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Cheddar Cheese Flavour Studies. I. Production of Volatiles and Development of Flavour During Ripening.

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FOOD

/METHANOL

Cheese was tested every two months over a one year period for volatile content. The methanol content increased from 20 nanograms/5ml (.004 mg./liter) to 60 nanograms/5ml (.012 mg./liter) after eight months of ageing. During this same time period ethanol went from .02 mg./liter to .22mg./liter or from 100 to 1100 nanograms/5ml. Therefore one liter of diet soda has the methanol equivalent of approximately two tons of the highest methanol cheese tested.

M. O H

In Cheese

I. Production of volatiles and development of flavour during ripening

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SUMMARY. Cheddar cheeses were pressed from a batch of curd each month over period of 1 year to study the production of headspace volatiles and the development of flavour in ripening cheese. At 2-monthly intervals, samples taken from selected sites in the cheese were presented to a trained panel for assessment of quality and intensity of Cheddar flavour. Using a headspace technique samples of vapours from bore holes in different parts of the cheese were analysed for H_2S , methanethiol, acetone, butanone, methanol, ethanol and 2-pentanone. Most of the compounds increased in concentration during the ripening period, but ethanol and butanone varied in concentration in an unsystematic way. Possible precursors of the headspace volatiles are discussed.

Various workers have studied the ripening process in Cheddar cheese in an attempt to obtain relationships between concentrations of volatile compounds in the cheese and flavour. Kristoffersen, Gould & Harper (1959) analysed cheeses ripened for 1-22 months to determine changes in concentrations of fatty acids, amino acids, NH_3 and H_2S . Lawrence (1963*a*) also studied changes in concentration of H_2S in ripening Cheddar, but was unable to find a direct relationship between its concentration in the cheese and flavour. Manning, Chapman & Hosking (1976) in their limited study of ripening Cheddar were also unable to find a direct relationship between H_2S and flavour, but were able to show a correlation between methanethiol (CH_3SH) and intensity of Cheddar flavour. Among the compounds studied by Walker & Keen (1974) and Keen & Walker (1974) were the methyl ketones, diacetyl and 2-butanol; Walker & Keen concluded that a mixture of methyl ketones *per se* is unlikely to impart a typical Cheddar flavour to cheese, but from a consideration of individual threshold values, 2-heptanone, 2-pentanone and 2-nonane probably make a direct contribution to Cheddar flavour.

The purpose of this study was to determine how volatiles present in Cheddar cheese headspace are produced, how they are distributed in the cheese and whether their concentrations can be related to the development of flavour. By making cheeses at monthly intervals throughout the year it was possible to study 'seasonal' effects. Part I of this study is concerned with the production of volatiles and development of flavour; volatile-flavour relationships and seasonal effects will be reported in future publications. Over 150 cheeses were examined using a headspace technique

the introduction of artifacts implicit in other methods of extraction of volatiles. 10% of the cheeses were also evaluated for Cheddar flavour by a trained panel.

EXPERIMENTAL

Cheeses

Open vat. Cheeses were manufactured each month between January and December 1974 with bulk milk from one of the Institute's herds. The milk was heat-treated at 72.5 °C for 17 s and cooled to 30 °C before cheese-making using *Streptococcus cremoris* NCDO 924 as starter. The cheeses were made by the standard procedure followed in the NIRD Experimental Dairy (Chapman & Burnett, 1972). Curd from one vat was pressed into 12 cheeses each weighing approximately 4.5 kg, in the traditional cylindrical shape. Each cheese was coated with microcrystalline wax and stored at 13–14 °C.

Aseptic. In addition to the open vat cheeses described above, on 2 occasions, in March and August, cheeses were made under aseptic conditions using low-count pasteurized milk (Mabbitt, Chapman & Sharpe, 1959; Chapman, Mabbitt & Sharpe, 1966). The curd from each vat was pressed into 8 cylindrical cheeses weighing approximately 4.5 kg.

Flavour evaluation

Samples from the open vat cheeses were evaluated by the Institute flavour panel at 2, 4, 6, 8, 10 and 12 months of age, and from the aseptic cheeses at 2 monthly intervals up to 16 months; one cheese from each batch was examined on each occasion.

