as influenced by temperature, as compared to a conventional octan-1-ol/water system. Alkan-1-ols were selected for their relative chemical stability and involvement as ester formation substrate. Findings from this study may be combined with existing partitioning data and principles to improve understanding of substrate availability to flavor-generating reactions in milkfat-containing systems.

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MATERIALS AND METHODS

Partitioning between Octan-1-ol and Water. Partitioning in octan-1-ol/water systems was conducted by the shake-flask method, according to Leo et al. (*3*). Three test vials were prepared at 0, 10, 20, 30, 40, 50, and 60 °C. Each phase was presaturated with its complementary phase prior to partitioning experiments. Water (HPLC grade, Fisher Scientific Co., Pittsburgh, PA) was combined with octan-1-ol (99%+ HPLC grade, Sigma-Aldrich Co., St. Louis, MO) in a separatory funnel. The funnel was inverted repeatedly for 2 min and then allowed to separate gravimetrically, overnight, at each experimental temperature (Fisher Scientific Co.). Ethanol, propan-1-ol, butan-1-ol, pentan-1-ol, hexan-1-ol, and heptan-1-ol (all 99%+, Sigma-Aldrich Co.) were dissolved in water or octan-1-ol to form a standard solution for partitioning experiments. Solutions were prepared at low concentrations (<10 mM) to minimize deviations from an ideal solution.

Partitioning studies were conducted in 20 and 40 mL (actual capacity ~23 and ~43 mL, respectively) glass vials with Teflon-lined/silicone septa caps (National Scientific Co., Duluth, GA). A solution containing a dissolved alkan-1-ol was combined with the complementing solvent phase, followed by mixing for 2 min of gentle repeated inversion by hand. The selected phase volumes kept headspace to an absolute minimum. Phases were allowed to separate in a refrigerated incubator.

Partitioning between Milkfat and Water. The lipid phase of the model system was anhydrous milkfat (Grassland Dairy Products, Inc., Greenwood, WI), which consists of 99.8% milkfat, 0.1% solids nonfat, and 0.1% water. This composition is virtually the same as the center of a milkfat globule (*11*).

Milkfat partitioning tests were conducted at 10, 20, 30, 40, 50, and 60 °C. At each temperature, three or more test vials (spanning an ~10-fold concentration range) were prepared by dissolving alkan-1-ols into water, which was presaturated with milkfat and held at the study temperature. An additional three or more test vials were prepared by dissolving the alkan-1-ols in milkfat. Before milkfat and aqueous phases were combined, liquid milkfat held at 50 °C was pipetted into glass vials and held at the final partitioning temperature for at least 16 h. This allowed the milkfat to temper to the final temperature prior to the addition of the aqueous phase. Achieving equilibrium conditions was accelerated by stirring with a 2.5 cm magnetic stir bar (Fisher Scientific Co.). Vials at 40−60 °C were stirred for 1 h, those at 20−30 °C for 4 h, and those at 10 °C for 96 h. After mixing, the vial was set to allow for phase separation for 1 h in an incubator at each experimental temperature.

To improve the quantitative precision at lower experimental temperatures, the initial concentration of alkan-1-ols in the partition vials was corrected according to the analyzed percent recovery of a standard solution. The standard solution prepared for partitioning was divided between the partition vial and a standard vial. The standard vial did not contain any milkfat, but was otherwise prepared and analyzed in exactly the same way as the partition vials. Side-by-side analysis removed errors associated with day-to-day variations in the analysis.

Equilibration was evaluated by analysis of a sample over time until plotted milkfat and water partitioning measurements (Log K_{MW}) achieved an asymptotic equilibrium. In a two-phase system, equilibrium is established when there is no net transfer of analyte from one phase to the other. Because the initial location of the solute is irrelevant to equilibrium concentrations, dissolution in either phase should achieve the same Log K_{MW}. Dual experiments were conducted by dissolving the alkan-1-ol in water in one set and in milkfat in the other set of experiments and then measuring after mixing treatments to verify that the same Log K_{MW} was found regardless of the initial dissolving phase.

