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Recent Developments in Cheese Flavour Research

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Cheese Flavour 연구의 최근 경향

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요 약

근래 유제품의 향기성분의 증강법은 효소를 사용하여 향취강도를 증가하는 방법이 개발되고 있는데 효소촉매의 생화학적 반응은 결정적으로 식품의 특징적인 향생산에 기여한다. 본 연구자료는 치즈향의 발효적 추출을 통해 식품원재료에서 생성되는 것과 같은 천연향을 얻기 위한 연구의 최근 경향을 보여준다.

치즈풍미에 특징적인 성분이 결정적인 영향을 미치지 않는 Cheddar 치즈와 각기 methyl ketone과 octenol이 flavour impact component로 작용하는 Roquefort 치즈와 Camembert 치즈에 대하여 치즈향의 성분, 이의 형성과정 및 제조방법 등에 관하여 고찰하였다.

INTRODUCTION

In this paper, the composition and formation of cheese flavour and the different types of cheese flavour concentrate production are described. Cheddar as a hard cheese was taken as an example of a cheese flavour without impact compounds. Roquefort, an internally mould-ripened semi-soft cheese and Camembert, an externally mould-ripened soft cheese were discussed as cheese flavours with impact compunds.

An overview about the formation of the most important flavour components from the main substances found in milk is given in table 1.[1] The enzymatic and the microbial fermentations work with the same substrates and the same enzymes or microorganisms as in cheese manufacture, thereby trying to maximize flavour strength and minimize processing time.

II. Cheddar cheese

Cheddar is a hard cheese popular in the most of English-speaking countries. Its main features are acidificaiton in the curd, salting before moulding, and pressing[2]. Lamparsky and Klimes[3] gave the following summary:

- Lactococci starter cultures are not only responsible for the acidification, but also for flavour development.
- Cheddar ripening is characterized by anaerobic conditions because of a wax layer on the cheese surface.
- The flavour substances are formed through the action of native milk enzymes and of microbial enzymes, which collaborate in a complicated and not well understood system and are able to degrade all curd components (fats, proteins, carbohydrates).
- The balance between the various substances is decisive for the flavour.

1. Cheddar Cheese Flavour

Ney[1] describes cheddar flavour with regard to volatiles in the following way:

- most important: free fatty acids C_2 up to C_{10} as well as iso-C_4 and iso-C_5
- relatively important: methyl ketones
- important: esters, hydrogen sulfide, amides, α-keto acids, aldehydes, amines

Tabe 2 shows the 161 volatiles found in Cheddar up to 1983[4,5]. According to Mcgugan et al.,[6] besides the lipophilic volatiles there are non-volatile hydrophilic components responsible for Cheddar flavour. The concentration of flavour components are continuously changing during cheese ripening[7,8]. There can thus be shifts in the importance of single substance or substance classes relating to cheese flavour.

1) Flavour Impact Compounds: None

The number and diversity of the flavour substances isolated from Cheddar are comparable to those found in other types of cheese. The difference is the absence of impact components. Whereas they were found in Blue cheese (methyl ketones) and Camembert (octenol), they have not been found in Cheddar cheese up to now. Thus, the Component Balance Theory[9] is not yet defeated.

This theory says that Cheddar flavour is made up by a number of components, which keep a certain flavour balance with no single substance being solely responsible for the characteristic flavour.

2) Fatty Acids

There are very inconsistent data about the free fatty acid (FFA) content of Cheddar and their contribution to flavour. According to Manning and Robinson,[10,11] the FFA are not essential for Cheddar flavour, but contribute to its overall flavour. According to Adda[12], a correlation between the FFA content and flavour intensity exists only for Blue Cheese, as their flavour thresholds are only exceeded in those cheeses.

On the other hand, Ney[1] called the FFAs as "main cheese flavour carriers" and important flavour precursors. However, there is unanimity that too high a FFA concentration leads to a rancid taste (e.q. Aston and Dulley[13]). Because of that, the FFAs are mainly sources of problems and not quality factors[12].

The FFAs in cheese are formed by milk fat lipolysis on the one hand and by microbial degradation of carbohydrates and proteins on the other hand[13]. The medium and long chain FFAs (C_6 to C_{18}) are formed during the ripening and are characteeristic for Cheddar flavour. As they are found in the same relations as in milk, their presence seems to be due to an unspecific hydrolysis of the milk triglycerides. The fatty acids C_2 to C_6 can be formed by the activity of lactic acid bacteria from casein hydrolysates and are thus not quantitatively derived from the milk fat.