Nine cores 10-cm long and 2-cm diam. were removed from a flat surface of the cheese, and the portion from which each one was taken was marked on a plan of the cheese surface. Since each core was divided into an upper and a lower portion, the position within the cheese, from which a sample was taken for a particular panel member was known. The cheeses were evaluated by a panel of 14–18 members, who were asked to score the cheeses for Cheddar flavour quality (0, none–8, excellent) and intensity (0, none–8, very strong). Since a flavour profile of Cheddar cheese has not been determined, Cheddar quality is assessed by a panel against standard cheeses selected by experts to be of high quality and free from flavour defects. The panel assess the Cheddar flavour quality according to the presence or absence of desirable flavour notes, these notes being accepted attributes of the flavour of the standard cheeses. Panel members assessed the Cheddar flavour quality independently of intensity of flavour or texture. Panel members were also required to identify flavour defects and to record their intensities (0, none–4, very strong). Each panel member received one sample from the cheese in random order.

To prevent deterioration of the cheese due to oxidation or contamination, the bore holes were filled with molten wax shortly after the cores had been removed.

Samples for gas chromatographic analysis

headspace within bore holes has been described elsewhere (Manning *et al.* 1976). The cheeses were sampled at 7 bore holes arranged symmetrically across a flat surface of the cheese, the position of each bore hole being marked on a plan of the cheese surface.

Since it was necessary to allow the cheeses to warm from 13 °C, the temperature at which they were ripened, to room temperature before sampling, headspace analysis was only made once on each cheese. As a result, cheeses examined on subsequent occasions, although from the same batch, were different cheeses.

Duplicate samples in the range 0.25–1 ml of cheese headspace were taken for the analysis of S compounds and a single 5-ml sample for analysis of the non-S compounds. Volumes of up to 7 ml could be removed without significantly altering the composition of the vapour in the bore hole.

Two Pye 104 gas chromatographs (Pye-Unicam Ltd, Cambridge, England) were used for the analyses, one equipped with the standard flame ionization detector (FID), and the other with a sulphur-specific flame photometric detector (FPD).

The gas chromatograph equipped with the FID was used to analyse all of the headspace volatiles except H_2S , which is not detected by the FID, and CH_3SH which was usually present in too low a concentration to be detected. A 1.5 m \times 4 mm i.d. glass column packed with Carbowax 20 M on Universal B support 60–85 mesh was used and the gas chromatograph was operated isothermally at 50 °C, with a N_2 carrier gas flow-rate of 40 ml/min. A 1.75 m \times 4 mm i.d. glass column packed with Carbopack B-HT-100 mesh 40–60, a H_2 -treated, deactivated, graphitized carbon absorbent (Supelco Inc., Bellefonte, Pa., USA) was used to separate the S compounds. The gas chromatograph was operated isothermally at 60 °C with a N_2 carrier gas flow-rate of 40 ml/min. Since sensitivity of the FPD is dependent upon the flow-rate of H_2 and O_2 , the flow-rates were maintained throughout the experiment at 75 ml/min and 8.7 ml/min respectively.

Calibration of FID

The FID was calibrated with acetone, butanone, 2-pentanone, methanol and ethanol. Each compound (1 μl) was injected through a septum into a 5-l flask which was left for 10 min to ensure that the compounds were entirely in the vapour state. Between 0.1 and 1 ml samples of vapour were then removed from the flask and injected onto the GC column. Linear relationships were obtained between quantity of vapour injected and peak height. Further additions of individual calibrating compounds to the 5-l flask resulted in proportional increases in peak heights from equal volumes of vapour injected onto the column.

Calibration of FPD

The gases used for the calibration were H_2S , 99.6 % (British Drug Houses) and CH_3SH , 99.5 % (B.D.H. Chemicals Ltd, Poole, Dorset, England). The method of obtaining solutions of H_2S and CH_3SH for calibrating the FPD is illustrated in Fig. 1. With the valve, B, on, the cylinder closed, the glass tube, C, together with balloon, G, was evacuated through the 3-way stop-cock using a water pump. When