Quantitation of Solute Concentration. Aqueous phase samples (100 μL) were drawn from partitioning vials and combined with an equal volume of internal standard (isoamyl alcohol solution). To limit the volume of water introduced onto the column and improve resolution, 1500 μL of acetone was also added to samples containing butan-1-ol, pentan-1-ol, hexan-1-ol, or heptan-1-ol. Assays for volatile partitioning experiments were conducted on a gas chromatograph (6890N, Agilent Technologies Inc., Palo Alto, CA) with a mass selective detector (5973N, Agilent Technologies Inc.). One microliter samples in solvent were injected into a split/splitless inlet at 250 °C. Chromatography was performed on a capillary column (Rtx-Wax, 30 m, 0.25 mm i.d., 0.5 μm stationary phase, Restek Inc., Bellefonte, PA). The carrier gas was ultrahigh-purity helium gas (99.999% pure, Praxair, Inc., Danbury, CT). The mass spectrometer operating conditions used were an ion source temperature of 230 °C, an ionization voltage of 70 eV, and a scan range of m/z 21−350 at 2.76 scans s^{-1}.

For samples containing butan-1-ol, pentan-1-ol, hexan-1-ol, and heptan-1-ol, the inlet was employed in the splitless mode, carrier flow was set to 1 mL min^{-1}, and the oven temperature was programmed from 40 to 100 °C at 5 °C min^{-1} and from 100 to 200 °C at 20 °C min^{-1}, with no hold times. Ethanol and propan-1-ol samples were injected into a 35:1 split ratio. Carrier flow was 1.2 mL min^{-1}, and the column temperature program started at 50 °C, with a 10 °C min^{-1} ramp to 100 °C, followed by a 20 °C min^{-1} ramp to 200 °C.

Quantitation was based on a calibration curve prepared from authentic standards at five concentrations (ranges were 0.816−16.3 mM for ethanol, 0.517−10.3 mM for propan-1-ol, 7.79−593 μM for butan-1-ol, 5.68−852 μM for pentan-1-ol, 4.56−684 μM for hexan-1-ol, and 4.11−617 μM for heptan-1-ol). Resulting chromatographic peaks were integrated with selected ion (m/z) signal to minimize background noise. Concentrations were corrected relative to the internal standard to correct for variations in injection volume.

Solid Fat Content. The percentage of solid fat in milkfat was measured by pulsed NMR (Minispec pc120, Bruker Optics, Inc., Karlsruhe, Germany). Prior to analysis, the system was calibrated with 0, 32.3, and 73.2% solid standards (Bruker Optics, Inc.). Samples of milkfat, presaturated with water and held completely liquid at 50 °C, were filled into 10 × 177 mm glass tubes (Corning, Inc., Corning, NY). The tubes were held overnight in a refrigerated incubator at 0, 10, 20, 30, or 40 °C. Solids were determined by pulsed NMR measurements of the milkfat tube according to AOCS official method Cd 16b-93 (*12*), with a modification of the tempering methods to match the conditions of partitioning studies.

RESULTS AND DISCUSSION

Calibration curves were constructed relative to the internal standard, isoamyl alcohol, from a series of five concentrations. Results confirmed a linear response ($R^2 > 0.99$). Partitioning measurements (Log P) in an octan-1-ol and water system were taken to verify methodology. Measured results were comparable to reference measurements [±0.04 of Sangster (*5*)].

Due to difficulties in analysis of the hydrophobic phase, it is not an uncommon practice to analyze only one phase and assume that the other phase can be calculated by difference (*13*−*15*). Calculation by difference assumes that all of the alkan-1-ols are present in either the aqueous or octan-1-ol phase. This does not account for any concentration present at the interface between phases. Analysis of the octan-1-ol phase was conducted in preliminary experiments, and ~100% recovery was verified.

Temperature-Dependent Partitioning of Alkan-1-ols in Octan-1-ol and Water. The alkan-1-ol series, from ethanol to heptan-1-ol, were partitioned in triplicate vials of octan-1-ol and water, at temperatures from 0 to 60 °C. The mean Log P values are listed in **Table 1**. Each methylene (−CH_2−) group added onto the *n*-chain primary alkan-1-ol predictably increased the hydrophobicity of that compound, hence, the Log P value.

All of the alkan-1-ols partitioned more into the octan-1-ol phase with increasing temperatures ($P < 0.01$). This is in agreement with empirical observations that the solubility of many aliphatic compounds in water decreases with temperature (*3*). The nature of this correlation may be due to the hydrophobic effect of dissolved apolar groups in water (*16*). According to Leo et al. (*3*), mixtures of alkan-1-ols and water increased free energy (+ΔG) despite exothermic conditions (−ΔH), indicating a larger loss in entropy (−ΔS). Water is hypothesized to arrange into flickering "ice-like" clusters in the presence of an apolar solute group. This more orderly structuring (−ΔS) is partially