Butyric acid, acetic acid and lactic acid as well as their esters with various alcohols are formed by fermentation, while butyric acid can be formed by

Table 1. Biochemical pathways in cheese flavour formation[1]

Origins	Intermediates	Products
Lactose	→ Pyruvate	→ Lactic Acid
		→ Diacetyl
		→ Acetaldehyde → Ethanol
		→ Acetic acid
		→ Carbon dioxide
Protein	→ Peptides →	Keto acids
	Amino acides	→ Amines
	→ α-	
→ Acids → Alcohols		
→ Phenols		
→ Hydrogen Sulfide		
→ Ammonia		
Fats	→ Fatty acids	→ Amides
		→ Aldehydes
		→ Primary alcohols
		→ Methyl ketones
		→ Sec. Alcohols
		→ Esters
		→ Lactones

enzymatic hydrolysis, too.

Lamparsky and klimes[3] could confirm the findings of Ney and Wirotama[14] that isobutyric acid, n-valeric acid and iso-valeric acid were present in Cheddar. These fatty acids and branched chain caproic acids are not found in milk fat and seem to originate from protein degradation, where they are formed by deamination of α-amino acids.

The only α-keto acid found is pyruvate[3], which is a key substance of Cheddar flavour formation, but not of the flavour itself. On the contrary, several α-hydroxy-acids were found originating from amino acid metabolism. Ney[1] however, found 13 α-keto acids, which are formed by transamination of free amino acids.

3) Carbonyls and Alcohols

Lamparsky and klimes[3] described the presence of the primary alcohols of C_6, C_7, C_8, C_{10} and C_{12} in Cheddar. Unlike other authors, including Van Straaten and Maarse[4], they did not find the corresponding carbonyls. The presence of these substance seems to depend to a high degree on age and ripening status of the cheese, because the reducing conditions during maturation cause the conversion of the aldehydes into the corresponding primary alcohols. The secondary alcohols of C_4 to C_{12} are formed in a comparable reaction by reduction of methyl ketones.

There are different opinions about the significance of carbonyls for Cheddar flavour. Whereas for exemple Walker[15] succeeded in creating a Cheddar flavour by adding methyl ketones, fatty acids and thioacetamide to bland cheese, carbonyls are of no importance to Cheddar flavour according to Patton[16].

4) Amino Acids and Amines

According to Aston and Dulley[13], for a long period it was held that the free amino acids were responsable for the background flavour of Cheddar without directly contributing to flavour. Mcgugan et al.,[6] however, concluded from their work that the volatiles were responsable for flavour quality, whereas the water soluble fraction, which contained the free amino acids, determined flavour intensity. The same opinion was held by Puchades, Lemieux and Simard[17], who succeeded in accelerating Cheddar maturation by adding lactobacilli, and by Hayashi, Revell and Law[18], who added bacterial aminopeptidase and proteinase. Both processes led to a higher level of free amino acids. Law et al.,[19] reported that the formation of amino acids as flavour precursors is the most important task of starter lactococci.

Ney[1] reviewed the data about the presence of free amino acids and amines in Cheddar without giving quantitative data. Adda[12] showed the large numbers of results as contradictory. There only seems to be consent concerning the role of amino acids as flavour precursors.

5) Sulphur Compounds

There is a controversial discussion about the significance of volatile sulphur compounds like thiols, mercaptans and sulfides, which are formed by degradation of the amino acids methionine and cysteine. Lamparsky and Klimes[3] did not find any sulphur compounds in mild Cheddar cheese concentrates, areason being perhaps the high volatility of these substances.

Manning and Robinson[11], however, attribute Cheddar flavour mainly to an extremely volatile fraction of 20 substances like hydrogen sulfide, methanethiol and dimethylsulfide.

Manning[10] emphasized the significance of sulphur compounds for Cheddar flavour that could not be characteristic without H2S and methanethiol.

Manning[10] and Kristoffersen[20] drew the conclusion that the low redox potential during Cheddar ripening led to the development of free sulphur compounds. According to Kristoffersen, enzymatic reactions were responsible for the formation of active sulfhydryl groups.

6) Aromatic Substances

Lamparsky and Klimes[3] mention various derivatives of benzoic acid and phenylacetic acid, which were formed from phenylalanine. Other important substances were tyrosine-derived phenols.

7) Lactones

Beside the α-lactones, which are mentioned in the TNO-List[4], Lamparsky and Klimes[3] found various γ-and δ-lactones like jasmine lactone and δ-7-deceno-lactone, which might be formed in cheese following the same pathways as in plants.

