

Agglomeration of Ethyl Esters in Model Spirit Solutions and Malt Whiskies

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(Received 8 October 1993; revised version received 10 March 1994; accepted 4 May 1994)

Abstract: Medium- and long-chain ethyl esters were found to have a limited solubility when model spirits and malt distillates were diluted to 230 ml ethanol per litre for sensory analysis. At concentrations above this critical level the concentration of esters in solution remained constant, with the excess esters forming agglomerates. The saturated nature of the solution reduced the volatility of other esters, with a proportion of these compounds being partitioned into the agglomerate. The distribution was dependent on the mole fraction of each ester and its activity coefficient. Dissolution of wood extractives caused a number of changes in the solubility parameters of organic compounds; activity coefficients were increased and both the concentration and activity at which agglomeration occurred were decreased. This resulted in lower solution concentrations with increased concentrations in agglomerates. The reductions in free solution concentration could result in lower headspace concentrations, and thus have a direct effect on the flavour of the matured spirit.

Key words: flavour, volatiles, maturation, oak, malt whiskies.

INTRODUCTION

Freshly distilled whiskies and brandies generally have unacceptable sensory characteristics and are traditionally matured in oak casks for several years to produce a premium product (Philp 1989). Maturation reactions are complex but involve extraction of wood components, evaporation of low-boiling-point solutes from the distillate and interactions of wood and distillate components (Nishimura and Matsuyama 1989). Dissolution of wood components is thought to be of prime importance. It is also known that the character of a matured Scotch whisky is closely related to the contents of non-volatile compounds (Piggott *et al* 1992; Clyne *et al* 1993) and it has been possible to predict sensory scores for mature characteristics from quantifications of non-volatile compounds (Piggott *et al* 1993). Maturation also involves a reduction in the perception of undesirable, or immature, characteristics. However, losses of ethanol and water through the caskwood increase the spirit concentrations of many volatile components during maturation (Reazin 1983; Nishimura *et al* 1983). It has recently been reported that matured distilled spirits contain agglomerates or micelles that relate

to sensory properties, and that these form when the spirits are diluted with water for sensory assessment, normally at 220 or 230 ml ethanol per litre (Hardy and Brown 1989; Perry 1989).

Ethyl esters, particularly acetate, hexanoate, octanoate, decanoate and dodecanoate (Salo *et al* 1972) make a major contribution to whisky odour. Both addition or depletion of such esters may have negative effects on aroma intensity (Jounela-Ericksson 1981). Dilution for sensory analysis will greatly affect the solubility of compounds such as these ethyl esters, which are less soluble in water than in ethanol. Ethyl esters are amphiphilic with a polar head group and a hydrocarbon chain tail. These may thus form agglomerates or micelles in aqueous solutions (Tanford 1980), and model solutions containing ethyl esters in aqueous ethanol gave similar 'micellar' diameters as those found in matured spirits (Paterson *et al* 1994). Further, in studies with a redistilled brandy it was observed that tannic acid and oak extract significantly reduced the activity of ethyl esters, with reductions in extraction reaching a maximum for 12–14 carbons (Piggott *et al* 1992).

The activities of solutes may be determined directly by measuring the vapour pressure for the compound of interest. However, chromatographic quantifications of

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headspace concentrations are more readily obtained (Grant and Higuchi 1990). These can be compared with data from headspace concentrations above the pure solute (unit activity). Activity can then be related to the solution concentration by the following equation:

$$a = fX_a$$

where a is the activity, f the activity coefficient and X_a the concentration of the solute expressed as mol fractions. For an ideal solution $f = 1$.

As a first step to understanding how solute interactions, particularly those between volatile and non-volatile constituents, influence the sensory properties of distilled beverages, we have examined the activity of ethyl esters in solutions and the effects of ester-ester interactions and dissolution of wood extracts.

EXPERIMENTAL

Materials

Ethanol was HPLC grade (Rathburn Ltd, Walkerburn, UK) and water was distilled and filtered using a Millipore-Q system. Ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate and ethyl hexadecanoate were 99% pure (Sigma Chemical Company, Poole, Dorset, UK). Limousin oak extract was supplied by International Flavours and Fragrances (Haverhill, Suffolk, UK) and redissolved overnight in aqueous ethanol (water/ethanol, 65 : 35 v/v) and filtered to give a solution with a pH of 5.5.