Table 1. Log *P* Partition Coefficients of Alkan-1-ols between Octan-1-ol and Water As Influenced by Temperature

	temperature								
alkan-1-ol	0 °C	10 °C	20 °C	30 °C	40 °C	50 °C	60 °C	P^a	linear regression slope of ΔLog *P* (°C^{-1})
ethanol	–0.483 (0.153)[b]	–0.367 (0.153)	–0.352 (0.153)	–0.336 (0.153)	–0.127 (0.153)	–0.188 (0.153)	–0.125 (0.153)	0.002	0.00592
propan-1-ol	0.103 (0.092)	0.181 (0.092)	0.196 (0.092)	0.265 (0.092)	0.366 (0.039)	0.318 (0.039)	0.371 (0.039)	0.001	0.00446
butan-1-ol	0.692 (0.065)	0.740 (0.065)	0.787 (0.033)	0.842 (0.024)	0.907 (0.028)	0.930 (0.024)	0.873 (0.042)	0.004	0.00373
pentan-1-ol	1.35 (0.03)	1.40 (0.03)	1.44 (0.03)	1.48 (0.03)	1.49 (0.03)	1.52 (0.03)	1.51 (0.03)	0.001	0.00266
hexan-1-ol	2.07 (0.03)	2.13 (0.03)	2.17 (0.03)	2.20 (0.03)	2.21 (0.03)	2.24 (0.03)	2.23 (0.03)	0.001	0.00272
heptan-1-ol	2.55 (0.03)	2.60 (0.03)	2.63 (0.03)	2.66 (0.03)	2.64 (0.03)	2.68 (0.03)	2.68 (0.03)	0.003	0.00193

[a] Probability (*P*) of no correlation between temperature and Log *P*. [b] Ninety-five percent confidence interval is represented by the mean ± the values in parentheses.

Table 2. Log K_{MW} Partition Coefficient of Alkan-1-ols between Milkfat and Water As Influenced by Temperature

	temperature									
alkan-1-ol	10 °C (W)[a]	10 °C (MF)[b]	20 °C	30 °C	40 °C	50 °C	60 °C	P^c	ΔLog K_{MW} (°C^{-1}) based on entire fat volume	ΔLog K_{MW} (°C^{-1}) corrected to liquid fat volume
ethanol	–1.68 (0.64)[d]	*e*	–1.07 (0.12)	–1.48 (0.23)	–1.14 (0.12)	–1.25 (0.12)	–1.13 (0.06)	0.238	0.00729	0.00375
propan-1-ol	–1.06 (0.40)	*e*	–0.742 (0.046)	–0.788 (0.053)	–0.625 (0.043)	–0.547 (0.040)	–0.498 (0.015)	0.007	0.0102	0.00665
butan-1-ol	–0.655 (0.046)	–0.338 (0.089)	–0.347 (0.013)	–0.102 (0.051)	–0.109 (0.022)	0.0238 (0.0577)	0.0381 (0.0290)	0.009	0.0131	0.00955
pentan-1-ol	0.101 (0.026)	0.215 (0.109)	0.344 (0.043)	0.384 (0.038)	0.543 (0.037)	0.586 (0.012)	0.645 (0.017)	0.002	0.0103	0.00679
hexan-1-ol	0.691 (0.024)	0.828 (0.042)	0.933 (0.034)	0.985 (0.041)	1.11 (0.02)	1.17 (0.01)	1.21 (0.02)	0.003	0.00980	0.00621
heptan-1-ol	1.32 (0.03)	1.40 (0.01)	1.54 (0.02)	1.58 (0.02)	1.63 (0.03)	1.68 (0.02)	1.73 (0.01)	0.007	0.00720	0.00375

[a] Determined partition coefficient when alkan-1-ol was initially dissolved in water. [b] Determined partition coefficient when alkan-1-ol was initially dissolved in milkfat. [c] Probability (*P*) of no correlation between temperature and Log K_{MW}. [d] Ninety-five percent confidence interval is represented by the mean ± the values in parentheses. [e] Unable to obtain measurements for ethanol or propan-1-ol dissolved in milkfat at 10°C.

offset by stronger hydrogen bonding of water ($-\Delta H$). As temperatures approach 0 °C, the overall loss in entropy associated with dissolution of apolar groups will reduce, which may explain the increase in alkan-1-ols partitioning into water at lower temperatures (lower Log *P*).

