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Original article

Effect of the association of surface flora on the sensory properties of mould-ripened cheese

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Summary — In cheese, flavour and taste are, in great part, generated by the starters during the ripening stage. Proteolysis and lipolysis are the first steps of the elaboration of a large number of taste and odour compounds directly involved in the sensory quality of cheeses. The pathways used by the microorganisms to produce flavour compounds are still unclear in many cases. It would be useful for the starter-producing industry to have screening criteria permitting diversification of the starter quality, and for the cheese industry to know which strain to associate to obtain cheeses with specific sensory properties. The production of experimental cheeses with different associations of surface microorganisms (*Penicillium camemberti*, *Geotrichum candidum*) followed by a sensory profile and chemical analysis was used. It is the first time that the effect of strain associations on sensory quality of cheeses has been investigated in depth. The aim of this study is to determine the impact of such associations on the organoleptic qualities of cheeses and to find compounds associated with defined sensory properties.

Camembert cheese / *Penicillium camemberti* / *Geotrichum candidum* / sensory quality / volatile compound

Résumé — Effet de l'association de flores de surface sur les propriétés sensorielles de fromages à pâte molle et croûte fleurie. L'arôme et la saveur des fromages se développent, en grande partie, grâce à l'action des ferments d'affinage. La protéolyse et la lipolyse sont les premières étapes

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de l'élaboration d'un grand nombre de composés sapides et aromatiques ayant une action directe sur les qualités organoleptiques des fromages. Les voies métaboliques mises en œuvre chez les microorganismes dans la genèse de ces composés sont, pour la plupart, encore mal connues. Il serait particulièrement intéressant pour les producteurs de ferments de posséder des critères de sélection leur permettant de diversifier la qualité des souches commercialisées et pour les industriels fromagers, de connaître quelles sont les souches à associer, afin d'obtenir un produit aux qualités organoleptiques spécifiques. La production expérimentale de fromages avec différentes associations de flores de surface (*Penicillium camemberti* et *Geotrichum candidum*), suivie d'une étude sensorielle et chimique, a été réalisée. C'est la première fois que l'effet de l'association de souches sur les qualités organoleptiques de fromages est ainsi étudié. Le but de ce travail est de mieux comprendre les impacts de telles associations sur les qualités organoleptiques des fromages, et de cerner les composés responsables de critères sensoriels particuliers.

Camembert / *Penicillium camemberti* / *Geotrichum candidum* / qualité sensorielle / composé volatil

INTRODUCTION

Microbial associations are widely used in the food industry to preserve or to diversify food products. For the last century, most of the studies in microbiology have dealt with pure cultures of microorganisms, while most of the fermented foods derive from the transformation of raw materials by mixed cultures of microorganisms. It is only recently that some studies have investigated the impact of microbial associations on the quality of the fermented products (Spicher et al, 1981, 1982; Laleye et al, 1990; Berdagué et al, 1993; Imhof et al, 1994; Perez-Zuniga et al, 1994; Spinnler et al, 1995). The quality of food products is determined by the quality of the raw material and also by the process parameters. Control of the technology using microbial association requires a better knowledge of the flavouring properties and behaviour of strain associations.

Product quality is measured either by instrumental analysis (physical, chemical) or by sensory analysis. The correlations between sensory and instrumental analysis are necessary to determine which compounds are responsible for sensory characteristics, and show how a change in the process could modify the sensory properties. Thus, it is useful to relate chemical or physical data and sensory analysis to efficiently alter the process parameters with the objective of improving the quality.

It would be useful for the starter-producing industry to have more screening criteria available to improve starter quality, and for the cheese industry to know which strains to associate to obtain cheeses with specific sensory properties. In this study, a simple model involving associations of four strains of *Penicillium camemberti* (*Pc*) and three strains of *Geotrichum candidum* (*Gc*) is considered. This association is currently used in Camembert cheese-making to improve the sensory quality (Mourgues et al, 1983). The aim of this study is to determine the impact of such an association on the organoleptic qualities of Camembert-type cheese.