Whisky samples were provided by Chivas Brothers Ltd (Keith, UK). A single batch of new malt distillate was diluted to 634 ml ethanol per litre and filled into four types of casks: new charred American white oak (type 1); used Bourbon (type 2); used Bourbon used for at least one maturation of Scotch (type 3); and a used Bourbon reused repeatedly until judged unable to mature new distillate (type 4). Casks were maintained in a bonded warehouse and pooled spirit from each cask type was analysed after 3 years ageing.

Method

Glass vials (20 ml) were fitted with PTFE lined silicone rubber septa in plastic screw caps. Standard concentrations of esters were prepared in aqueous ethanol (65 : 35 v/v) and 3.5 ml placed in a vial and diluted with 6.5 ml of water to a final ethanol concentration of 230 ml litre⁻¹. Vials containing pure solute (10 ml) were also prepared. Samples were equilibrated in a water bath at 30°C for at least 30 min and a 2.5 ml sample of headspace gas withdrawn using a 5 ml gas-tight syringe, heated to 50°C. Only one headspace injection was made per vial and samples were analysed in quadruplicate using a Carlo Erba HRGC 5300 Mega series gas chromatograph (Fisons Instruments Ltd, Crawley, UK) with

an FID linked to a TRIO computing integrator. A 0.5 mm × 12 m BP1 column (df = 1) (SGE UK Ltd, Milton Keynes, UK) was used with a carrier gas flow rate of 5 ml min⁻¹. The column temperature was 60°C for 1 min after injection, increasing to 240°C at 18°C min⁻¹. The detector temperature was 250°C. The injector was a cold-on column injector fitted with an external gas-tight septum. Ethanol headspace injections followed the same procedure but using a 100 µl syringe and an oven temperature of 40°C.

RESULTS

Single esters

The initial studies were determinations of activity coefficients and the concentrations at which agglomeration occurred for each ester. For even-numbered carbon esters from ethyl decanoate to hexadecanoate, plots of activity against solution concentration followed the general form shown in Fig 1(a). In each, there was an initial linear relationship between the activity and the solution concentration delineated by the origin (A) and a transition point (B). This was followed by a second

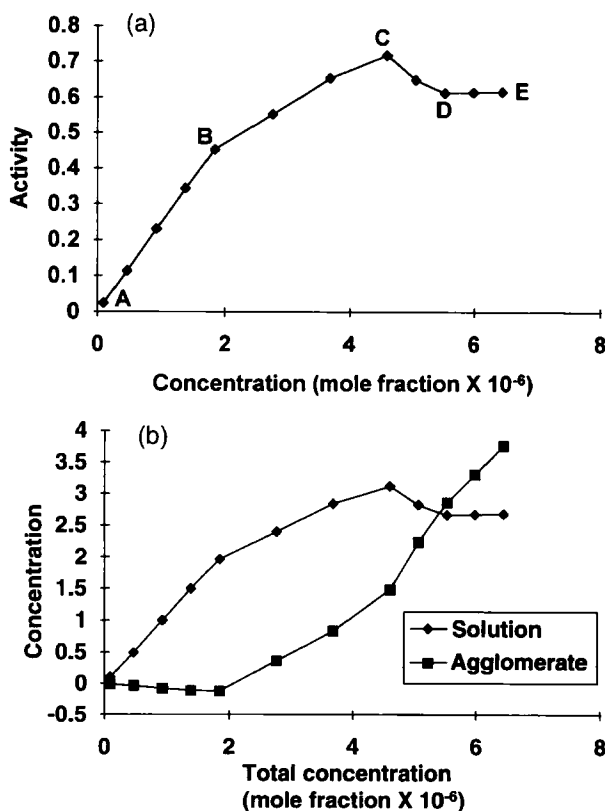


Fig 1. (a) Typical plot of activity versus total concentration. For each ester, concentrations were selected to give at least five points on the linear portion (AB) to calculate the activity coefficient. The average of the activities of the plateau region was used to estimate the agglomeration concentration (CMC). (b) Plot of concentration of free ester in solution and amount forming agglomerates against overall concentration.

linear increase to a maximum (C), followed by decline to a third transition point (D) and a plateau (terminating at E). With ethyl decanoate, activity reached a maximum of 0.7 whereas for the longer chain esters plateau values were between 0.9 and 1.0. For each ester, five concentrations within the initial linear portion (AB) were selected. Plotting of activity against concentration provided an activity coefficient (f) for each ester. The concentrations at which agglomeration occurred were determined from the average of the activities in each plateau (DE) and the activity coefficient. The distribution of esters between the solution and associated phases (Fig 1(b)) was calculated from the activity coefficient.

