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Production of Phenyl Ethyl Alcohol and its Esters During Ripening of Traditional Camembert

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ABSTRACT

During raw milk camembert manufacturing, the production of phenyl ethyl alcohol and its esters reached a maximum after 1–2 weeks of ripening. Thereafter the concentration decreased to a level of about 1 ppm at the end of ripening. Spraying of cheese surfaces with a phenylalanine solution increased the observed maxima, but did not markedly change the concentrations at the end of ripening. The detection thresholds of phenyl ethyl alcohol, phenyl ethyl acetate, phenyl ethyl propanoate, determined in a model curd, were 8, 20, 18 ppm (odour thresholds) and 9, 18, 17 ppm (taste thresholds), respectively. The amounts present in raw milk camembert ripened for a long period were lower than these mean thresholds, but corresponded to the detection thresholds of the most sensitive panelists.

INTRODUCTION

Traditional camembert cheese manufactured with raw milk has a marked aroma in which trained panelists can detect different notes.

The characteristic flavour of this cheese is due to the presence of 1-octen-3-ol (a mushroom-like flavour; Dumont *et al.*, 1974*b*, 1976), sulphur compounds (garlic flavour; Dumont *et al.*, 1976; Cuer *et al.*, 1979) and ammonia. Furthermore, some panel members detect a floral flavour. In an earlier study on neutral volatile compounds, Dumont *et al.* (1974*a*) showed that the main volatile compounds in the cheese appeared to be methylketones and the corresponding secondary alcohols, but noted also

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the presence of a substantial amount of 2-phenyl ethyl alcohol (2-PE) and 2-phenyl ethyl acetate (2-PEA). These two compounds produce a floral flavour and might contribute to the final flavour of a raw milk camembert (Adda *et al.*, 1978).

The purpose of the present study was to determine, more accurately, the role of 2-PE and its esters in camembert aroma. Odour and taste thresholds of 2-PE, 2-PEA and 2-phenyl ethyl propanoate (2-PEP) were evaluated in a model curd after amounts of these compounds were estimated in traditional cheese. Furthermore, an attempt was made to enhance the production of these compounds during ripening by spraying the cheese surface with the amino acid precursor, i.e. phenylalanine.

MATERIAL AND METHODS

Odour and taste thresholds of 2-PE and its esters

Thresholds were determined in a model curd using a 5-fold concentrated skim milk retentate to which 20 g milk fat was added for 100 g final product. 2-PE and its esters (Fluka AG, GC purity 99%) were added at the same time as rennet and the mixture was moderately stirred for 1 min. After 1/2 h the curd was cut and portioned out (25 g) in tasting glasses covered with watch-glasses. Samples were equilibrated for 30 min at 20°C before the session. The panel of judges consisted of 25 members selected among 35 persons of the laboratory staff, after a period of training to the recognition of odour and taste of the compounds in the model curd. The training sessions took place thrice a week, preferably in the morning, in a room specifically designed for sensory evaluation. Multiple paired-sample tests were conducted according to the procedure described by Guadagni *et al.* (1973, 1974). Each pair included a spiked and unspiked randomized sample. At each session two pairs were presented, the first having the higher concentration of added substance. Each judge was asked to test and qualify the odour and taste of the samples and to detect the spiked sample. For these threshold measurements, decreasing logarithmic concentrations were used and at each session the lower previous concentration was included in the first pair. So, each judge tested each concentration twice.

The choice of a paired test with forced selection results in a symmetry of errors (Sauvageot, 1982). The calculation could then be made on true correct responses, i.e. responses corrected for chance according to the formula:

$$P = 2p - 1$$

where $p = R/N$ proportion of correct responses; $N =$ total number of responses; $R =$ number of observed correct responses.

The above-chance correct responses were converted into Z-scores. Using the unweighted least-square solution, the regression lines between Z-scores and the logarithm of the concentrations were calculated. The thresholds were measured from regression lines, considering these values as the concentrations producing a frequency of 50% correct above-chance (i.e. a Z-score = 0) as suggested by ASTM (1968) and Guadagni *et al.* (1973).