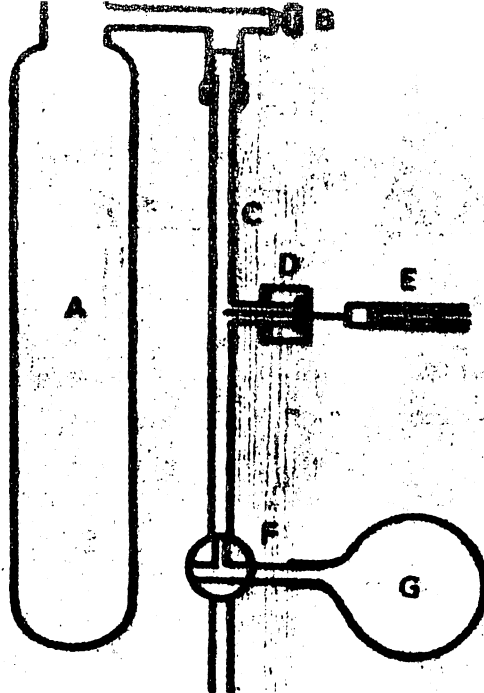


Fig. 1. Apparatus for removing samples from gas cylinders in the preparation of calibration solutions. A, gas cylinder; B, control valve; C, glass tube; D, septum; E, gas syringe; F, 3-way stop-cock; G, rubber balloon.

The valve on the cylinder was opened until the balloon was slightly inflated, and then closed, ensuring that the glass tube was flushed with the pure gas. One ml of gas was transferred to a glass jar almost full of absolute ethanol. With the jar inverted the gas was injected into the solution through a septum sealed into the cap. After mixing the contents of the jar, portions of 1–15 ml were diluted to 25 ml in a volumetric flask. One μ l samples of the dilute ethanol solutions were injected onto the Carbopack column. Plots of log peak height against log concentration were linear with a slope of 2 for both H_2S and CH_3SH .

The calibrations of the FID and FPD were checked at regular intervals during the 2 years of experiments.

RESULTS

Open vat cheeses

Panel evaluation. Scores allocated to cheeses after different periods of ripening are shown in Fig. 2. Since a batch of cheese was made every month over a period of 1 year, each point on the diagrams represents the mean score for 12 cheeses. It can be seen in Fig. 2(a) that maximum scores for Cheddar quality have been given to cheeses ripened for 8–10 months, whereas the intensity of Cheddar flavour continues to increase up to 10–12 months, as seen in Fig. 2(b).

The presence of flavour defects, noted by the panel, in cheeses ripened for different periods, is shown in Fig. 3. Only the most common flavour defects are shown in the Figure, together with changes in total defect, calculated by summation of all the individual defects, with ripening time. For young cheeses, ripened for less than 4 months, the main defect is bitterness, whereas for cheeses ripened for 8 months or more, fruitiness and rindiness are the major flavour defects.

