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Studies on the Transfer of Some Flavour Compounds to Milk

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To determine what flavour compounds could be transferred to milk *via* the digestive route, series of aliphatic alcohols, aldehydes, ketones and esters were fed into the rumen of a cow. The milk samples were analyzed by means of a gas chromatograph. Alcohols with an odd number of carbon atoms, lower ketones and esters (except those methyl and ethyl esters of fatty acids with an odd number of carbon atoms tested) were found to enter the milk to such a degree that they gave distinct flavour defects to milk. Aldehydes, with one exception, and some unsaturated alcohols and ketones were transferred to milk only in traces or not at all.

Flavour threshold values of some aliphatic alcohols, ketones and esters in milk are given.

It is a well known fact that certain fodder plants and silages can give rise to characteristic flavour defects in milk. For example an onion-like off-flavour was noticed in milk after feeding cows with some *Allium*-species, *Melilotus albus* gave an aroma of coumarin and *Trifolium pratense* a clover-like taste. Likewise some fodder plants of the family *Cruciferae* gave typical off-flavours to milk. Sometimes a grass-like off-flavour appears when the cows are moved to pasture feeding in the springtime. Silages can also impart an extremely bad off-flavour to milk if repulsive flavour substances have been formed in the silage by the effect of micro-organisms, as happens if the pH is not sufficiently low.

The milk flavours can be classified into two groups: those flavour compounds which are transferred from fodder to milk *via* the organism and those which are formed in the organism (in the rumen and/or in the metabolic processes in the liver or mammary gland) from carbohydrates, amino or fatty acids and other chemical compounds in the fodder.

Recently Dougherty and co-workers [1,2] have performed some experiments with cows having ruminal and tracheal fistulas to determine what chemical compounds could be transmitted to milk through the lungs or the rumen or both. They found that if sufficient amounts (usually such enormous amounts as 25—50 ml) of the substances tested (esters, alcohols, ketones, aldehydes,

lower fatty acids or dimethylsulphide) were introduced into the rumen or vapourized into the lungs, all of them, except the fatty acids, were transmitted by both routes to the milk. There was also no apparent difference between the transmission by the two pathways, except in respect to the speed of the transmission. When the substances were introduced into the lungs, the flavour was detected in the milk sooner than when the substances were introduced into the rumen. Likewise the off-flavour appeared sooner, when the gases from the rumen were permitted to enter the lungs than when they were blocked from entering the lungs. The milk samples were judged by an experienced flavour-panel.

When using this method, it is possible to test only one compound at a time, and the amount of substance has to be very large if the flavour threshold concentration of the substance in milk is fairly high. Because many of the substances in such high concentrations are more or less toxic, the experimental conditions are unphysiological and the results may not be fully reliable. It is likewise not possible to detect in the organoleptic tests whether some chemical changes have occurred in the substances given, since the off-flavours observed were often characterized as "fruity", "cowy", "malty" or "grassy" etc. and might not be caused alone by the compound given.

When we wish to investigate the transfer of flavour substances to milk *via* the digestive route, by giving the cow such amounts of the substances in question, which are not wholly unnatural, the only way is to use sufficiently sensitive methods for the determination of these substances in milk.

Table 1. Flavour threshold values (FTV) of some aliphatic compounds.

Substance	FTV in μg/l
Ethanal	1200
Propanal	430 *
Butanal	190 *
Pentanal	130 *
Hexanal	49 *
Heptanal	120 *
Octanal	430 *
Nonanal	220 *
Decanal	240 *
Propanone	$1-2.5 \times 10^5$; 5×10^5 **
Butanone	2.5×10^4; 5×10^4 **
Pentan-2-one	500
Hexan-2-one to undecan-2-one	250–500
Oct-1-en-3-one	10 ***
Butan-1-ol to hexan-1-ol	500
Methyl butanoate	500
Ethyl butanoate	25
Ethyl pentanoate	25
Methyl and ethyl hexanoate	75
Methyl and ethyl octanoate	500

