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CHANGES IN THE LEVELS OF 2-PHENYLETHYLAMINE IN CHEESE AND CHOCOLATE DURING PROCESSING AND STORAGE

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ABSTRACT

Amines from cheese and chocolate were extracted with solvent and separated from non-basic material on an ion-exchange column. The amines were reacted with trifluoroacetic anhydride after the addition of aminotetrahydronaphthalene as an internal standard. The derivatives were analysed on a SCOT column by gas chromatography. The levels of phenylethylamine increased in cocoa beans during fermentation and roasting. Some samples of cheese contained phenylethylamine, whereas others prepared aseptically did not.

INTRODUCTION

A recent publication (Koehler & Eitenmuller, 1978) on the analysis of 2-phenylethylamine in some foods, prompts us to report on some similar results obtained in these laboratories during 1975. A part of this work has already been published (Chaytor *et al.*, 1975). The present report gives the method for quantitative analysis of 2-phenylethylamine.

The relationship between the consumption of certain foods and the incidence of migraine has been known for nearly 200 years (Fothergill, 1784). Although the subject of dietary migraine continued to be of interest, it was not until recently that controlled scientific investigations were made on the subject (Smith *et al.*, 1970).

2-Phenylethylamine has been shown to initiate migraine attacks in sufferers (Sandler *et al.*, 1974). Methods for the extraction of amines from foods have been published (Chaytor & Saxby, 1975) and 2-phenylethylamine has been positively identified in plain chocolate and some cheeses (Chaytor *et al.*, 1975).

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EXPERIMENTAL

Method

Isopropanol (400 ml), dichloromethane (200 ml), aqueous 4 M potassium carbonate solution (150 ml) and finely grated sample (150 g) are added to a 1-litre separating funnel. The mixture is homogenised for 30 min with a Silverson mixer–emulsifier (laboratory model) fitted with a 1-in tubular disintegrating head and running at full speed.

The homogenate is left to settle for about 30 min. The organic solvents, containing all basic and neutral components of the sample, separate as a discrete phase above the homogenate. These are pumped through a filter tube and through a phase-separating paper into the reservoir above the ion-exchange column of Amberlyst 15.

The sample passes down the column at a rate of about 250 ml/h. The column is washed successively with three solvent mixtures: (a) 90 ml isopropanol–dichloromethane (2:1); (b) 50 ml isopropanol; and (c) 80 ml acetone–water (65:15).

All the basic material is removed from the column by elution with 250 ml 1 M hydrochloric acid (in acetone). The elution takes 1 h to complete.

The eluate is evaporated to dryness on a vacuum rotary evaporator, set initially at 30 °C until most of the organic solvent has been removed and then at 60 °C to remove the aqueous phase.

The residue is washed with ether which is decanted and discarded. Trifluoroacetic anhydride, dissolved in dry ether, is added to the residue suspended in dry ether. Contact between the residue and the anhydride is maintained for at least 30 min at room temperature. A solution of 1-amino-5,6,7,8-tetrahydronaphthalene in dry ether (500 μg in 500 μl) is added as an internal standard together with further trifluoroacetic anhydride. The solution is cautiously washed with 2·5 M potassium bicarbonate solution, rigorously dried over sodium sulphate and evaporated to about 0·2 ml. The analysis is undertaken by gas chromatography on a SCOT column (50 ft × 0·02 in i.d.) of diethylene glycol succinate operated isothermally at 140 °C. Detection is by flame-ionisation.

A calibration graph is obtained by fortification of samples of Coberine or cottage cheese with phenylethylamine and determination of the ratio of peak areas due to the amine and the added internal standard. The calibration graph for Coberine is shown in Fig. 1.

Figure 2 shows a typical chromatogram which is obtained from a chocolate extract.

Samples

Some cheeses were obtained directly from manufacturers and others were bought from local supermarkets. In the former case, the date of production was known, but cheeses bought locally were arbitrarily assumed to be two months old.