A wide variation in panel scores was found for samples taken at different sites

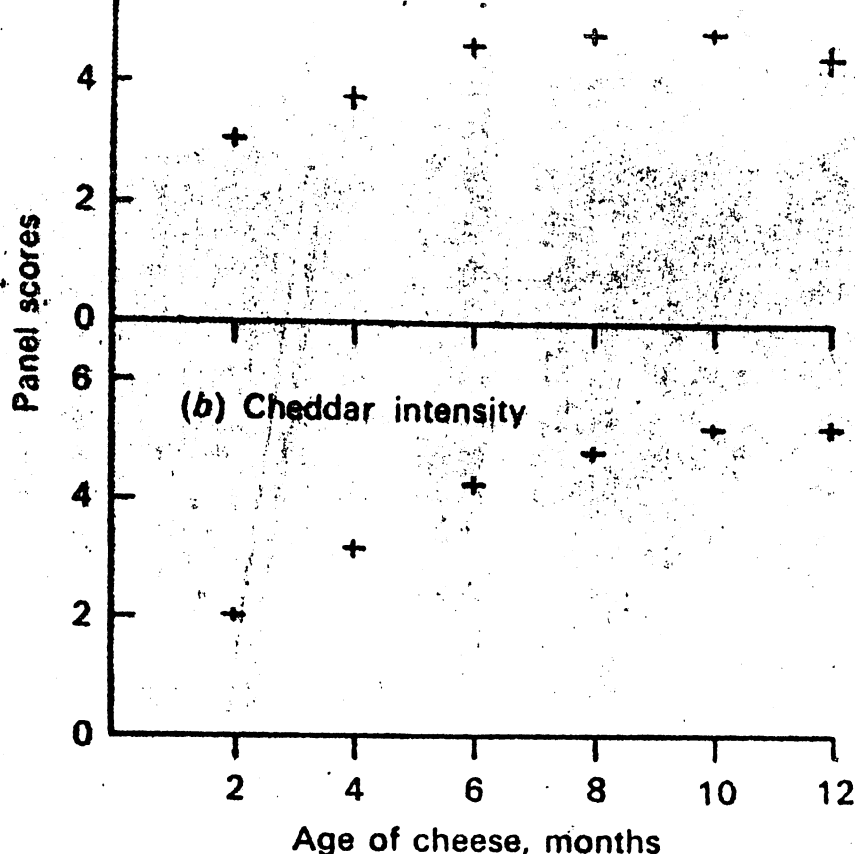


Fig. 2. Panel scores for Cheddar flavour quality (a) and intensity (b) of cheeses ripened for different periods: horizontal lines represent mean scores of 12 cheeses and vertical lines the standard error of the mean.

within the cheese, and calculated coefficients of variation for panel scores were in the range 10–60 %, although over 75 % of the values were in the range 20–40 %. Major flavour defects, such as fruitiness and rindiness, were found to be located in particular areas within the cheese, although bitterness, when present, was normally found distributed evenly throughout the cheese.

Volatile analysis. Concentrations of volatiles are given as calculated means of analyses of headspace samples from 7 sites across the surface of each cheese. Coefficients of variation for concentration across the surface of the cheese ranged from 5 to 80 %, although the majority were in the range 5–30 %. The largest variations were found with ethanol, butanone and H_2S , and the least with CH_3SH for which over 75 % of the values had a coefficient of variation of less than 15 %. Coefficients of variations up to about 5 % could be accounted for by errors in headspace sampling (Manning, 1976).

Concentrations of the sulphur compounds CH_3SH and H_2S in cheeses at different stages of ripening are shown in Fig. 4(a) and (b) respectively. Although each point in the diagrams is a mean of 12 results, it was found that the general trend of the curves was representative of each batch of cheese, although the absolute values of the concentrations varied. Only results obtained from cheeses not previously examined by the panel were used to show the relationship between H_2S concentration and period of ripening, because the concentrations were always lower in cheese which had been bored prior to headspace analysis. Changes in concentrations of methanol, acetone and 2-pentanone in the cheese headspace with age are shown in