* Lillard *et al.*[4]; ** Patton and Josephson;[8] *** Stark and Forss.[7]

In a previous paper [3] from this laboratory a semiquantitative gas chromatographic micromethod was described for the determination of some flavour compounds in liquid foodstuffs. By this method it is possible to demonstrate the presence of as little as 5 μg of added flavour compound in one liter of milk (0.005 ppm), which concentration is considerably lower than the flavour threshold concentration of most aliphatic compounds in milk (Table 1). Since the percentage recovery from milk which contains fat decreases very rapidly with the increase of the molecular weight of the flavour compounds we have performed several distillations with known quantities of substances added to obtain the relevant percentage recovery for various types of organic compounds. The average values are presented in Table 2. As can be seen, the

Table 2. Percentage recovery of flavour compounds added to milk (4—5 % fat).

Total number of carbon atoms	Percentage of aldehydes, ketones and esters	Percentage of alcohols
4	5—15	35—45
5	35—45	65—75
6	55—65	75—85
7	70—80	65—75
8	75—85	45—55
10	55—65	—
12	15—25	—

maximum recovery (70—85 %) is obtained with aldehydes, ketones and esters having 7 or 8 carbon atoms and by alcohols having 5—7 carbon atoms. The low boiling compounds (b.p. $<$ 80°C) are lost almost completely in the concentration procedure with pentane-ether extraction, and the higher-boiling compounds are held in the fat.

Most of the feeding experiments in this study were performed with cows, which were fed with an odourless purified diet [9-11] (cellulose, starch and sucrose, urea and ammonium salts as the nitrogen source). The substances tested (1—2 g) were dissolved in dilute (25—50 %) ethanol or acetone (in the case of methyl and butyl esters to prevent the trans-esterification with ethanol) and fed through an armoured plastic tube into the rumen. Milk samples (250 ml) were collected in pure thin-walled polythene tubes before this feeding and after it at 1—2 hours' intervals. The tubes were immediately closed, chilled to —25°C and analyzed later. By choosing substances of sufficiently different retention times, it is possible to feed several compounds at the same time. In our experiments we have fed a mixture of various homologous organic compounds (boiling range 80—300°C). The results are presented in Table 3.

Table 3. Transfer of some aliphatic compounds to milk *via* the rumen.

Substance	Amount fed, g	Concentr. at the maximum, µg/l milk	Total amount transferred to milk, µg	Percentage of total amount fed
Alcohols:				
n-Pentan-1-ol	2	380	250	0.013
n-Hexan-1-ol	2	30	20	0.001
n-Heptan-1-ol	2	120	100	0.005
n-Octan-1-ol	2	?	—	—
n-Nonan-1-ol	2	75	100	0.005
cis Hex-3-en-1-ol	2	45	25	0.0013
trans Hex-3-en-1-ol	3	25	15	0.0005
dl Oct-1-en-3-ol	1	20	15	0.0015
Aldehydes:				
2-Methyl-propanal	2	1500	600	0.03
Hexanal	2	15	10	0.0005
Heptanal	2	20	10	0.0005
Octanal	2	30	10	0.0005
Ketones:				
Pentan-2-one	2	1300	1000	0.05
Hexan-2-one	2	1100	800	0.04
Heptan-2-one	2	750	250	0.013
Octan-2-one	2	490	150	0.0075
Nonan-2-one	2	150	50	0.0025
Decan-2-one	2	60	30	0.0015
Undecan-2-one	2	40	25	0.0013
Octan-3-one	2	50	25	0.0013
Oct-1-en-3-one	2	—	—	—
Esters:				
Methyl esters of C_6—C_{10} fatty acids	à 2	—	—	—
Ethyl esters of C_5, C_7, C_9 fatty acids	à 2	—	—	—
Ethyl butanoate	2	120	120	0.006
Ethyl hexanoate	2	60	60	0.003
Ethyl octanoate	2	120	100	0.005
Ethyl decanoate	2	120	100	0.005
Butyl 2-methyl-propanoate	1	300	160	0.016
Butyl butanoate	1	160	120	0.012
Butyl 3-methyl-butanoate	1	140	90	0.009
Butyl pentanoate	1	200	150	0.015
Butyl hexanoate	1	260	170	0.017
Butyl octanoate	1	110	75	0.0075

Most of the substances tested were transferred to the milk, the maximum concentration being reached in general after 2 h. The higher molecular weight compounds tend, however, to reach this point somewhat later (after 4 h). The concentration then rapidly decreases so that after 8—10 h only about 10 % of the maximum concentration is present.