Cheeses

Controlled and experimental camembert cheeses were made in a Normandy dairy factory. The curd was moulded and dry-salted according to traditional manufacturing and ripening methods. Samples were taken at regular intervals during the 30-day cheese ripening period.

To increase 2-PE production, the surfaces of some cheeses were sprayed with a solution of phenylalanine at 2, 5 and 7 days after cheese making. One millilitre of solution (28 mg/ml) was uniformly distributed over the whole cheese surface, i.e. about 84 mg phenylalanine per cheese after three sprayings. Control cheeses were sprayed with the same volume of water.

Determination of the quantity of 2-PE and its esters in cheese

The analysis was made according to techniques already described by Dumont *et al.* (1974*a*) with the following main steps.

Frozen cheese was finely grated prior to vacuum extraction (10^{-4} torr). Volatile substances were condensed in traps cooled with liquid nitrogen. Trap contents were brought to pH 9·0 and the neutral compounds extracted by dichloromethane whereafter they were successively concentrated on an adiabatic column and Dufton's microcolumn until about 50 μl extract were obtained.

The dichloromethane extract was separated with a Girdel 3000 gas chromatograph (Delsi Instruments, Suresnes, France) fitted with a flame ionization detector and a capillary pyrex glass column (54 m × 0·4 mm id, SE-54 immobilized stationary phase). After an isothermal period of 10 min at 30°C, the temperature was programmed from 30 to 140°C at a rate of 20°C/min. The column was operated with hydrogen carrier gas (1·5 ml/min). The peak areas and the retention times were measured using an integrator (Minigrator Spectra physics). Compounds were identified by matching retention time data with those of pure chemicals or internal control injections. 2-PE and its esters were quantified after determination of the yield of analysis by addition of pure substances either to the model curd (1, 10 or 100 ppm) or at different stages of extraction and concentration. It appeared that the overall yield for each of the three compounds was 10%.

So, we used a corrective factor of 10 for calculating the true amount in the cheese.

Free phenylalanine content of cheese

As phenylalanine was sprayed on the surface of the curd, analyses were only made in the external part (up to 8 mm under the rind).

A sample of 10 g cheese was ground at 40°C in 40 ml of distilled water using an Ultraturrax tissue macerator. The suspension was precipitated by trichloracetic acid at a final concentration of 12%. The acid was eliminated from the supernatant by ether extraction and the amino acid composition determined by an amino acid analyzer (Beckman Multichrom).

RESULTS

Detection thresholds of 2-PE and its esters

The taste threshold of 9·1 ppm for 2-PE was about half that of its esters (18·5 ppm for acetate and 16·8 ppm for propanoate) (Table 1). This trend, a little more marked, was also observed for the odour, i.e. a threshold of 7·6 for alcohol against 19·8 and 18 ppm, respectively, for acetate and propanoate.

For any of these compounds the taste and odour threshold values were almost the same, the differences being only 1·5 ppm for 2-PE, 1·3 ppm for acetate and 1·2 ppm for propanoate. However, phenyl ethyl alcohol was more readily detectable by smelling than by tasting (7·6 versus 9·1 ppm) whereas the reverse was true for the esters. Besides, as is usually the case in

TABLE 1
Taste and Odour Thresholds of Three Compounds in Cheese for 25 Panelists

Compounds	*Number of tested concentrations*	*Taste detection*		*Odour detection*	
		Threshold (ppm)	*Coefficient of correlation (r)*	*Threshold (ppm)*	*Coefficient of correlation (r)*
2-Phenyl ethyl alcohol (2-PE)	9	9·1	0·959	7·6	0·883
2-Phenyl ethyl acetate (2-PEA)	7	18·5	0·954	19·8	0·985
2-Phenyl ethyl propanoate (2-PEP)	6	16·8	0·953	18	0·952

threshold experiments, there were large variations between the panel members. Among the 35 persons selected in the preliminary training sessions, 5 had no flavour sensitivity even at 2-PE concentrations of 200 ppm, i.e. 20-fold higher than the threshold of the final group of panelists.