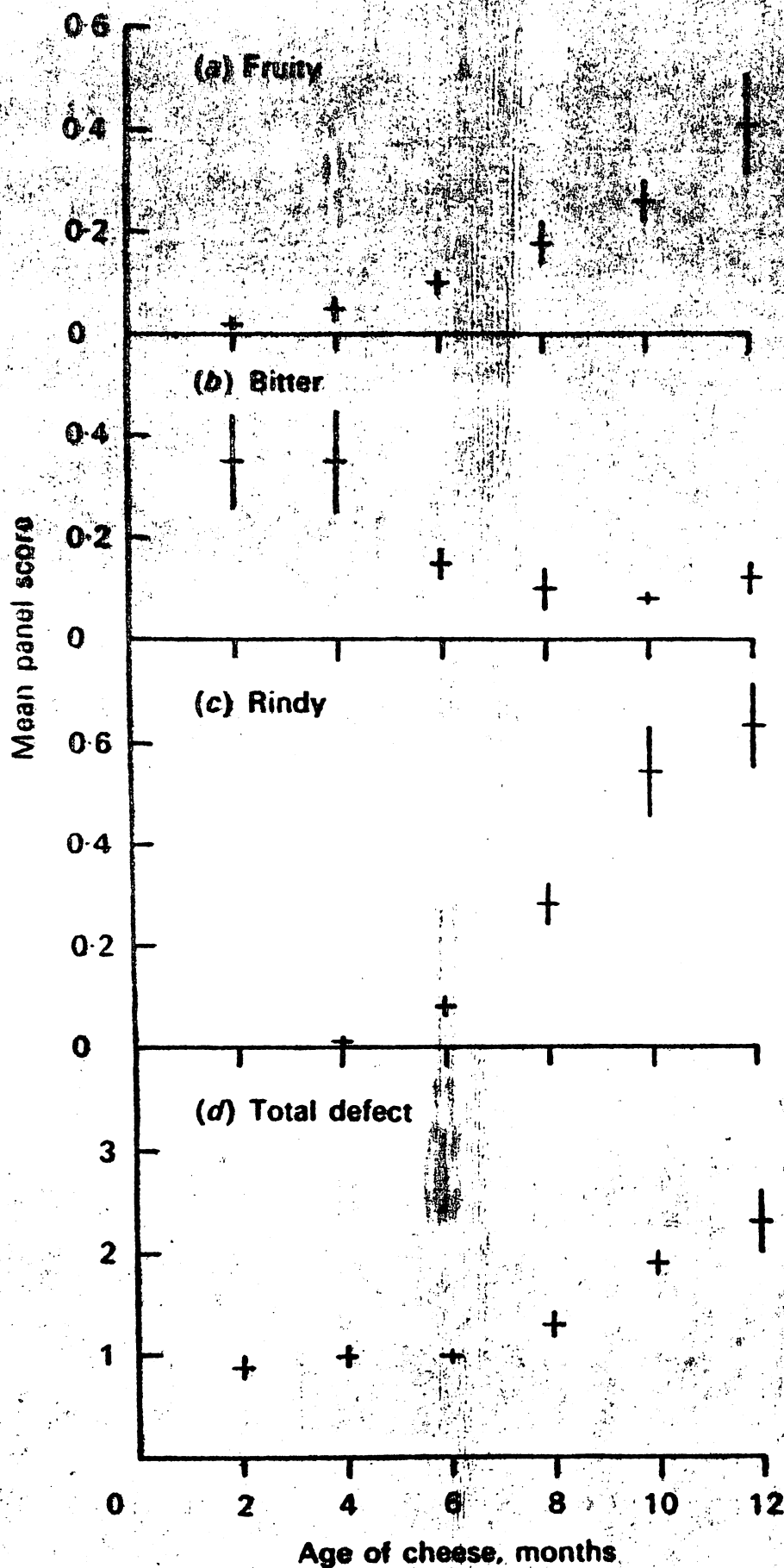


Fig. 3. Panel scores for different flavour defects in cheeses ripened for different periods: horizontal lines represent the mean scores of 12 cheeses and vertical lines the standard error of the means.