The 'aseptic cheese' was provided by the National Institute for Research in

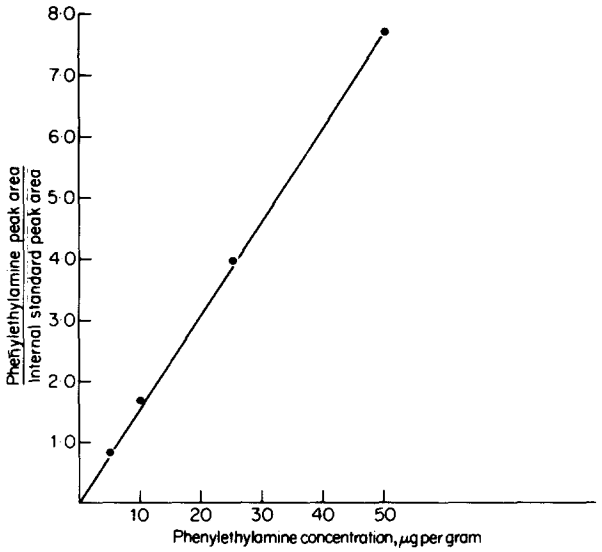


Fig. 1. Calibration curve for phenylethylamine in Coberine.

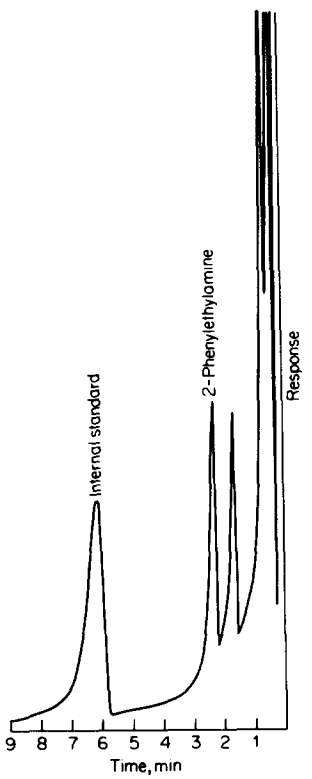


Fig. 2. Chromatogram of chocolate extract.

TABLE 1
LEVELS OF 2-PHENYLETHYLAMINE IN CHEESES DURING MATURATION

Cheese type	*Age months*	*Level ppm*	*Age months*	*Level ppm*	*Age months*	*Level ppm*	*Age months*	*Level ppm*	*Age months*	*Level ppm*
Wensleydale	2	6	5	13	7	10	8	16	9	16
Dutch Gouda	2	4	5	4	7	<4	8	<4		
English Cheshire	3	6	6	<4	11	18				
Blue Stilton	6	<4	8	<4	9	<4				
Leicester	3	<4	6	14	8	12				
Double Gloucester	4	8	9	7	9	<4				
Dutch Edam	2	10	6	14	7	14				
Mild English Cheddar	2	0	5	0						
Caerphilly	2	0	5	0						
Camembert	2	0	5	0						
St Paulin	2	0	5	0						
Dutch Jaarlsberg	2	0	5	0						
[a]English Cheddar No. 3	4	18	6	22						
English Cheddar No. 4	5	24	9	40						
Cottage cheese	2	0	9	0						
Aseptic cheese No. 1	<6	0	<8	0						
Aseptic cheese No. 2	<6	0	<8	0						
[a]English Cheddar No. 1	4	0								
[a]English Cheddar No. 2	4	0								
Danish Blue	2	0								

Cheeses stored at 4°C except where indicated.
[a] Cheeses stored at room temperature.

TABLE 2
CONCENTRATION OF 2-PHENYLETHYLAMINE IN COCOA BEANS OF DIFFERENT ORIGIN AND TREATMENT

Country of origin	*Sample*	*Concentration ppm*
Unknown	Unfermented beans	0
Unknown	Fermented, unroasted beans	<2
Unknown	Fermented, roasted beans	13
Trinidad	Unroasted shell	<2
	Unroasted beans	<2
	Roasted beans	2
Ghana	Unroasted beans	<2
	Low-roast beans	8·6
	Medium-roast beans	9·8
	High-roast beans	12
New Guinea	Unroasted beans	<2
	Roasted beans	<2
Venezuela	Unroasted beans	<2
	Roasted beans	<2
Ecuador	Unroasted beans	<2
	Roasted beans	<2

Dairying, Reading, and consisted of cheese which had been prepared under strictly aseptic conditions from a single starter.

Cocoa beans and chocolate samples were supplied by British manufacturers.

RESULTS

2-Phenylethylamine levels in cheese, cocoa beans and chocolate are given in Tables 1, 2 and 3.