Ethanol activity in solutions containing esters at high and low concentrations (regions D to E and A to B in Fig 1(a), respectively) were determined, but showed no significant differences.

Plotting the natural logarithm of the agglomeration concentration against the number of carbons in the ester (Fig 2(a)) produced a gradient indicating a change in the free energy of association of $-2724 \text{ J mol}^{-1} \text{ CH}_2$ (standard deviation (SD) = $193 \text{ J mol}^{-1} \text{ CH}_2$). For homologous series of ionic amphiphiles at constant ionic strength and most nonionic amphiphiles the free energy of micelle formation changes by about

$-2930 \text{ J mol}^{-1} \text{ CH}_2$. The corresponding change in free energy on removing an alkyl chain from water to hydrocarbon for a homologous series of n -alkanes is $-3430 \text{ J mol}^{-1} \text{ CH}_2$ (Tanford 1980).

Plotting the natural logarithm of the activity coefficient against number of carbons in the ester (Fig 2(b)) indicated an increase in the excess chemical potential of solution (Denbigh 1981) of $2675 \text{ J mol}^{-1} \text{ CH}_2$ (SD = $118 \text{ J mol}^{-1} \text{ CH}_2$).

Table 1 compares the agglomeration concentrations for the ethyl esters in aqueous ethanol ($230 \text{ ml litre}^{-1}$) with critical micelle concentrations for hexa-oxyethylene alkyl ethers in water (Shinoda 1978). In each case the ethyl esters gave lower results, despite the higher temperatures used in this experiment.

Ester mixtures

In the second set of experiments ester mixtures were prepared to examine the relationship between agglomerates and the activity of a second ester.

It was apparent that the activity coefficient for ethyl decanoate was significantly decreased ($P < 0.01$) by addition of a second ester at a concentration of 20 mg litre^{-1} (Table 2(A)). There were also significant ($P < 0.05$) differences between the effects of octanoate, dodecanoate and hexadecanoate. Table 2(B) shows the reduction in the activity of ethyl decanoate at 20 mg litre^{-1} in the presence of other ethyl esters at increasing concentrations. With ethyl octanoate no significant decrease in activity was observed at $< 20 \text{ mg litre}^{-1}$ where the sum of solute activities was less than 0.70. Thereafter, decreases in ethyl decanoate activity were balanced by increases in ethyl octanoate activity ($f = 0.025$ (SD = 0.006), $R^2 = 0.940$). For tetradecanoate and hexadecanoate an activity minimum of 0.20 was reached for ethyl decanoate. For 20 mg litre^{-1} solutions of esters the weight percentage forming agglomerates ranges from 0% for octanoate to 99% for hexadecanoate. This indicates that the presence of any second ester has a large effect. The structure and distribution of the second ester was of less importance.

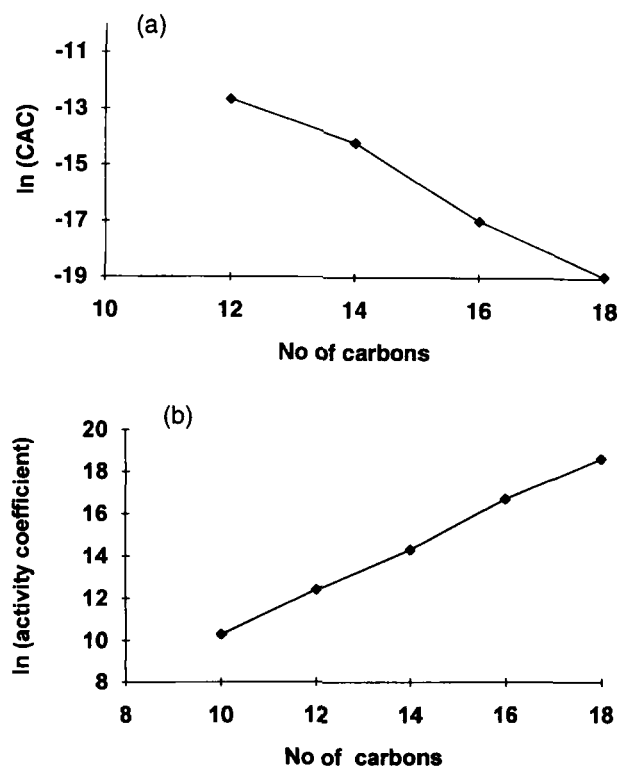


Fig 2. (a) Plot of the natural logarithm of the agglomeration concentration (ln CAC) against the number of carbons in the ethyl ester (gradient = -1.08 , SD = 0.08 , $R^2 = 0.990$). Agglomeration concentrations are in mole fractions. (b) Plot of ln (activity coefficient) against the number of carbons in the ester (gradient = 1.05 , SD = 0.02 , $R^2 = 0.999$).