A typical feeding test with a mixture of *cis* and *trans* hex-3-en-1-ol is seen in Fig. 1. As can be seen, the amount of the *cis* form, the so called "leaf alcohol",

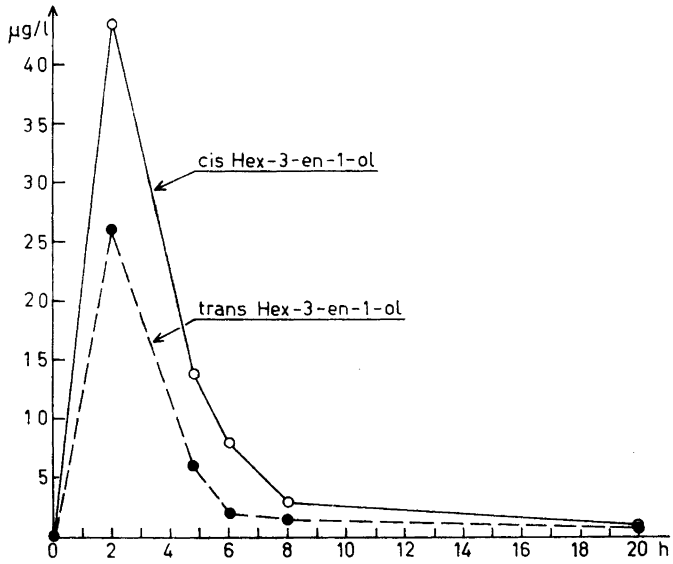

Fig. 1. Feeding test with a mixture (5 g) of *cis* (40 %) and *trans* (60 %) hex-3-en-1-ol. Ord.: transfer of both components to the milk, µg/l milk; absc.: time after feeding. 1. *cis* Hex-3-en-1-ol. 2. *trans* Hex-3-en-1-ol.

transferred to the milk is more than double that of the *trans* isomer (Table 3). Similar interesting features can also be observed in the feeding tests with normal aliphatic alcohols (Fig. 2) and ethyl esters of aliphatic fatty acids (Fig. 3). The alcohols with an odd number of carbon atoms are transferred to

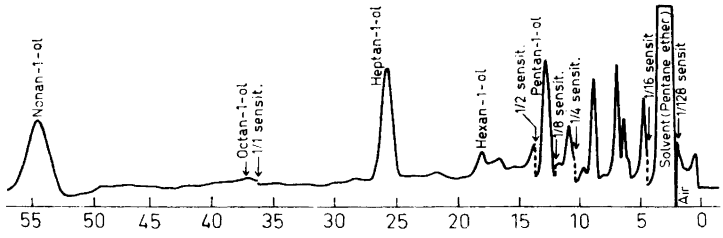

Fig. 2. Gas chromatogram of the milk obtained 2 h after feeding of *n*-alkan-1-ols (C_5—C_9), 2 g of each. Polyethyleneglycol column, 6 m, i.d. 4.5 mm, 175°C, 45 ml/min of N_2.