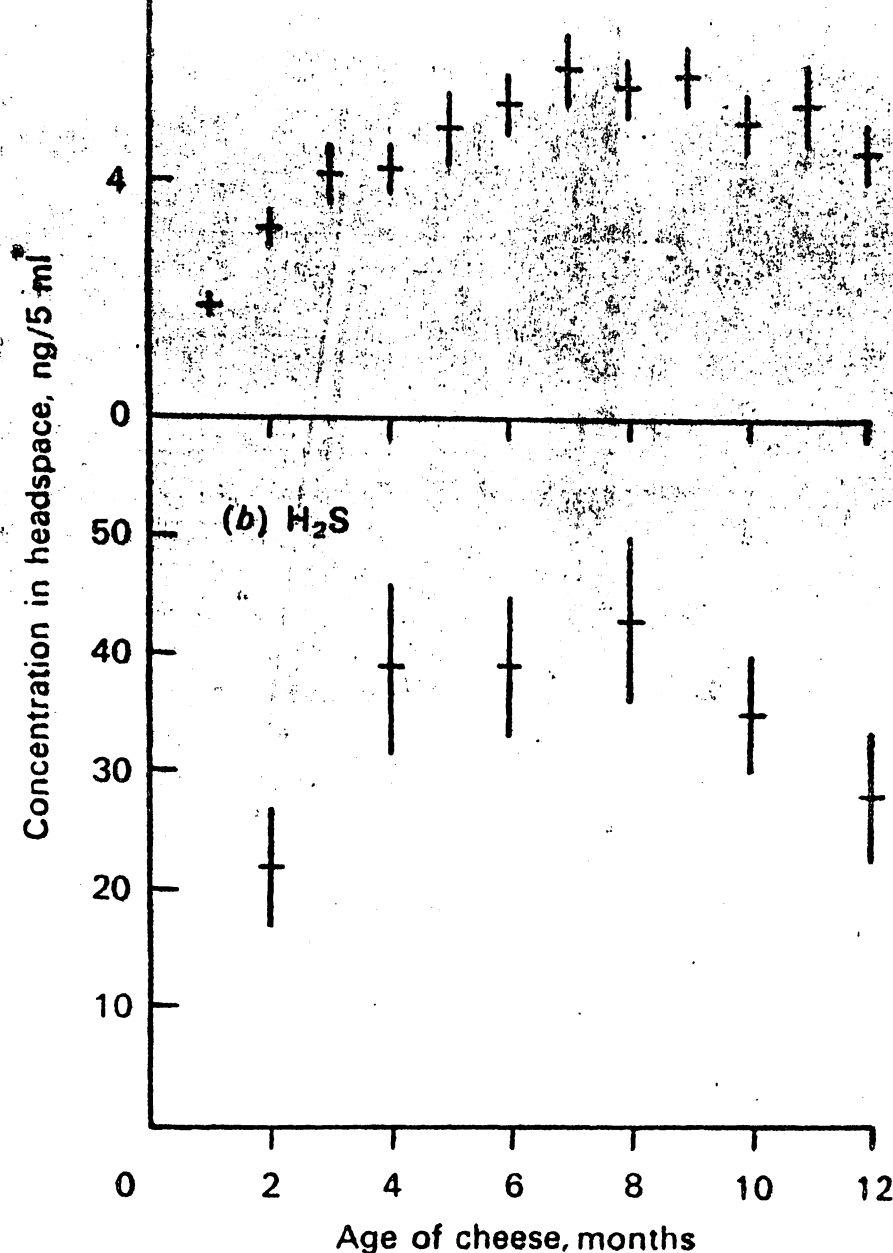


Fig. 4. Concentrations of (a) CH_3SH and (b) H_2S in cheeses ripened for different periods. horizontal lines represent the mean concentration of 12 cheeses and vertical lines the standard error of the means.

independent of the period of ripening. These 2 compounds were sometimes present in young cheese at the same concentration as in cheese ripened for a full 12 months. Butanone was present at concentrations in the range 100–200 ng/5 ml in some cheese, but virtually absent from others of the same age. A large variation in concentration of butanone (10–300 ng/5 ml) was often found within the same batch of cheese, whereas other batches contained consistently low levels (< 20 ng/5 ml).

Aseptic cheese

The 2 aseptic cheeses made in March and August showed sufficient difference in production of volatiles and development of flavour to prevent mean results from adequately describing either cheese. Panel scores and volatile concentrations at different stages of ripening are shown in Table 1. Some general comments are, however, valid. The aseptic cheeses did not attain as high scores for intensity of Cheddar flavour as open vat cheeses made in the same months (panel scores for

