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The partitioning of diacetyl between food oils and water

M. H. Archer, V. M. Dillon, G. Campbell-Platt & J. D. Owens

Department of Food Science and Technology, University of Reading, PO Box 226, Whiteknights, Reading, RG6 2AP, UK

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The distribution of diacetyl between water and oils has been determined in order to predict whether diacetyl can offer the same level of preservation in fatty foods as it can in non-fatty foods. The partition coefficients were found to be approximately 0·2 for rapeseed oil and 0·3 for olive, sunflower, and blended-vegetable oils. Experimentation showed that the partition coefficient could be used to predict the antimicrobial effect of diacetyl in an oil/medium system. The differences between the oil/water system tested and a food system are discussed.

INTRODUCTION

Diacetyl (2,3 butadione) is a volatile yellow liquid with a pungent butterscotch aroma. It is an important flavour component of dairy products such as ripened-cream (cultured) butter, sour cream, and Cheddar cheese and some non-dairy products. Diacetyl also has antimicrobial properties (Jay *et al.*, 1983; Narasimhan *et al.*, 1988) but, owing to its strong flavour, it is not widely used as a food preservative. A food-preservation system incorporating diacetyl is currently under investigation in our laboratory. Its potential application is not hindered by the flavour of diacetyl. There was concern, however, that diacetyl's effectiveness as a preservative might be impeded by its solubility in fat.

In foods such as water-in-oil emulsions, micro-organisms grow in the water phase or at the oil/water interface. As a consequence of this, preservatives that completely migrate into the oil phase do not inhibit the growth of micro-organisms within fatty foods. When a preservative is mixed with oil and water, it becomes distributed in the two phases according to its solubility in each. The ratio of its solubility in oil to its solubility in water is called the *partition coefficient*. It is determined by measuring the concentration of preservative in each phase after an oil/water/preservative system has reached equilibrium.

The most efficient preservatives have low partition coefficients, with very small proportions distributed in the oil phase. Propionic acid, for example, has a partition coefficient of 0·17, whereas 4-hydroxybenzoic acid propyl ester has a very high partition coefficient, 87·5 (Lueck, 1980). It was important to know the magnitude of diacetyl's partition coefficient in order to assess whether diacetyl can be an effective preservative in fatty foods as well as non-fatty foods. The extent of migration of diacetyl into the fat phase in a food would depend on the food composition and structure. Our experiments were therefore designed to determine the maximum possible proportion of diacetyl which might, under some conditions, migrate into the oil phase. If the partition coefficient was found to be high, then the preservative system might have to be restricted to non-fatty foods. Alternatively, it may be feasible to increase the diacetyl concentration in fatty foods to compensate for the proportion located in the fat. The partition coefficient can be used to predict the additional amount of diacetyl required in fatty foods.

MATERIALS AND METHODS

Colorimetric determination of diacetyl concentration

The diacetyl concentration in the water phase was measured by the colorimetric reaction between diacetyl, creatine and 1-naphthol in alkali (Hill *et al.*, 1954; Mattessich & Cooper, 1989). The solution of diacetyl to be tested was diluted as necessary and 1·00 ml added to 0·65 ml saturated aqueous solution of creatine and 0·35 ml of an aqueous solution containing 30 g/litre NaOH and 35 g/litre 1-naphthol. The reaction mixture was left to stand at room temperature (22°C) for 8 min, after which its absorbance was read at 520 nm. The diacetyl concentration was determined by reference to a calibration graph, which was linear for diacetyl solutions between 0·04 mM and 0·14 mM.

Determination of the partition coefficient for diacetyl in oil-and-water mixtures

Diacetyl (Sigma D3634) was diluted in purified (Purite R050 reverse-osmosis and ion-exchange) water to give concentrations of 0·25, 0·22, and 0·18 mM, as determined by the colorimetric test. Equal volumes of diacetyl solution and oil were added to 7-ml Bijoux bottles to leave a headspace of 5 cm^3. Rapeseed oil, olive oil, sunflower oil and blended-vegetable oils were each tested with the three different concentrations of diacetyl, in duplicate, at room temperature (22°C). The mixtures were mixed for 1 min on a Whirlimixer to form an emulsion, and 1·5 ml was then transferred to 1·5-ml microcentrifuge tubes. The mixtures were centrifuged for 10 min at 15 000 r/min to break down the emulsion. A sample of the water phase was removed with a Pasteur pipette, and its diacetyl concentration was determined by the colorimetric test.

The partition coefficient of diacetyl was calculated for each emulsion as the diacetyl concentration in the oil phase divided by the concentration in the water phase. It was assumed that all the diacetyl lost from the aqueous phase had migrated into the oil phase and that none was lost by evaporation. The six results for each oil were averaged to give a mean partition coefficient.

Prediction of the effect of diacetyl on the growth of *Salmonella typhimurium* LT2 in an oil/medium mixture

Medium GYA2 contained (mg/litre purified water): glucose, 1000; vitamin-free casamino acids (Difco 028801), 200; yeast extract (Difco 0127-01), 100; L-histidine, 10860; glutamic acid, 5880; K_2HPO_4, 208; KH_2PO_4, 150; $CaCl_2.6H_2O$, 50; $MgSO_4.7H_2O$, 200; NaCl, 100; $(NH_4)_2SO_4$, 500; $EDTANa_2.2H_2O$, 19·05; $ZnSO_4.7H_2O$, 6·6; $MnSO_4.4H_2O$, 1·71; $FeSO_4.7H_2O$, 1·5; $CoCl_2.6H_2O$, 0·483; $CuSO_4.5H_2O$, 0·471; $Na_2MoO_4.2H_2O$, 0·453; H_3BO_3, 0·687. The pH was adjusted to pH 5·5 with 10 M HCl. The medium was dispensed into Universal bottles and autoclaved at 121°C for 2 min.