Temperature-Dependent Partitioning of Alkan-1-ols in Milkfat and Water. Similar methods were employed with the milkfat and water systems. The mean Log *P* values are listed in **Table 2**. Agitation with a magnetic stir bar and greater time (up to 4 h) were required before equilibrium was achieved. Contrary to experiments at 20−60 °C, the apparent equilibrium endpoint at 10 °C was dependent on the phase in which alkan-1-ols were dissolved; extended time (96 h) and agitation did not result in identical Log K_{MW} calculations ($P < 0.05$). Results indicate that alkan-1-ols dissolved in partially solid milkfat are dramatically less available to partition into the aqueous phase. Likewise, when dissolved in water, alkan-1-ols encounter less available liquid milkfat, resulting in a disparity between apparent equilibrium endpoints. These results suggest several possibilities: (1) alkan-1-ols become entrapped in solid milkfat, thus reducing the availability for partitioning; (2) liquid milkfat is entrapped in the aggregation of crystals, thus reducing the availability of any alkan-1-ols dissolved in entrapped liquid milkfat while also reducing the level of liquid milkfat available for solvation; or (3) due to a very reduced rate of diffusion through the crystal network, the equilibrium endpoint was not achieved. Regardless, the deviation in partition results suggests that high levels of solid milkfat may have some effect on substrate availability in actual food systems. The weaker network of crystallized fats at 20 °C may have been insufficient to extend these effects above 10 °C.

Partitioning results are listed in **Table 2** along with a 95% confidence interval. Partitioning measurements of alkan-1-ols between milkfat and aqueous phases deviated significantly from those of octan-1-ol and water, although similarities in their trends were present. Longer chain alkan-1-ols preferentially partitioned more into the lipid phase. Overall, there is an approximately 1 log decrease in partition coefficients of the milkfat/water versus the octan-1-ol/water systems; this difference varies somewhat with temperature.

Octan-1-ol acts as a stronger solvent for alkan-1-ols than milkfat. Hydrogen bonding between hydroxyl groups of alkan-1-ols and octan-1-ol results in a more favorable enthalpy ($-\Delta H$) than the minimal interactions with milkfat. Further experimentation is needed to determine how the deviation would vary between Log *P* and Log K_{MW} for other compounds. Develop-

ment of fragmental constant models for milkfat based on adjustments to Log *P* constants may be feasible with a greater understanding of how solute structure relates to Log K_{MW}.

Rekker and Mannhold (*6*) presented a modification to Log *P* fragment constant models for an aliphatic hydrocarbon (Log K_{AHC}) phase. Calculated Log K_{AHC} partition values for ethanol through heptan-1-ol are −2.06, −1.44, −0.83, −0.22, 0.39, and 1.00, respectively. In all cases the observed Log K_{MW} lies between Log *P* and Log K_{AHC}. This result is not surprising, as the ester linkages of triacylglycerols are likely to have an enthalpic effect from alkan-1-ol hydroxyl group interactions, which lies intermediate to that of octan-1-ol and the minimal interactions with an aliphatic hydrocarbon phase. More nonpolar compounds (e.g., alkanes, aromatics) have Log K_{AHC} values that are greater than their Log *P*. According to this trend, alkanes would partition more favorably into milkfat than octan-1-ol.

Both octan-1-ol and milkfat partitioning experiments show a positive correlation with temperature ($P < 0.01$), except for ethanol in milkfat. Additionally, the temperature effect demonstrates an apparent leveling at temperatures approaching 60 °C. Linear regression slopes ranged from 0.00720 to 0.0131 Δ Log K_{MW} $°C^{-1}$ (**Table 2**); however both sets of curves exhibit an apparent changing slope. The effect of temperature is likely to be entropy associated. Compared to octan-1-ol results, the steeper slope of temperature dependence for milkfat models suggests that entropy has a greater impact on partitioning when milkfat is the hydrophobic phase. Lower temperatures favor dissolution into the water phase, as the entropy of forming "iced in" ordering becomes more favorable. This same phenomenon is present with a milkfat phase; however, the temperature dependence becomes even greater. Dissolution into milkfat has a minimal impact on enthalpy, as little interaction energy is realized. As a consequence, entropy becomes a proportionally larger influence on partitioning than enthalpy, which results in greater temperature dependence. The leveling observed at 50−60 °C may be a result of competing phenomena associated with solubility of apolar groups in water and the enthalpy realized from polar interactions.