TABLE 1

Critical micelle concentrations (mol fraction $\times 10^{-6}$) for hexa-oxyethylene alkyl ethers at 20 or 25°C (Shinoda 1978) and agglomeration concentrations for ethyl esters at 30°C

<i>i</i>	<i>CiE6</i>	<i>EtCi</i>
8	240 ^a	—
10	21 ^b	3.2
12	2.1 ^a	0.67
14	—	0.042
16	0.024 ^a	0.0059

^a At 25°C.

^b At 20°C.

TABLE 2A
The effect of a second ester (20 mg litre⁻¹) on the activity coefficients of ethyl decanoate and dodecanoate

<i>Added ester</i>	<i>Activity coefficient × 10⁶ (Mean ± SD)</i>	<i>Intercept (Mean ± SD)</i>	<i>R²</i>
<i>Decanoate</i>			
—	0.233 ± 0.016	0.02 ± 0.02	0.984
Octanoate	0.140 ± 0.006	0.10 ± 0.01	0.998
Dodecanoate	0.108 ± 0.007	0.06 ± 0.02	0.988
Tetradecanoate	0.091 ± 0.010	0.04 ± 0.03	0.948
Hexadecanoate	0.080 ± 0.050	0.05 ± 0.02	0.987
<i>Dodecanoate</i>			
—	1.60 ± 0.37	0.07 ± 0.06	0.902
Decanoate	0.31 ± 0.06	0.02 ± 0.05	0.969
Tetradecanoate	0.23 ± 0.06	0.03 ± 0.05	0.971
Hexadecanoate	0.21 ± 0.04	0.09 ± 0.05	0.973

Table 2A also gives the activity coefficients of ethyl dodecanoate in the presence of a second ester at a concentration of 20 mg litre⁻¹. These data also support the assertion that it is the presence, rather than structure, of the second ester that is of primary importance. Moreover, it is clear that the linearity of activity extended well beyond the agglomeration concentration for ethyl dodecanoate.

Figure 3 shows the effect of increasing concentrations of ethyl decanoate on a solution of ethyl dodecanoate and hexadecanoate, each at a concentration of 20 mg litre⁻¹. The linear increase in the activity of ethyl decanoate is balanced by decreases in the activities of ethyl dodecanoate and hexadecanoate, such that there was no significant change in the sum of the activities of the three esters. A similar experiment (Fig 4) was performed in which ethyl octanoate was added to a solution of three esters; ethyl decanoate, dodecanoate and hexadecanoate, each at 20 mg litre⁻¹. A decrease in hexadecanoate activity was observed but neither decanoate nor dodecanoate activity was affected.

Effect of wood extract on solutions of single esters

Activity coefficients and agglomeration concentrations for ethyl decanoate and dodecanoate were determined in the presence of oak wood extract at a range of concentrations (Table 3). Wood extract at concentrations up to 5 g litre⁻¹ significantly increased the activity coefficient of ethyl decanoate but decreased the agglomeration concentration. At higher concentrations of wood extract the activity coefficient was decreased, but the intercept shifted significantly from the origin. Similar results were observed with ethyl dodecanoate, although these effects were observed at lower concentrations of wood extract. There was also a marked decrease in the activity at the agglomeration concentration.

Effect of wood extract on solutions of more than one ester

The effect on activity coefficients of both ethyl decanoate and dodecanoate in the presence of a second ester

TABLE 2B
The effect of increasing concentrations of a second ester on the activity of ethyl decanoate (20 mg litre⁻¹)

<i>Added ester</i>	<i>Rate of decrease^a (Mean ± SD)</i>	<i>Intercept (Mean ± SD)</i>	<i>R²</i>
Octanoate ^b	-0.024 ± 0.008	0.484 ± 0.035	0.903
Dodecanoate ^c	-0.062 ± 0.018	0.384 ± 0.026	0.800
Tetradecanoate ^d	-0.083 ± 0.002	0.350 ± 0.002	0.999
Hexadecanoate ^e	-0.162 ± 0.001	0.353 ± 0.001	1.000

^a Rate of decrease of activity per μ mol fraction added ester.