Conversely, in the group of 25 persons participating in the threshold determination tests, 4 exhibited a 10-fold lower perception threshold than the group average value. Their threshold level of 2-PE was about 1 ppm and 2 ppm for the esters. At any concentration (even the highest concentration), all these compounds had a floral odour with a typical wilted rose, rosewater trait in the presence of 2-PE rather than in that of its esters, which were more characterized by terms like hyacinth, perfume, viny.

With respect to taste, a chemical note was detected at high concentrations, e.g. sweet, perfume-like, pharaceutical, acidulated, fruity, bitter. At concentrations close to their perception threshold, the flavour became more and more floral.

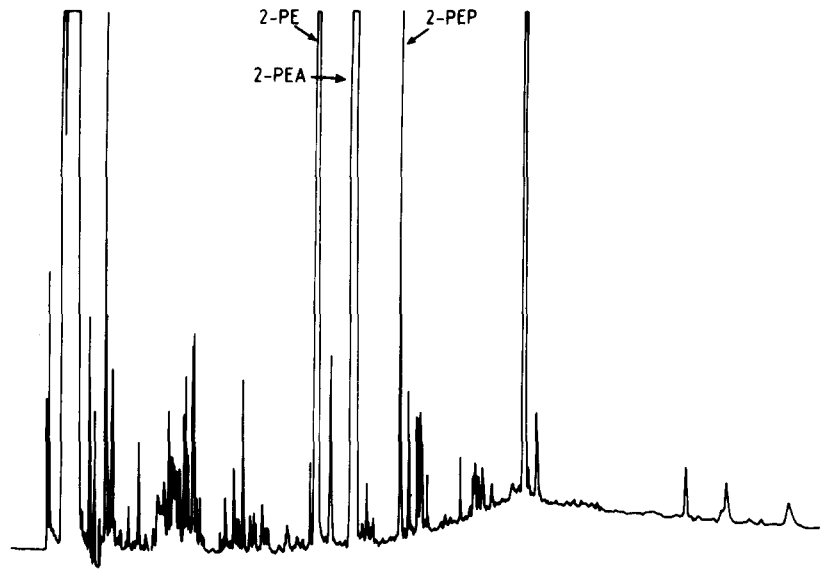
Production of 2-PE and its esters during ripening

The proportion of 2-PE and 2-PEA relative to that of the other neutral volatile cheese components is clearly shown in the two chromatograms obtained from camembert after 7 and 30 days of ageing (Fig. 1a and 1b). Already after 7 days, 2-PEA constituted the major compound of the cheese (4·6 ppm) followed by 2-PE (1·15 ppm). The amounts of these compounds then exceeded those of methylketones and the corresponding secondary alcohols. After 30 days ripening (Fig. 1b), these two compounds were still present in rather large amounts (about 1 ppm) although not representing the major cheese volatile compounds.

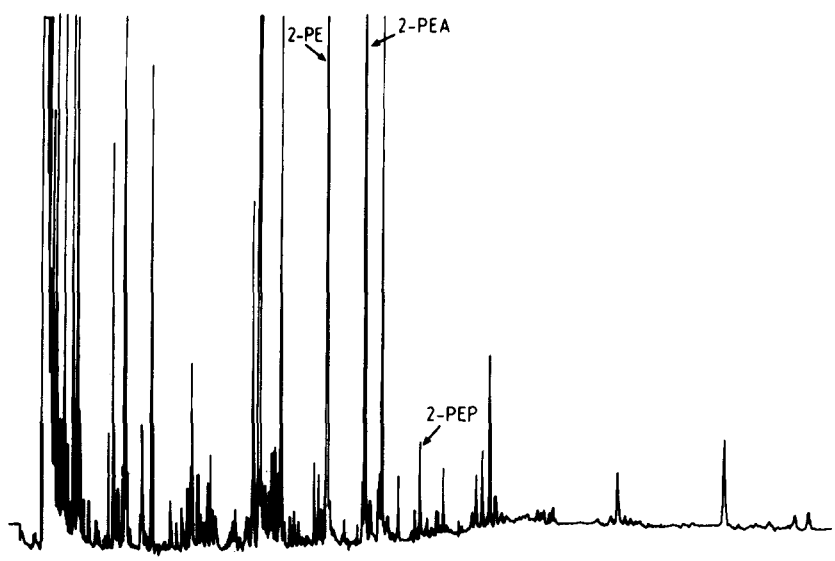
Figure 2 shows the changes in 2-PE contents and its esters during ripening of a traditional raw milk camembert. It may be noticed that the amount of acetate increased during the first week of ripening whereafter it decreased and remained at a concentration of about 1 ppm till the end of the process. 2-PE appeared much more slowly than 2-PEA in the cheese and reached its maximum concentration after 2 weeks of ripening (1·7 ppm). Between 12 and 18 days, the amount of 2-PE slightly exceeded that of 2-PEA whereafter it decreased and became stabilized around 1 ppm.