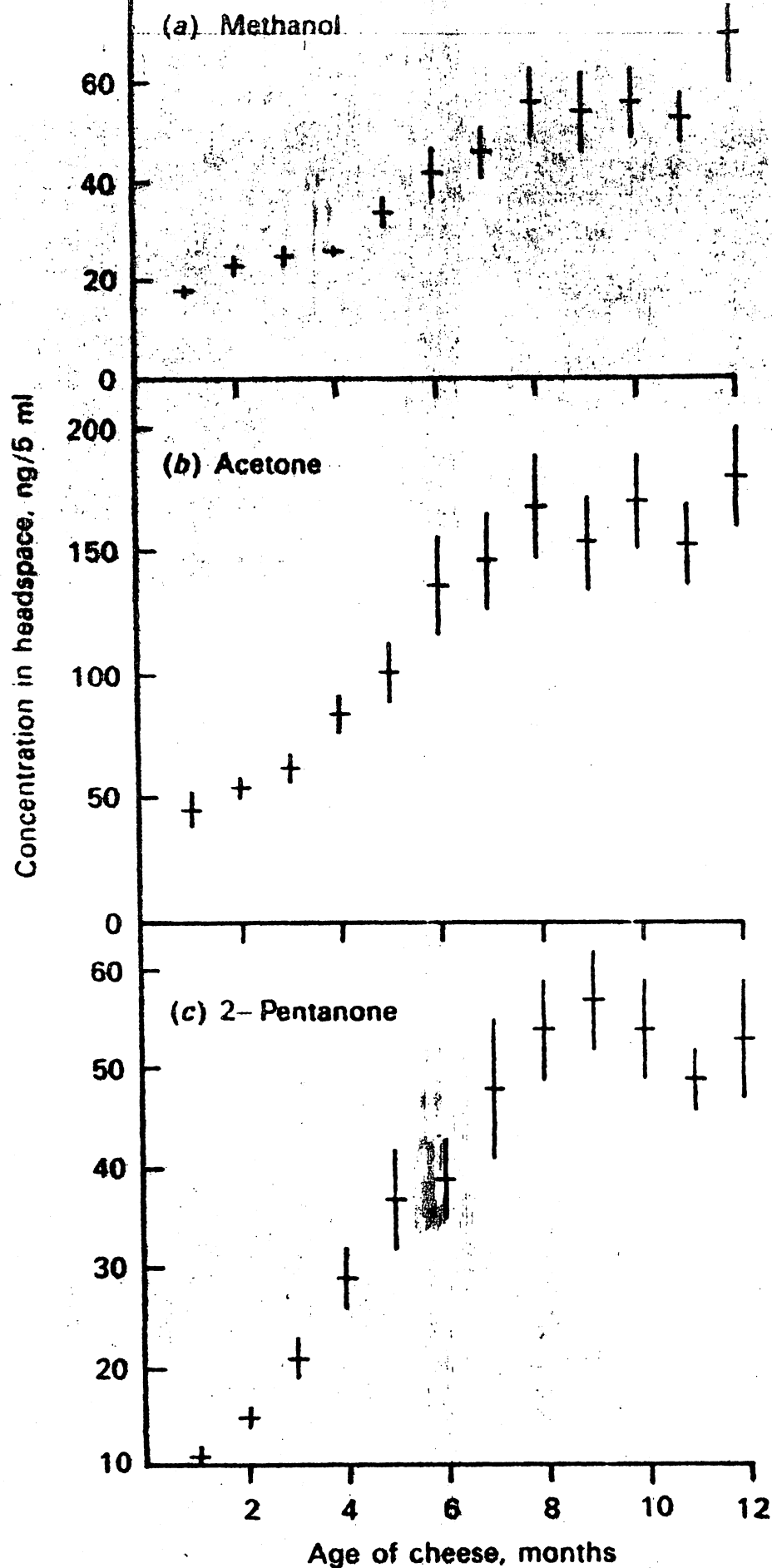


Fig. 5. Concentrations of (a) methanol, (b) acetone, (c) 2-pentanone in cheeses ripened for different periods: horizontal lines represent mean concentrations of 12 cheeses and vertical lines the standard error of the means.

Table 1. Panel scores and concentrations of headspace volatiles for aseptic cheeses made in March (M) and August (A): panel scores for open vat cheeses made in March and August are in parentheses for comparison

Age, months	Cheddar flavour score				Concentrations of volatiles, ng/5 ml headspace									
	Quality		Intensity		Methanethiol		Hydrogen sulphide		Methanol		Acetone		2-Pentanone	
	M	A	M	A	M	A	M	A	M	A	M	A	M	A
2	3.8 (3.8)	3.4 (3.1)	2.0 (2.1)	2.2 (1.8)	2.9	3.8	9	25	0	0	39	28	20	1
4	3.9 (3.9)	4.0 (4.4)	2.5 (3.4)	3.3 (3.8)	3.5	4.9	11	22	29	0	145	36	54	1
6	3.5 (4.2)	4.2 (4.6)	2.8 (3.9)	3.6 (4.2)	3.5	4.6	11	15	26	22	72	51	51	3
8	5.1 (5.2)	4.3 (5.0)	4.8 (5.4)	4.7 (5.2)	4.7	4.0	6	13	24	22	98	80	33	7
10	4.7 (5.2)	3.7 (4.6)	4.3 (5.6)	4.1 (5.4)	5.8	3.8	9	6	37	28	134	82	56	4
12	4.6 (5.1)	4.3 (3.1)	5.4 (5.8)	5.0 (4.3)	4.1	4.0	7	6	31	36	109	108	71	4
14	4.6 (—)	4.0 (—)	5.7 (—)	4.4 (—)	3.6	3.4	3	5	55	27	184	153	93	7
16	4.5 (—)	3.3 (—)	5.3 (—)	4.2 (—)	3.8	3.5	3	9	52	39	227	135	118	6

more slowly.

All the main flavour defects observed in open vat cheeses were also detected at similar stages of ripening in the aseptic cheeses, and were generally confined to localized areas within each cheese. Ethanol was also found in concentrations ranging from 100 to 1100 ng/5 ml in both batches of aseptic cheese, but as with the open vat cheeses, concentrations did not vary systematically with age of cheese. A major difference between the aseptic and the open vat cheeses was in the concentration of butanone. Although high concentrations of butanone were present in all of the open vat cheeses at some time during ripening, it was virtually absent from the August aseptic cheese and at very low concentrations in the aseptic cheese made in March.