^b Concentration range 20–50 mg litre⁻¹, no significant decrease up to 20 mg litre⁻¹.

^c Concentration range 5–30 mg litre⁻¹.

^d Concentration range 5–20 mg litre⁻¹, no further decrease at higher concentrations.

^e Concentration range 5–15 mg litre⁻¹, no further decrease at higher concentrations.

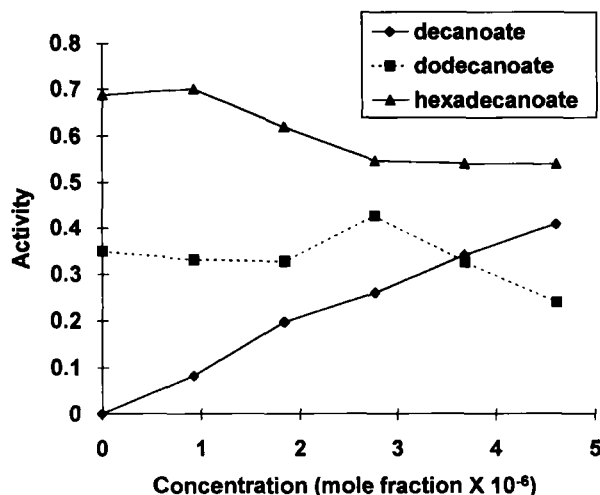


Fig 3. The effect of increasing concentrations of ethyl decanoate on a solution of ethyl dodecanoate and ethyl hexadecanoate (20 mg litre⁻¹ each).

and wood extract at a concentration of 2.5 g litre⁻¹ was also investigated (Table 4). As previously (Table 2A), addition of a second ester resulted in significant decreases in the activity coefficient with the nature of the second ester being of less importance. Again the linearity of the relationship extended well beyond the agglomeration concentration.

The effect of wood extract on the distribution of esters in a mixture of decanoate, dodecanoate and hexadecanoate, each at 20 mg litre⁻¹, was also studied (Fig 5). It was apparent that there was a displacement of ester from solution to agglomerates that reached a

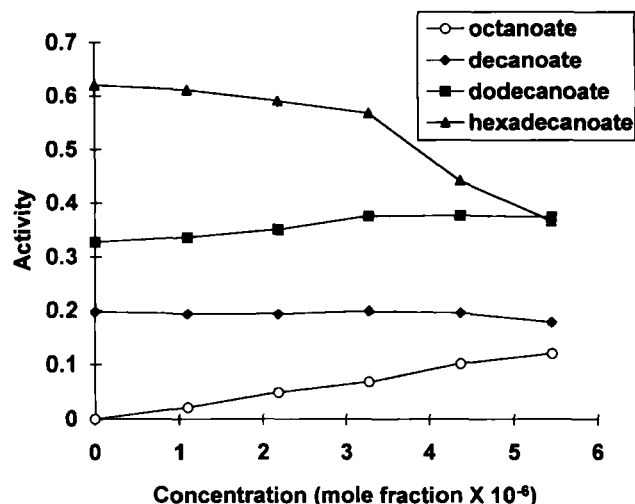


Fig 4. The effect of increasing concentrations of ethyl octanoate on a solution of three esters (ethyl decanoate, dodecanoate and hexadecanoate, 20 mg litre⁻¹ each).

maximum at 2.5 g litre⁻¹. At concentrations above 3.75 g litre⁻¹ the concentration of esters in solution was increased. For wood extract the effect was greatest for ethyl decanoate and least for ethyl hexadecanoate, in contrast to that observed with addition of ethyl octanoate.

Distribution of esters in matured spirit samples

The calculated agglomeration concentrations for ethyl dodecanoate, tetradecanoate and hexadecanoate (Table 1) were related to the concentrations observed in a malt

TABLE 3
The effect of increasing concentrations of wood extract on the solution thermodynamics of ethyl decanoate and dodecanoate