A very small amount of 2-phenyl ethyl propanoate was found in all analyzed cheeses. Its production maximum occurred after 7 days (0·15 ppm).

A quantification of PE and 2-PEA was made in two other raw milk camembert series after 40 days of ripening. In one of these, the amount of each compound was 1 ppm and in the other one there was 6 ppm of 2-PE and 1·5 ppm of 2-PEA. There seemed thus to be variations in these contents according to cheese making.



(a)



(b)

Fig. 1. Chromatographic profiles of cheese extracts after (a) 7 and (b) 30 days of ripening. Position of 2-phenyl ethyl alcohol (2-PE), 2-phenyl ethyl acetate (2-PEA) and 2-phenyl ethyl propanoate (2-PEP) peaks is indicated on the Figures. Figure 1a represents 14 g of cheese. Figure 1b represents 10 g of cheese.

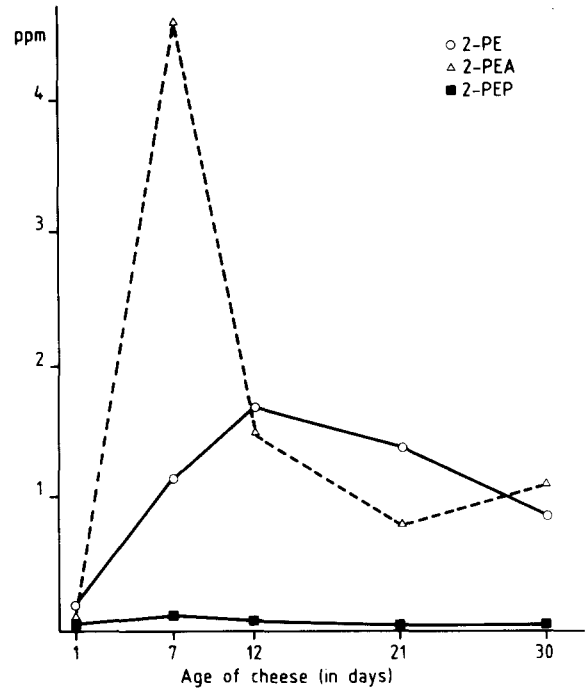


Fig. 2. Development of 2-phenyl ethyl alcohol (2-PE), 2-phenyl ethyl acetate (2-PEA) and 2-phenyl ethyl propanoate (2-PEP) during ripening of traditional camembert. Amounts are expressed in ppm (mg/kg cheese).