Diacetyl (Sigma D3634) was added to 20 Universal bottles containing Medium GYA2 to give 1·1, 1·2, 1·4, 1·6, and 1·8 mM with four replicates of each. An equal volume of sterile sunflower oil (5 ml) was added to half of the bottles, and the mixtures were Whirlimixed for 1 min and left to stand for 15 min at room temperature (22°C). *Salmonella typhimurium* LT2, NCIMB 10248, was then inoculated into the aqueous phase in each bottle to give a cell density of approximately 3×10^4 cells/ml of medium. After remixing, the mixtures were incubated at 30°C. Control mixtures of: (i) oil and medium, (ii) medium and culture, and (iii) oil, medium, and culture were also prepared.

After 24 h, the mixtures were visually examined for turbidity in the aqueous phase.

Table 1. Distribution of diacetyl between oil and water

Oil	Partition Coefficient
Rape seed	0·21 ± 0·01
Olive	0·29 ± 0·03
Sunflower	0·32 ± 0·02
Blended vegetable	0·31 ± 0·02

Values are the means (± SD) of six determinations.

RESULTS AND DISCUSSION

The partition coefficients measured for diacetyl (Table 1) were considered to be sufficiently low to recommend the use of a diacetyl preservation system in fatty foods as well as non-fatty foods. The partition coefficients were very similar for all the oils tested. This supports the assumption of Lubienieck-von Schelhorn (1964) that partition coefficients are similar between water and most types of oil.

The partition coefficients were calculated by assuming that diacetyl was not lost by evaporation; however, some would have been lost into the headspace during the emulsion-forming stage. Land and Reynolds (1981) measured the headspace concentration of diacetyl above solutions of diacetyl in oil or water. This allows calculation of the approximate amount of diacetyl lost to the 5-ml headspace left in the Bijoux bottles. For example, the 0·22 mM ($1·78 \times 10^9$ μg/litre) diacetyl solution would have a diacetyl concentration of approximately 10 μg/litre in the headspace, which is equal to a total of $6·17 \times 10^8$ mMole diacetyl in 5 cm^3. The amount would be slightly higher above oil, but, even so, its effect on the partition coefficient would still be negligible.

The results in Table 2 show that it was possible to predict the effect of diacetyl on the growth of *Salmonella typhimurium* in an oil/medium system. The diacetyl concentration remaining in the medium in oil/medium mixtures was calculated by using a partition coefficient of 0·3. For example, when oil was added to medium containing 1·4 mM diacetyl, then it was assumed that three parts (3/13) of the diacetyl migrated to the oil phase and ten parts (10/13) remained in the medium to give a final concentration of 1·1 mM (mMole/l medium). The effect of the concentration remaining in the medium on the growth of

Table 2. The effect of diacetyl on the growth of *Salmonella typhimurium* LT2 in medium and oil/medium mixtures

Diacetyl added to medium (mM)	No oil	Oil/medium system		
	Growth in medium	Growth in medium	Calculated diacetyl in medium (mM)	Predicted growth
0	+	+	0	+
1·1	+	+	0·8	+
1·2	−	+	0·9	+
1·4	−	+	1·1	+
1·6	−	−	1·2	−
1·8	−	−	1·4	−

+ = growth; − = no growth observed after 24 h.

Salmonella typhimurium was predicted to be the same as the effect of that concentration in a medium-only system. The experimental results exactly matched the predicted results (Table 2). The control mixtures also produced satisfactory results.

It is important to consider the differences between the oil/water system and a real food. The experiments were conducted by using oils; however, many foods contain crystalline fats. The partition coefficients for preservatives in crystalline fat/water systems are generally lower than those for oil/water systems (Lubieniecki-von Schelhorn, 1964). The migration of preservatives through solid or semi-solid foods is also much slower than that through liquids. The presence of certain proteins in a food may bind diacetyl (Land & Reynolds, 1981) and prevent its migration, which thus, in effect, would reduce its partition coefficient. Although these food properties can all result in a reduced partition coefficient, the presence of some food components can increase the partition coefficient. The presence of sodium chloride would be expected to increase migration to the fat phase since it reduces the solubility in the aqueous phase (Lueck, 1981). Lubienieck-von Schelhorn (1964) found a decreased partition coefficient for sorbic and benzoic acids when sodium chloride was added to the water phase, and Land and Reynolds (1981) reported that diacetyl was more volatile (and hence less soluble) with the addition of sodium chloride. The influence of sugar on the partition coefficient, however, is unclear. Lueck (1980) states that sugar, like sodium chloride, will reduce the solubility in water, but Lubienieck-von Schelhorn (1964) found that the presence of sugar did not alter the partition coefficient for sorbic or benzoic acids, and Land and Reynolds (1981) found that glucose had a negligible effect on the volatility of diacetyl. Although Medium GYA2 contained both sodium chloride and glucose, the predictions based on the partition coefficient found for an oil/water system proved to be sufficiently accurate for the oil/medium system. This indicates that small amounts of sodium chloride and glucose do not significantly alter the partition coefficient for diacetyl. When the preservation system, incorporating diacetyl, has been fully developed, it should be tested in a range of fatty foods of different composition to confirm that the partition coefficient for diacetyl in foods is similar to that found in the oil/water system.

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