Wood extract concentration (g litre ⁻¹)	Activity coefficient $\times 10^6$ (Mean $\pm$ SD)	Intercept (Mean $\pm$ SD)	AGC ^a	R ²
Decanoate				
0	0.23 $\pm$ 0.02	0.02 $\pm$ 0.02	3.28	0.984
2.5	0.41 $\pm$ 0.09	0.04 $\pm$ 0.11	2.30	0.955
5.0	0.49 $\pm$ 0.05	0.04 $\pm$ 0.06	1.17	0.989
7.5	0.10 $\pm$ 0.02	0.13 $\pm$ 0.04	<0.09	0.909
Dodecanoate^b				
0	1.60 $\pm$ 0.07	0.07 $\pm$ 0.07	0.67	0.983
1.2 (AB)	2.13 $\pm$ 0.04	0.00 $\pm$ 0.08	0.43	0.974
1.2 (BC)	0.52 $\pm$ 0.02	0.37 $\pm$ 0.12		0.999
2.5 (AB)	2.41 $\pm$ 0.31	-0.01 $\pm$ 0.04	0.14	0.968
2.5 (BC)	0.73 $\pm$ 0.17	0.30 $\pm$ 0.12		0.904
5.0 (AB)	1.75 $\pm$ 0.12	-0.01 $\pm$ 0.02	0.09	0.990
5.0 (BC)	0.61 $\pm$ 0.06	0.20 $\pm$ 0.06		0.955
7.5	0.74 $\pm$ 0.05	0.11 $\pm$ 0.02	<0.01	0.985

^a Agglomeration concentration (mol fraction $\times 10^6$).

^b AB/BC refer to linear portions of graph in Fig 1(a).

TABLE 4

The effect of a second ester (20 mg litre⁻¹) plus wood extract (2.5 g litre⁻¹) on the activity coefficients of ethyl decanoate and dodecanoate

Added ester ^a	Activity coefficient $\times 10^6$ (Mean $\pm$ SD)	Intercept (Mean $\pm$ SD)	R ²
<i>Decanoate</i>			
Dodecanoate	0.081 $\pm$ 0.003	0.06 $\pm$ 0.01	0.999
Hexadecanoate	0.080 $\pm$ 0.015	0.07 $\pm$ 0.04	0.932
E12 + E16	0.062 $\pm$ 0.003	0.04 $\pm$ 0.01	0.993
<i>Dodecanoate</i>			
Decanoate	0.194 $\pm$ 0.022	0.12 $\pm$ 0.03	0.975
Hexadecanoate	0.188 $\pm$ 0.025	0.15 $\pm$ 0.05	0.983
E10 + E16	0.105 $\pm$ 0.014	0.05 $\pm$ 0.04	0.964

^a E10, ethyl decanoate; E12, ethyl dodecanoate; E16, ethyl hexadecanoate.

distillate aged for three years in either glass, new charred (type 1), used Bourbon (type 2), and used (type 3) and exhausted (type 4) Scotch casks (Table 5). From these results the proportion of each of the three esters in

the associated phase could be estimated. As predicted, ethyl dodecanoate, tetradecanoate and hexadecanoate were primarily in agglomerates. However ethyl decanoate was more abundant free in solution in glass-aged distillate and in the three casks that would be used for maturation of Scotch malt distillate. In the new charred wood, as used for Bourbon maturations, decanoate was primarily in the associated phase.

DISCUSSION

With solutions of individual esters, plotting activity against total solute concentration yielded a plateau as activity approached unity. There are two alternate hypotheses that may explain this observation. The first is that a surface film forms as the ester is excluded from solution, or alternatively excess ester forms agglomerates or micelles. However, measurements of ethanol activity showed no significant change with increasing ester concentration, which would be expected if a surface film were formed. Consequently it was concluded that the esters were forming agglomerates or micelles.

For the homologous series of esters the increase in free energy of association depended on the increase in length of the hydrocarbon chain. This behaviour is an important characteristic of the hydrophobic effect which is mainly responsible for the formation of micelles. In other studies on solutions of amphiphiles, the formation of micellar aggregates accounted for all or the major part of non-ideality in these solutions (Tanford 1980). For this series of ethyl esters, activity coefficients were always much greater than 1 indicating non-ideal solutions. For all ethyl esters the plot of activity against concentration gave a straight line through the origin, so the choice of whether solutions are ideal or not depends on the convention used for unit activity; either that in the headspace over the pure solute or that above an infinitely dilute solution (Denbigh 1981).