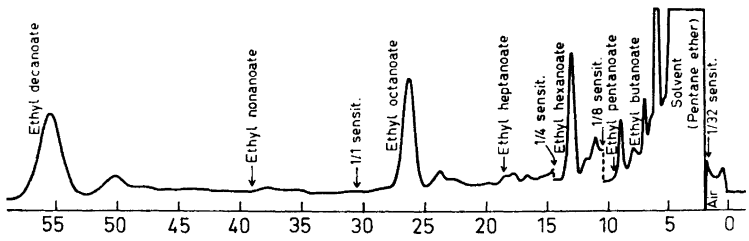

Fig. 3. Gas chromatogram of the milk obtained 2 h after feeding of ethyl esters of *normal* fatty acids (C_4-C_{10}), 2 g of each. The same column and conditions as in Fig. 2.

the milk to a considerably greater degree than the others. Of the ethyl esters only those with an even number of carbon atoms in the fatty acids enter the milk. An alternation like this is not observed with methylketones (Fig. 4) or

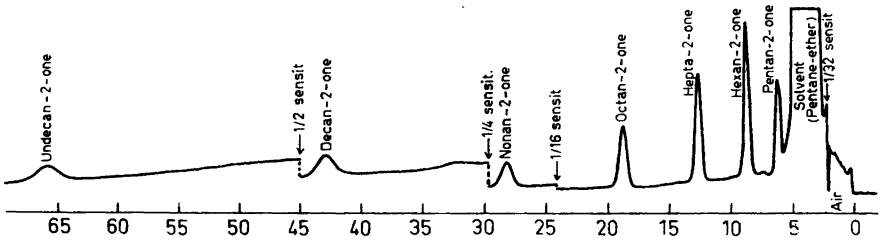

Fig. 4. Gas chromatogram of the milk obtained 2 h after feeding of *n*-alkan-2-ones (C_5-C_{11}), 2 g of each. Silicone grease column, 6 m, i.d. 4.5 mm, 175°C, 45 ml/min of N_2.

aldehydes. Also butyl pentanoate was transferred to the milk just as easily as the other *n*-butyl esters.

The percentage transfer varies within wide limits. The methyl esters of C_6-C_{10} fatty acids are not transferred to milk in detectable amounts and only traces of aldehydes, except 2-methylpropanal, were found in the milk. The ketones, alcohols (odd C-numbers) and esters are transferred to such a degree that they can give distinct flavour defects to milk, although the percentage transfer is as low as 0.01—0.05 %. Lillard *et al.*[4] have observed an additive effect if several flavour compounds are present at the same time, so that also lower concentrations than the threshold value can give flavour defects to milk. Nawar and Fagerson [5] pointed out the potential significance of sub-threshold amounts of some methyl ketones and concluded that a synergistic effect was obtained by combining the compounds. In the experiments of Guadagni *et al.*[6] the effect of mixtures containing substances of a single class or different classes was additive. For instance, a mixture of 10 saturated aldehydes containing each compound at only about 10 % of its threshold concentration gave an odour.

The unsaturated alcohol, oct-1-en-3-ol, a compound isolated from some clover species,[12] also was found to enter the milk. The maximum concentration,

20 μg/l, was obtained after 2—4 h. In organoleptic tests, however, no detectable off-flavour was observed. According to Stark and Forss [7] a possible precursor for the so called "metallic off-flavour" (identified as oct-1-en-3-one) is oct-1-en-3-ol, which was also isolated from dairy products ("mushroom compound"). Accordingly, it is possible that oct-1-en-3-ol is transferred from fodder to milk and the metallic off-flavour formed in milk and especially in dried milk might be formed by oxidation of this compound to the corresponding ketone. After feeding the cows with aftermath, a peak with the identical retention time as the synthetical oct-1-en-3-ol appears in the gas chromatogram of the milk. The corresponding ketone, oct-1-en-3-one, which was also isolated from the flowers of *Trifolium repens* and *Tr. hybridum* [13] did not enter the milk *via* the digestive route. Some unidentified lower molecular weight compounds appear, however, after the feeding of oct-1-en-3-one in the gas chromatogram of the milk. This ketone was found to be sensitive to acids and it disappears obviously very quickly in the rumen.

EXPERIMENTAL

The flavour threshold values were determined according to Patton and Josephson.[8]

The vacuum steam distillation and solvent extraction method for the isolation and concentration of the flavour compounds for gas chromatographic analyses is described in an earlier paper.[3] A "Fraktometer 116 E" of the Perkin Elmer Co. with FID and a sample inlet valve [3] were used in the experiments.

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