Confidence intervals in **Table 2** show the greater uncertainty of ethanol at all temperatures and of propan-1-ol at 10 °C due to nonideal phase volumes required to mix partially solid milkfat. The volume of each phase has an impact on the precision of the test. If the phase volumes can be manipulated to yield as close to equal moles per phase, then the error of calculating by difference can be minimized (*3*). At lower temperatures, ideal mixing conditions limited the maximum milkfat phase volume and thus also decreased the method precision. Examination of polar compound partitioning in milkfat should be conducted after the development of improved systems to analyze the milkfat phase for dissolved solutes. Analysis of both phases will remove errors associated with concentration determination by difference. Furthermore, the mass balance could be examined by analysis of both phases to determine if accumulation at the interface is a factor.

Effect of Solid Fat on Partitioning. The other effect of temperature in partitioning is partial solidification of the milkfat phase. As discussed, measurements at 10 °C were dependent on the initial dissolving phase; final partitioning values did not come to equilibrium. In each case Log K_{MW} is greater for samples dissolved in milkfat, suggesting that solid milkfat somehow entraps or limits entry or escape of volatiles.

Crystal formation generally excludes impurities into the liquid phase; however, crystal aggregates can form a three-dimensional network with liquid fat held in the network (*17*). A portion of the entrapped liquid fat may become inaccessible to the aqueous phase, depending on the extent of crystallization and the physical stresses to the crystal network. At 20 °C, milkfat was 13.7% solid, yet no effect of entrapment was seen. At 10 °C, 37.8% solid milkfat exerts an entrapment effect. Roberts et al. (*9*) also found that volatiles could be entrapped in solid milkfat in their flavor release studies. The potential also exists for adsorption to solid fat surfaces (*18*). This may or may not be a true equilibrium condition. Solid fat aggregations could limit substrate availability in real cheese, but the scale of the model likely forms aggregation networks that limit diffusion beyond what would be seen in actual milkfat globules. In a real cheese, the crystal network would not grow beyond the size of milkfat globules, thus limiting the volume of entrapment (*11*).

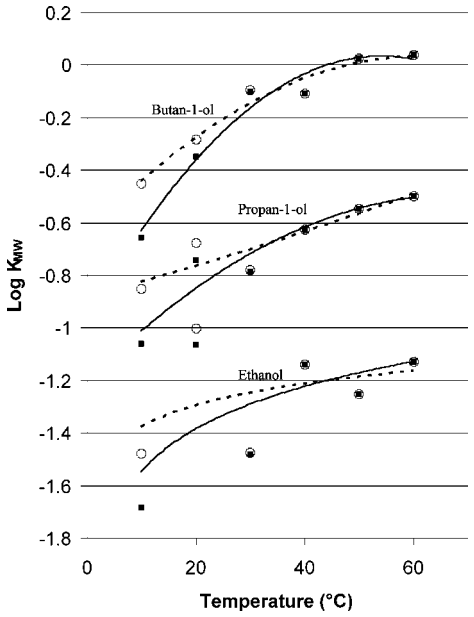

Figure 1. Partition coefficients of ethanol, propan-1-ol, and butan-1-ol between milkfat and water: (■) Log K_{MW} based on entire milkfat volume; (○) Log K_{MW} based on liquid milkfat volume only. Lines represent the best-fit solution of model data.

Milkfat crystallization reduces the liquid volume available to interact with solute. Assuming that crystalline milkfat is inert as a solvent, Log K_{MW} can be recalculated on the basis of only liquid-phase volume. According to NMR measurements, the observed milkfat was 59.8% solid at 0 °C, 37.8% at 10 °C, 13.7% at 20 °C, 2.00% at 30 °C, 0.02% at 40 °C, and 0% at 50−60 °C. Solid fat content is variable and dependent on seasonal and other factors (*19*). Using solid fat content measurements, Log K_{MW} was recalculated and plotted in **Figures 1** and **2**. In contrast to the original steep slopes at 10−30 °C, the alkan-1-ols maintained a more similar slope throughout the entire temperature range when calculated on the basis of the liquid portion only. Linear regression for corrected Log K_{MW} values versus temperature is listed in **Table 2** alongside the previously determined uncorrected values. The slope ranges from 0.00375 to 0.00955 Δ Log K_{MW} $°C^{-1}$, which still indicates a greater effect of temperature as compared to octan-1-ol models; however, the change in slope is now more consistent with octan-1-ol. The more constant slope over the observed temperature range for milkfat systems may relate to its dependence on entropy as the key determinant of partitioning. Beyond the role of entropy, partitioning in the octan-1-ol system is influenced by a greater effect from hydrogen-bonding interactions. Ad-