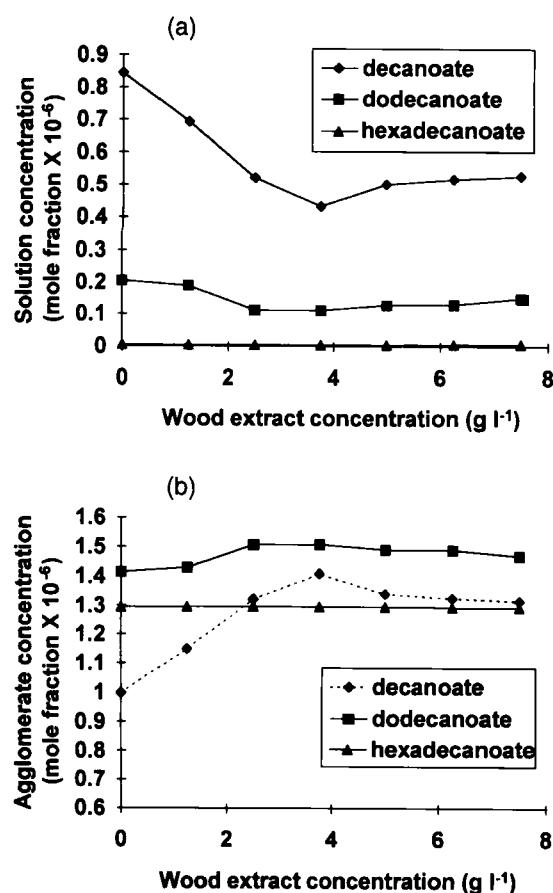


Fig 5. (a) Changes in the free solution concentrations of ethyl decanoate, dodecanoate and hexadecanoate (20 mg litre⁻¹ each) in the presence of increasing concentrations of wood extract. (b) Changes in the agglomerate concentrations of ethyl decanoate, dodecanoate and hexadecanoate (20 mg litre⁻¹ each) in the presence of increasing concentrations of wood extract.

The increase in free energy of association for the homologous series of esters is close to that reported for the formation of micelles. However, results from dynamic light scattering indicated that 'micellar' diameters for ethyl esters in 230 ml litre⁻¹ ethanol were extremely large at approximately 500 nm (Paterson *et al* 1994) and gave a cloudy solution. The agglomeration concentration may therefore represent the cloud point concentration for that ester at 30°C and DE (Fig 1(a)) represents the saturation concentration of singly dispersed molecules (Shinoda 1978). Comparison with hexa-oxyethylene alkyl ethers indicates that the ethyl esters have much lower solubilities as the extent of H-bonding to water is considerably reduced.

For ethyl esters, the increase in activity coefficient with hydrocarbon chain length gave an increase in the excess chemical potential of solution of 2675 J mol⁻¹ CH₂. Using the reasoning for changes in the free energy of association, then changes in the excess chemical potential also provided a measure of hydrophobic interaction for that solute. A further consequence is that, for increasing ester chain lengths, decreases in the agglomeration concentration are balanced by increases in ester activity coefficients and for most esters this results in agglomeration when solute activity approaches unity. Consequently, each of the homologous series of esters displays the same maximum activity, or chemical potential in 230 ml litre⁻¹ ethanol, above which agglomeration occurs. This limit is exceeded by many malt whiskies.

For mixtures of ethyl octanoate and decanoate, no change in the activity coefficient of either ester was observed at activities less than 0.7. Thus the activity coefficients calculated for single esters in solution can be used to calculate the free solution concentration of each ester in a mixture. At values greater than this, increases in the activity of one ester were balanced by decreases in that of the second. In solutions in which a portion of ester has formed agglomerates, observed reductions in activity coefficients must relate to ester being transferred to these agglomerates. Although there are significant differences between esters, such effects are less marked than those arising from the presence of any ester. It can thus be concluded that the presence of a saturated solution is more important than either the structure or quantity of ester present. Increasing the proportion of ester as agglomerates resulted in a decrease in the activity of a shorter chain ester maintained at constant concentration. Such changes may be the result of the decreases in the relative mol fraction of the shorter chain ester. The rate of decrease in ethyl decanoate activity increased with the chain length of a second ester. As the minimum activity observed was similar for each ester, changes in rate may relate solely to the activity coefficient of the second ester.

In mixtures of three and four esters, increasing the concentration of the ester with the shortest hydrocar-

bon chain resulted in decreases in the activity of the other esters. The ester with the largest hydrocarbon portion was then displaced preferentially. Thus, it can be concluded that the maximum activity calculated for single esters in solution is closely related to the sum of ester activities in a mixture.

Previous studies have reported interactions between many classes of volatile flavour compounds and purines (King and Solms 1982; Seshadri and Dhanraj 1987). These interactions were attributed to plane-to-plane stacking resulting from hydrophobic and π -electron interactions. Compounds lacking an extended π -electron system were unable to interact. In our studies on esters the driving force for interaction appears to be the hydrophobic effect, suggesting that other amphiphilic compounds may also be involved. Further, the presence of agglomerates may result in the solubilisation of hydrophobic compounds in spirit solutions.