MATERIALS AND METHODS

Biological material

Four strains of *Pc* (Pc1, Pc2, Pc3, Pc4) were used alone or in association with three strains of *Gc* (Gc1, Gc2, Gc3). Sixteen associations were tested. Those strains were obtained from the SBI-Systems Bio-Industries collections (La Ferté-sous-Jouarre, France).

Cheese-making

Sixteen experimental model cheeses were produced using the four *Pc* strains alone or in mixed culture with the three *Gc* strains (99% Pc, 1% Gc). Twenty Camembert cheeses were made for each model with pasteurised and standardised milk, and each fabrication was repeated three times. Cheeses were ripened for 12 days at 15 °C before packaging and stored for 16 days at 8 °C.

Sensory profile

Eighteen judges selected from outside the research centre were trained for 45 h (Molimard, 1994; Issanchou et al, 1995). A list of 19 flavour attributes, seven taste and after-taste attributes and one mouthfeel attribute was established after tasting all the experimental Camembert cheeses and evaluating some commercial Camembert cheeses (table I). For the measurement, four prod-

Table I. Descriptors used by panelist and references.
Descripteurs utilisés par les dégustateurs et leurs références.

Descriptors	*References*
Flavour attributes	
Flavour intensity	Overall intensity of flavour
Milky	Whole milk UHT (Candia®)
Creamy	Fresh thick cream (Carrefour®)
Buttery	Diacetyl, 5 ppm
Cancoillotte cheese	Cancoillotte cheese (Landel®)
Processed cheese	Vache qui Rit®
Fermented	Very ripe cheese flavour
Cowshed	Cowshed straw
Rancid	Butyric acid, 5 ppm
Cabbage	Dimethyldisulfide, 50 ppb
Ammonia	Ammonia Prolabo®, 3 drops in 500 mL
Dead leaves	Wet earth, earth after rain
Mushroom	Oct-1-en-3-ol, 0.1 ppm
Blue cheese	Roquefort cheese (Société®)
Mouldy	2,2,6-Triethylcyclohexan-1-ol, 10 ppb
Cardboard	Wet cardboard
Nutty	Decoction of nut powder (Malilé®)
Metallic	Ferrous sulfate, 0.005 g/L
Plastic	Styrene, 0.1 ppm
Taste, after-taste and mouth sensation attributes	
Acid	Lactic acid, 2 g/L
Bitter	L-Leucine, 5 g/L
Salty	Sodium chloride, 2 g/L
Acid after-taste	
Bitter after-taste	
Metallic after-taste	
Pungent	

ucts were tasted per week and sensory analysis of each product was repeated twice. The sample presentation design was established using a latin square balanced for the order and carry-over effects (Schlich, 1994).

Chemical analysis

Nitrogen fraction assay

Total nitrogen, soluble nitrogen at pH 4.6 and soluble nitrogen in phosphotungstic acid were analysed as described by Molimard et al (1994).

Volatile compound analysis

The cheeses were extracted with diethyl ether. This extract was concentrated to 50 mL. The mixture was then dialysed using a Nafion™ membrane, as described previously (Molimard and Spinnler, 1993). The apparatus was modified to improve the extraction yields by recycling of the solvent (Molimard, 1994). After a three-day dialysis, the extracts were concentrated to 200 µL. The compounds extracted were then identified on the basis of their retention indices and GC–MS results.

RESULTS AND DISCUSSION

Experimental Camembert cheeses: taste

A discriminant analysis of taste and mouthfeel attributes revealed that the experimental cheeses were mainly discriminated on bitterness. This attribute was linked to dimension 1, which constituted 79.8% of the information (Molimard, 1994; Molimard et al, 1994). The intensity of bitterness could be partly correlated with the concentration of peptides ($r = 0.48$, ddl = 46, $P = 0.07$). Cheeses inoculated with pure *Pc* were judged more bitter, especially those inoculated with the strain Pc2. They also showed the highest concentrations of soluble nitrogen at pH 4.6 and of peptides. *Gc* has a higher aminopeptidasic activity than *Pc*; it might be a pathway by which *Gc* could decrease bitterness. Total nitrogen concentration and soluble nitrogen concentration in phosphotungstic acid of the 16 experimental Camembert cheeses were not significantly different (Molimard, 1994; Molimard et al, 1994).