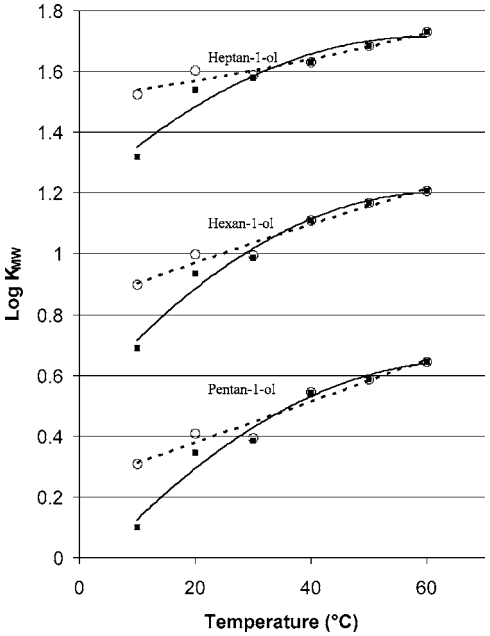

Figure 2. Partition coefficients of pentan-1-ol, hexan-1-ol, and heptan-1-ol between milkfat and water: (■) Log K_{MW} based on entire milkfat volume; (○) Log K_{MW} based on liquid milkfat volume only. Lines represent the best-fit solution of model data.

ditional experiments with individual known melting profile fats are needed to further resolve solid fat's role in adsorption and dissolution.

Partitioning results were determined by difference, under the assumption that alkan-1-ols would be present in either the aqueous or milkfat phase. Although preliminary tests in octan-1-ol systems suggested no significant accumulation at the interface, it is unknown if the longer chain alkan-1-ols (e.g., heptan-1-ol) accumulated at all in the interface between milkfat and aqueous phases. Analysis of solute concentration in the milkfat phase by solid-phase microextraction was not precise enough to determine if accumulation at the interface has an effect on Log K_{MW}.

Significance of Partitioning on Flavor Development. Although large deviations were seen between milkfat and octan-1-ol partitioning, the deviations behave in somewhat consistent patterns, especially when the solid volume is disregarded in partition calculations. Continued experimentation with other solutes may allow for models to utilize Log *P* data to estimate Log K_{MW} (*19*).

This study describes the distribution of compounds between milkfat and aqueous phases and the impact of temperature. Theoretically, the aqueous phase of cheese at 10 °C (e.g., ripening) would contain twice the concentration of heptan-1-ol than it would at 40 °C (e.g., cooking curd). However, a more polar compound, such as propan-1-ol, is less affected by this change in temperature, as cooling from 40 to 10 °C increases the concentration by 14%. Regular fat Cheddar cheese contains relatively similar volumes of milkfat and water, with the assumption that some water is unavailable due to bonding and hydration. Assuming no other influences, heptan-1-ol would partition ~5% into the aqueous phase of cheese, whereas 92% of propan-1-ol would be present in the aqueous phase. These calculations do not account for all of the factors that may influence substrate availability (e.g., kinetics of transfer and the impact of proteins, salts, and emulsions), but they do suggest that the relative availability of substrates should be examined in studies that explore substrate to flavor conversion.

Reduced fat cheese develops a flavor different from that of full fat cheese. Proteolysis is thought to relate directly to flavor development; however, reduced fat cheeses typically experience increased proteolysis without resulting flavor development (*20*). The change in lipid content affects the relative flavor release (volatilization) of polar and apolar compounds. The lipid phase may also play a role as a solvent for reaction flavors formed from amino acids. Reduction in fat may reduce the accumulation of nonpolar flavor compounds, which tend to accumulate primarily in the lipid phase.

This study focused on partitioning of solutes between milkfat and water and its possible role in substrate availability. Differences in a bulk phase of fat versus a fat emulsion with a milkfat membrane should be considered; however, evidence from Landy et al. (*21*) and Carey et al. (*22*) suggests that emulsifying layers would not significantly affect partitioning under cheese conditions. Research is needed to evaluate the role of solid milkfat within an emulsion where crystal networks formed would more accurately model a cheese system.

Further research is needed on the effects of salt, pH, and protein on partitioning. Additionally, the evaluation of other compounds (e.g., nonpolar alkanes, ionizable acids, phenolics) is needed to extend correlations to existing Log *P* data. Greater understanding of partitioning will aid in synthesis models to evaluate flavor-forming pathways. Finally, real cheese experiments can test flavor control, with full consideration of how temperature and phase characteristics could affect bacterial and enzymatic activities.

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