Addition of Limousin wood extract was observed to change the solubility of esters in 230 ml litre⁻¹ ethanol. Activity coefficients were increased, but both the concentration and the activity at which agglomeration occurred decreased. Reductions in activity coefficients were observed for ethyl tetradecanoate and hexadecanoate with wood extract at any concentration and with ethyl decanoate and dodecanoate at 5 g litre⁻¹. As the activity at the agglomeration concentration decreased (Table 3), a further linear increase, with a significantly lower gradient, was observed. This relates to the transition in the plot of activity against concentration in single ester solutions (point B in Fig 1(a)). Dynamic light-scattering results indicate that dissolution of wood extract results in a decrease in the agglomerate diameter for ethyl esters in 230 ml litre⁻¹ ethanol (Paterson *et al* 1994) and it can be postulated that the expansion of BC in Fig 1(a) is accompanied by the formation of smaller and stabler structures.

In mixtures of two esters, despite the increased activity coefficients of the individual esters, addition of wood extract resulted in decreases in the activity coefficient. This suggests that the wood extract enhances the partitioning of the second ester into the agglomerates. The effect is a reduction in ester concentration in solution in the presence of the wood extract. When mixtures of three esters were analysed, addition of wood extract was found to decrease the ester in solution to a minimum with the greatest effect being on the ester with the smallest hydrocarbon group.

Estimating the distribution of ester between solution and agglomerate phases in matured distillate samples indicated that dodecanoate, tetradecanoate and hexadecanoate were primarily in the agglomerate phase. Little difference was observed between cask types, though for ethyl decanoate a significantly higher proportion was in the agglomerates for the new charred casks. However, values in Table 5 were calculated from the activity coefficients of esters in 230 ml litre⁻¹

TABLE 5

Fatty acid ethyl ester concentrations (C) and percentage forming agglomerates (%A) in a malt distillate aged for 3 years in four types of cask (concentrations are given as mg litre⁻¹ at nosing strength (230 ml ethanol litre⁻¹))

Ester ^a	Distillate ^b C (%A)	Type 1 ^c C (%A)	Type 2 ^d C (%A)	Type 3 ^e C (%A)	Type 4 ^f C (%A)
C10	19 (42)	42 (68)	22 (37)	23 (36)	19 (28)
C12	16 (87)	17 (85)	17 (86)	19 (86)	17 (80)
C14	3 (92)	3 (99)	3 (97)	3 (98)	3 (97)
C16sat	11 (99)	10 (99)	11 (99)	11 (99)	10 (99)
Total	49	72	53	56	49

^a C10, ethyl decanoate; C12, ethyl dodecanoate; C14, ethyl tetradecanoate; C16sat, ethyl hexadecanoate.

^b Distillate stored in glass for 3 years.

^c Type 1, new, charred barrel.

^d Type 2, used Bourbon barrel.

^e Type 3, used Scotch barrel.

^f Type 4, exhausted barrel.

ethanol. Variation in concentrations of non-volatile components arising from the different cask types were not included in the equation.

From this and a second study of aged spirit samples using dynamic light-scattering (Paterson *et al* 1994) it is clear that an agglomerate phase was present in model solutions studied previously (Piggott *et al* 1992). A plausible explanation for differences in extraction resulting from the presence of tannic acid and wood extract may lie in the stability of the esters in the agglomerate phase. It is also possible then that, in matured whisky samples, the stability of agglomerates is related to the concentrations of wood-derived, non-volatile compounds. The influence of such compounds on release of aroma compounds into the beverage head-space or retronasal cavity of the human taster is also under investigation.

CONCLUSIONS

An important conclusion from this study is that the use of odour units to assess the contribution of esters to the aroma of a distilled beverage cannot be justified. As odour threshold concentrations are normally determined from solutions of single compounds, extrapolation of data obtained to spirits with an agglomerate phase will lead to an overestimate of the contribution of any ester to the aroma.

Although this study centred around the behaviour of esters in solutions, whiskies contain numerous other amphiphilic or hydrophobic compounds, such as alcohols, aldehydes, ketones and organic acids. The effect of the presence of ester agglomerates and the appearance of non-volatiles derived from extraction of the wood during maturation could influence the distribution of

such compounds between solution and agglomerate phases and thus between the beverage and its head-space.

ACKNOWLEDGEMENT

This research is supported by the Agricultural and Food Research Council of the UK and Chivas Brothers Ltd, Keith, Scotland.

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