Experimental Camembert cheeses: flavour

Sensory analysis

A principal component analysis on the covariance matrix of the means of the three replicates of cheese production presents the results in sensory map form. Figure 1A shows that the first component, which represented 45.8% of the information, opposed milky, creamy, buttery and mushroom flavours to the cardboard, plastic, rancid and fermented flavours. The second component (26.7% of the information) was mainly determined by cabbage, Cancoillotte cheese and cowshed notes. The upper right quarter of the map is characterised by blue cheese, ammonia, cowshed, fermented, rancid notes and total flavour intensity. The upper left quarter of the map is characterised by Cancoillotte cheese, buttery, milky and creamy notes. The lower right quarter of the map is characterised by dead leaves, mouldy, plastic and cardboard notes and the lower left quarter by processed cheese and nutty notes.

All the products made with Pc1, were characterised by plastic, cardboard, mouldy and dead leaf notes. The strain Pc2 gave products with blue cheese, rancid, fermented and cowshed notes, and strains Pc3 and Pc4 gave processed cheese, nutty, milky, buttery and creamy notes to the products (fig 1B). The cheeses made with pure cultures of *Pc* were characterised by plastic, cardboard, dead leaf and mouldy notes. The addition of strain Gc2 developed cowshed, cabbage, fermented notes and flavour intensity. Gc1 and Gc3 developed creamy, buttery, milky and mushroom notes. Strain Gc2 was able to increase the cabbage, cowshed, fermented notes and flavour intensity of cheeses made with strains

Pc1, Pc2, Pc3 and Pc4, while strain Gc3 developed, with all the *Pc* strains, creamy, milky and buttery flavours.

Chemical analysis

Sixty-four neutral compounds were quantified. Fourteen of them showed significant differences between the strains ($P < 0.01$); only five of these were identified (3-methylbutan-2-ol, 2-phenylethanol, oct-1-en-3-ol, undecan-2-ol and benzaldehyde). Over 30 acid compounds were quantified, 13 of which showed significant differences between the products ($P < 0.01$), 11 of which were identified (isovaleric, pentanoic, hexanoic, ethylhexanoic, octanoic, nonanoic, decanoic, undecanoic, dodecanoic, tetradecanoic and phenylpropandioic acids).

We found significantly more oct-1-en-3-ol, benzaldehyde, 3-methylbutanol and 2-phenylethanol in the cheeses produced with the strain Pc1 ($P < 0.001$). The strains Pc2 and Pc4 seemed to have a significant lipolytic activity and gave products showing a more abundant fatty acid content. The strain Gc2 produced an important quantity of isovaleric acid.

Relations between sensory and chemical analysis

With the strain Pc1, which produced a plastic flavour, we did not observe a higher styrene concentration than with the other strains. This could be linked to the extraction method which did not allow a complete extraction of the very hydrophobic compounds such as styrene. However, styrene was produced from phenylalanine and other catabolites of phenylalanine, such as 2-phenylethanol and benzaldehyde, showed significant correlations with plastic flavour ($r = 0.38$ and $r = 0.35$, $P < 0.01$). Thus, these two compounds could be considered as indicators of the level of plastic off-flavour.

Considering the undesirable cardboard flavour, a significant correlation was observed with oct-1-en-3-ol ($r = 0.55$, $P = 0.0001$). Nevertheless, this compound does not induce any cardboard flavour but a characteristic mushroom flavour. However, two compounds are likely to have issued from the same catabolism (intrachain oxidation). (*E*)-2-Nonenal and oct-1-en-3-one have recently been described as dispensing cardboard flavour (Widder and Grosch, 1994). As oct-1-en-3-one has already been identified in Camembert cheese, oct-1-en-3-ol could be a 'marker' of this catabolism. Our results