DISCUSSION

It must be emphasized that cheeses studied for changes in flavour or in concentration of volatiles over a 12-month period were individual cheeses, although from the same batch of curd. Although there were differences in the composition of the different batches of cheese, they only affected the magnitude of the results, and the general pattern of behaviour did not differ substantially from Figs 2-5. With the exception of the 2 aseptic cheeses it was therefore possible to average the results obtained from a number of cheeses and still present an accurate picture of the production of volatiles and of the development of flavour in Cheddar cheese.

The tendency for the quality score to decrease after 10 months of ripening reflects the increase in total flavour defects which increase substantially after 8 months' ripening. It is clear that the slight development of some defects, e.g. fruitiness, was acceptable to some members of the panel, and it is possible that when evaluating the Cheddar cheeses, the panel found it difficult to differentiate Cheddar flavour intensity from total flavour intensity. It would appear from Figs 1 and 2 that the optimum ripening period to obtain maximum Cheddar flavour free from defects is about 8 months. Considering individual flavour defects, only bitterness decreased as the cheese matured, whereas the remainder, particularly rindiness and fruitiness, increased markedly with age.

The apparent changes in concentration of the H_2S with ripening time are different from those observed by Kristoffersen & Gould (1960) and Lawrence (1963*a*), and also appear to differ from those previously observed by the author (Manning *et al.* 1976). The apparent decrease in concentration of H_2S in the later stages of ripening might be due to its diffusion out of the cheese, although the possibility remains that it may be reacting with the compounds in the cheese (Badings, 1967).

Returning to the apparent discrepancies between the present results for H_2S and CH_3SH and those published previously (Manning *et al.* 1976), it was stated at that time that the results could only be regarded as tentative since the cheeses examined were not only of different ages, but were also made at different times of the year. It was not known then if a seasonal effect existed or what its magnitude might be. Although it is intended to report the seasonal effect in detail at a later date, it must be pointed out that, if cheeses are selected from this present experiment having the same age and made at the same time of the year as those reported in the earlier

development of Cheddar quality (Figs 4a and 2a).

The source of CH_3SH in ripening cheese is almost certainly β -lactoglobulin (rich in sulphur amino acids) (Hutton & Patton, 1952), but the mechanism leading to the production of CH_3SH is not known, although it has been possible to show that it is not produced by starter organisms. Although most of the β -lactoglobulin is lost in whey, sufficient is retained by the curd to account for the low concentrations of CH_3SH present in cheese.

Of the remaining volatiles that vary systematically with ripening time the ketones, acetone and 2-pentanone are probably produced by decomposition of β -ketoacids formed during the synthesis of fat (Lawrence, 1963b). The derivation of methanol in Cheddar cheese is unknown, but although its concentration in headspace is never as high as that of ethanol, its concentration increases steadily throughout the 12 months ripening. Ethanol is almost certainly formed from acetaldehyde, being the final product of carbohydrate fermentation, but it is not produced in any systematic way during ripening. That the concentrations of butanone varied widely between cheeses made from the same batch of curd, but not systematically with age, combined with the fact that it was always at a low concentration or absent from the aseptic cheeses tends to confirm the observations of Keen & Walker (1974) and Walker & Keen (1974) that butanone arises from the action of adventitious bacteria which had entered the vat during cheese-making. The findings in this present work confirm those of McGugan *et al.* (1968) who found over 50 times more butanone in their open vat cheese compared with the cheese made in a closed vat under aseptic conditions.

The general behaviour of the aseptic cheeses was not different from that of the open vat cheeses, apart from the concentration of butanone as mentioned previously. Flavour developed more slowly in aseptic cheese than in open vat cheese and the aseptic cheese reached their maximum flavour intensity later. Reiter *et al.* (1967) also showed that flavour was slow to develop in aseptic cheese although a strong Cheddar flavour was eventually produced. It may be significant that the concentration of H_2S in the aseptic cheese never reached the high concentrations found in the open vat cheese. Since the flavour defects perceived in the open vat cheese were also found in the aseptic cheeses, it is clear that these defects do not result from contamination by non-starter organisms.

The concentrations of various volatile compounds varied so much across a cheese that it would be expected that if any of them contributed to its flavour, flavour would be very variable through the cheese. The large coefficient of variation calculated for panel scores suggest that this heterogeneity of flavour exists.

The size of the cheeses used for these experiments (4.5 kg) is not typical of commercial cheeses, but since rind formation extends less than 5 mm into the cheese, it is believed that the results are representative of larger cheese.

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