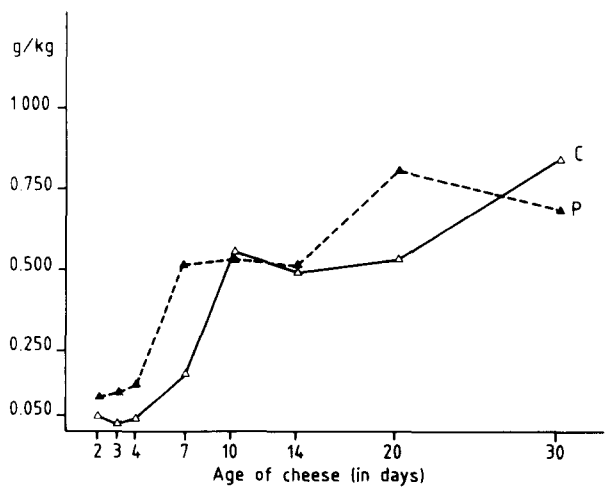
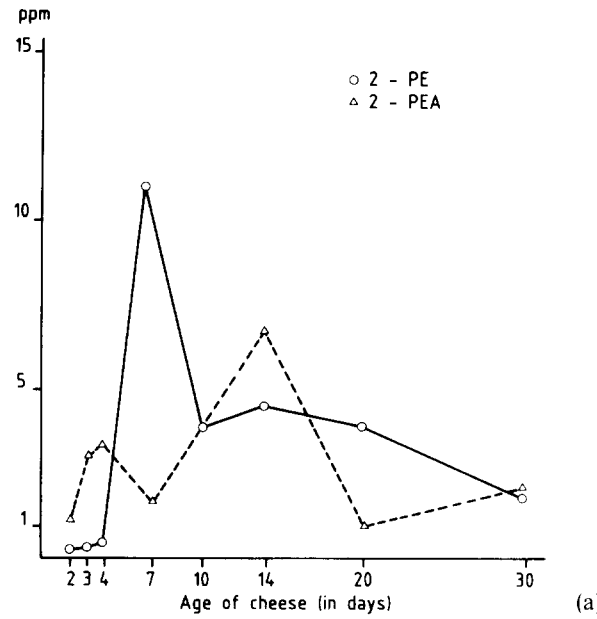
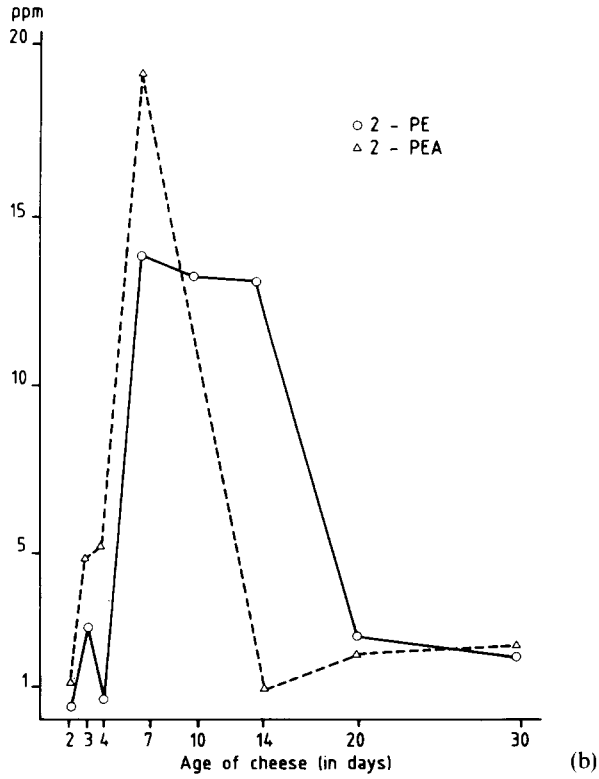


Fig. 3. Change in the concentration of free phenylalanine in the external cheese zone during ripening. P: cheese surface sprayed with phenylalanine. C: control (cheese surface sprayed with distilled water).



(a)



(b)

Fig. 4. Change in the concentration of 2-phenyl ethyl alcohol (2-PE) and 2-phenyl ethyl acetate (2-PEA) during ripening of cheese sprayed with water (Fig. 4a) or with a phenylalanine solution (Fig. 4b). Amounts are expressed in ppm (mg/kg cheese).

Stimulation of 2-PE production

Free phenylalanine concentrations measured after 30 days of ripening (Fig. 3) were analogous to those observed by Do Ngoc *et al.* (1971) in raw milk camembert, but higher than those measured by Ismail & Hansen (1972) in Danish camembert. In control cheese, the concentrations were lower than 50 mg/kg during the first 4 days, but reached 150 mg/kg after 7 days. The concentration was about 3-fold higher in cheese sprayed with a phenylalanine solution (Fig. 3), but the difference between the two cheeses disappeared after 10 days.

Figures 4a and 4b show the production kinetics of 2-PE and its esters with or without phenylalanine spraying on the cheese surfaces. Production maxima of 2-PE were observed after 7 days and those of 2-PEA after 7 or 14 days. These maxima were about 2-fold higher in cheese sprayed with phenylalanine. However, at the end of the ripening process the values were almost the same in control and test cheese.