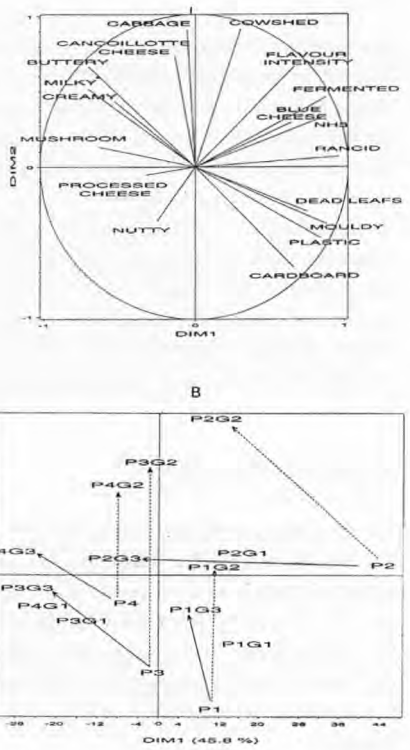

Fig 1. Principal component analysis of the sensory data performed on the mean of the three replicates of cheese-making. **A**: Score of the flavour attributes in the sensory space defined by the two first components. **B**: Location of the products in the sensory space. P: *Penicillium camemberti*. G: *Geotrichum candidum*. *Analyse en composante principale sur les données de l'analyse sensorielle, réalisée sur la moyenne des trois répétitions de fabrication des fromages.* ***A*** *: Notes des descripteurs de l'arôme dans l'espace sensoriel défini par les deux premières composantes.* ***B*** *: Localisation des produits dans l'espace sensoriel. P :* Penicillium camemberti. *G :* Geotrichum candidum.

showed that the use of *Gc* involved a significant decrease of these two undesirable flavours. As these results were obtained with each of the three strains of *Gc,* it could be a species effect.

However, a quality control does not only consist in avoiding off-flavours. The real quality of cheeses is related to specific flavour characteristics at the appropriate intensity. Our results showed that the flavour intensity was correlated with the blue-veined cheese flavours, and as a consequence with the lipolytic intensity. The strains Pc2 and Pc4, which gave products characterised by a high score of the blue-veined cheese flavour note, also gave products with a higher short chain fatty acid content, such as octanoic and hexanoic acids. However, no significant difference was observed between methylketone concentrations.

Cabbage and cowshed notes were known to be typical of Camembert cheeses made from raw milk or pasteurised milk and some strains of *Gc* in addition to *Pc* (Ribadeau-Dumas, 1984). Thus, we analysed the correlations between the intensity of cabbage flavour and the concentrations in volatile compounds. The cabbage note is strongly correlated to the concentration of isovaleric acid ($r = 0.77$, $P = 0.0001$). This acid has no cabbage flavour, but seems to have issued via the Strecker pathway from leucine. We now have to check whether methionine is degraded by the same pathway (as most of the cabbage flavours in cheese are related to methionine catabolism). Would the products of this catabolism be responsible for the cabbage flavour noted by the panel?

CONCLUSION

Lipolysis plays a major role in flavour development in cheeses. It seems that the terminal oxidation which produces methylketones is positive, as these compounds are related to flavour intensity. However, the intrachain oxidation, giving oct-1-en-3-ol, could be indesirable, especially when occurring extensively. Presumably strains with a low activity of both oxidation systems giving a low level of those flavour compounds were characterised as very mild cheeses. Some strains of *Gc* (Gc1 and Gc3) seem capable of limiting intrachain oxidation and developing creamy notes. However, other strains like Gc2 which induces cabbage notes are able to produce a strong and typical Camembert cheese flavour, similar to the raw milk Camembert cheese flavour. This ability may be due to a special amino-acid catabolism in such strains.

This approach could be extended to the improvement of the quality of fermented foods. A set of products analysed by both sensory and chemical analysis may provide clues concerning the metabolism by which the strains produce the major flavours. After determining the enzymes presumed to be involved in these metabolisms, it would be possible to develop screening tools to select the most appropriate starter strains. For example, this would permit selection of strains with specific flavouring properties to produce either traditional products with a defined flavour quality, or any new products.

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