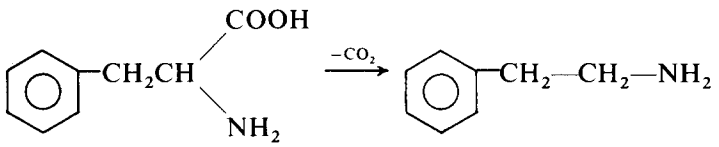
TABLE 3
CONCENTRATION OF 2-PHENYLETHYLAMINE IN CHOCOLATE CONSTITUENTS AND CHOCOLATE TYPES

Sample	*Concentration ppm*
Cocoa butter	0
Cocoa nibs	8·3
Finished chocolate	2·6
Plain chocolate A	<2
Plain chocolate B	3
Plain chocolate C	12
Plain chocolate D	2
Milk chocolate A	2·5
Milk chocolate B	5·9
Milk chocolate C	2
Milk chocolate before conching	<2
Milk chocolate after conching	<2

DISCUSSION

The levels of 2-phenylethylamine in all varieties of cheese range from 0 to 40 ppm. It is of interest to note that the highest values are considerably above those found during investigations into chocolate, in which the highest observed value was 12 ppm. There is a particular interest in the fact that chocolate is always considered to be a more severe migraine potentiator than cheese and suggests that chocolate contains a further migraine precipitant, which may not be present in cheese.

A possible precursor for 2-phenylethylamine is the amino acid phenylalanine.



Investigations (S. Berridge, pers. com.) into the amino acid composition of Cheddar cheeses during ripening show that a typical cheese contains about 1000 ppm of phenylalanine after 6 months' storage. Hence, it is clear that a cheese would contain more than sufficient phenylalanine to yield the quantities of phenylethylamine detected. However, all Cheddar cheeses exhibited gradual autolysis and phenylalanine was found in all the cheeses examined.

It would therefore appear that certain cheeses lack a specific enzyme necessary for the conversion of phenylalanine to phenylethylamine. Cheeses prepared from a single starter and produced under strictly aseptic conditions failed to produce phenylethylamine; this further emphasises the need for a specific enzyme in the formation of the amine. Table 1 also indicates that cheeses which exhibited no phenylethylamine at the beginning of the storage experiment, did not start to produce the amine on extended storage. Once again the absence of a specific enzyme is suggested. Furthermore the cheeses labelled Cheddar Nos. 1, 2 and 3 were also made from single starters, though the progress of the production was not necessarily performed under aseptic conditions. Two of these cheeses contained no phenylethylamine.

Most cheeses which do contain 2-phenylethylamine, show either a fairly steady concentration or a slight increase in concentration of the amine during storage. Several cheeses, after storage for 6–9 months at 4 °C exhibited mould growth on cut edges, whilst the three cheeses stored at room temperature became ammoniacal and fishy. The evidence suggests that 2-phenylethylamine (if present at all) is formed in the early stages of cheese manufacture and subsequent microbial activity may only enhance the concentration, if the necessary enzyme is present in the early stages.

The levels of phenylethylamine found in this study lie within the range found by Koehler & Eitenmuller (1978).

The concentration of 2-phenylethylamine in cocoa beans of different origin and

treatment is shown in Table 2. Before fermentation the beans contain no detectable amount of phenylethylamine. The process of fermentation leads to a low concentration of phenylethylamine, which increases several fold during roasting. It seems probable that the source of 2-phenylethylamine is again phenylalanine, which decarboxylates most readily under the influence of heat in the roasting stage.

Table 4, adapted from Rohan (1964) and Pinto & Chichester (1966), shows the levels of phenylalanine in cocoa beans at various stages of production. It shows that the concentration of phenylalanine rises to a maximum after fermentation and then decreases during roasting; this is consistent with the formation of 2-phenylethylamine from phenylalanine by decarboxylation. During the latter process, the concentration of phenylalanine falls by 1000 ppm, so that even if only about 1 % is due to decarboxylation, about 10 ppm of 2-phenylethylamine would be formed.

TABLE 4

CONCENTRATION OF PHENYLALANINE IN COCOA BEANS

Type	*Level in mg/100 g*
Unfermented	28
Fermented	403
Unroasted	432
Roasted	332

Tables adapted from Rohan (1964) and Pinto & Chichester (1966).

The remainder of the phenylalanine probably undergoes a Maillard reaction with reducing sugars yielding compounds which contribute to the flavour.

The results in Table 2 for Ghanaian cocoa beans show that the higher roasting yields higher levels of phenylethylamine. The table also shows that cocoa beans of different origin can differ considerably in their content of phenylethylamine.

Table 3 shows that the phenylethylamine is associated with the non-lipid portion of the cocoa bean, since none is found in cocoa butter.

The level of phenylethylamine varies from about 2 to 12 ppm in plain chocolate and 2 to 6 ppm in milk chocolate. These figures are in good agreement with those obtained by Schweitzer *et al.* (1975).

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