As regards the control cheese, 2-PE and 2-PEA production maxima and their levels at the end of ripening (Fig. 4a and b) were substantially higher than in the previous manufacturing (Fig. 2). This might be due to water spraying on the cheese surface or more probably they merely reflect variations from one manufacturing to another. Indeed, as camembert cheese is made from raw milk, one has to realize that substantial variations can occur between cheese making performed at different periods of the year.

Amounts of 2-PEP remained very low (below 0·1 ppm) throughout the ripening process.

DISCUSSION

It has been well established (Powers & Quinlan, 1973; McNulty, 1975) that the sensory odour threshold of an aromatic compound depends on the medium. This is the reason why our determinations of odour and flavour thresholds were made in a curd. The 2-PE threshold determined here (7·6 ppm) was, however, very close to that found by Salo (1970) and Salo *et al.* (1972) in a water/alcohol medium (7·5 ppm). The findings of Powers & Quinlan (1973) were a little more different from our values and those of Salo; he found odour thresholds of 41·1 ppm in water, 33·4 ppm in 10% alcohol and 174·4 ppm in corn oil. As in our study, Powers & Quinlan (1973) also found large individual variations in 2-PE detection values with extreme thresholds ranging from 0·18 to 400 ppm in water and from 25 to 600 ppm in oil. The lowest thresholds obtained by Powers & Quinlan (1973) were similar to those of Sheldon *et al.* (1971), i.e. 0·2 ppm in water and 0·07 ppm in skimmed milk.

With regard to 2-PEA, 0·65 ppm for the odour threshold determined by Salo (1970) in a water/alcohol medium was rather different from our value obtained in a lipid/protein/water medium, i.e. 19·8 ppm.

After a long ripening period, amounts of 2-PE and 2-PEA ranged around 1 ppm. These concentrations were lower than the mean detection threshold of our panel, but they corresponded to the threshold of the most sensitive panelists. Furthermore, considering that both had a floral flavour, their effects might be additive. 2-PE and 2-PEA concentrations in cheese were thus large enough to explain the floral touch detected by some panel members.

Dumont *et al.* (1974*b*) only estimated the amounts of these compounds in 'young', 'mature' and 'very mature' camembert cheeses and found a rather large quantity of 2-PEA in young cheese. Our findings confirmed this early formation and showed that the production of 2-PE and 2-PEA took place during the first 2 weeks of ripening whereafter it regressed and reached a lower level during late ripening. Lee & Richard (1984) observed that, among the different species of microorganisms developing in raw milk camembert, only yeasts were able to produce 2-PE from phenylalanine. This production occurred probably through the Ehrlich Neubauer pathway with phenyl pyruvate and CO_2 as intermediates (Lee & Richard, 1984). Conversely, other microorganisms isolated from camembert, e.g. *Geotrichum candidum*, *Arthrobacter*, *Moraxella* spp., *Brevibacterium linens*, did not produce 2-PE. Schmidt & Lenoir (1978) observed the development of yeasts at the surface of traditional camembert during the first 10 days of ripening. Their ability to produce 2-PE as well as the analogy between their kinetics of development and the kinetics of 2-PE and 2-PEA production suggested that these microorganisms were responsible for the production of these compounds. Such a decrease in 2-PE and 2-PEA concentrations after 2 weeks of ripening could be due to the breakdown of these products by other microorganisms of the surface flora. Thus, Lee *et al.* (1985) observed that *Brevibacterium linens* was able to break down the benzene ring of phenylalanine.

During the beginning of the ripening period, 2-PE and 2-PEA production was enhanced by phenylalanine spraying on the cheese surface probably because of the superficial development of yeasts. At this ripening stage the amounts of free phenylalanine were rather low (about 150 ppm after 7 days) but 20- to 30-fold higher than the amounts of 2-PE and 2-PEA (about 5 ppm).

Despite a surplus of free phenylalanine added onto the cheese surface, it was only possible to obtain a twofold concentration of these compounds in cheese. However, this did not lead to larger concentrations at the moment of cheese consumption as the levels were the same as those of control cheeses at the end of ripening.

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