2. Formation of the Flavour Components

1) Carbohydrate Degradation

The degradation of the milk or curd carbohydrates, which nearly consist of 100% lactose, is due to microbial enzymes. The key substance for flavour formation is pyruvate, which reacts with substances like acetoine, 2-butanone, and butanol.

Lactic acid is not flavour volatile, but belongs to those substances which form the basic cheese flavour. The acids lead to a decrease in pH which is responsible for cheese mass structure and thus for flavour, too. Besides, lactic acid leads to the development of a negative redox potential, which is a main condition of flavour development[12].

Other flavour substances resulting at least partly from carbohydrate degradation are α-ketoglutarate, acetaldehyde, diacetyl, formic acid, acetic acid, propionic acid, and butyric acid (Moskowitz and Labelle[21]; Lamparsky and Klimes[3]). However, these substances can also lead to the formation of off-flavours (Puchades et al.[17]).

2) Fat Degradation

Triglyceride degradation is brought about by microbial and native milk lipases. As the distribution patterns of the fatty acids C_6 to C_{18} in Cheddar and in milk are nearly identical, the hydrolysis is thought to be unspecific. Butyric acid, however, is additionally formed by microbial processes. As an optimal flavour is not formed with vegetable fats, the type of the oil/water interface might be of importance Adda[12].

3) Protein Degradation

The milk casein is degraded during the production process by the combined effect of different proteolytic enzymes, that is to say the lactococci-proteases and the rennet protease.

According to Lowrie and Lawrence[22], casein is degraded by the rennin (calfesterase of the rennet) to non-bitter peptides of high molecular weight. These are hydrolyzed by the proteinases of the starter lactococci to low molecular weight peptides, which give a bitter taste because of their highhydrophobicity. The lactococci proteinases are active again in the third phase and lead to the formation of non-bitter peptides and amino acids. Without starter strains added, rennet is only able to degrade casein to high molecular weight peptides, and neither the unwelcome bitter peptides nor the desired flavour components are formed.

The bitterness of the cheese, which is an offflavour, can be reduced by correct selection of the process parameters. Besides formation and degradation of the bitter peptides by the microbial proteases, the reactions of the involved strains ("bitter" and "non-bitter" lactococci) and the parameters temperature, pH, and salt content have to be considered (Lowrie and Lawrence[22]).

Mills and Thomas[23] continue these reflections and make distinctions between the types of the active proteinases. Whereas rennin and the cell wall bound starter proteinases lead to the formation of bitter peptides, they are degraded by intracelluar peptidases which are set free by bacterial lysis. Low molecular weight peptides can also get into the cell plasma and be hydrolyzed there. At the end, milk protein degradation leads to the formation of free amino acids, amines, free fatty acids, and sulphur compounds.

3. Process of Cheddar Flavour Concentrates

The fundamental principles are mentioned in the following and illustrated by examples from patent literature. The expression "Cheddar Flavour" stands for "Basic Cheese Flavour", too, as no special sensory impression has been found that can be related to Cheddar flavour.

1) Synthetic Processes

Various substances in different combinations with others can be used for generating a synthetic Cheddar cheese flavour. Yamamoto et al.[24] describe a mixture of organic acids, secondary alcohols, esters, acetoin and diacetyl in vegetable oil. Dependent on the percentage of the individual substances, a Cheddar or a Blue Cheese flavour (including Roquefort) can be made. However, a sentence by Olson[25] is especially true for Cheddar, "The flavor profiles of cheeses are complex··· None are characterized sufficiently to permit duplicaiton of their complete flavor by mixture of pure compounds".

2) Enzymatic Processes

Enzymes are used in cheese production with the objective of flavour enhancement or accelerated ripening by use of lipolytic and/or proteolytic enzymes. These products show a significantly increased content of free fatty acids and non-protein nitrogen (Moskowitz[26]). A lipase of *Aspergillus oryzae* showed unique solubility characteristics in the curd and a high specifity for hydrolysis of triglycerides of intermediate chain length (Arbige et al.[27]). There are also attempts to increase the concentration of sole substances by enzyme addition to the curd, i.e. a methionase from *Pseudomonas putida* in order to increase the concentration of methanethiol (Lindsay and Rippe[28]).

The Enzyme Modified Cheeses (EMC) are to be mentioned, which are flavour concentrates produced by enzyme action on cheeses. Their advantage over fermented flavour concentrates made from substrates like milk components is the presence of a basic cheese flvour, their disadvantage is the time-consuming production, as their processing starts with cheese. In most of the cases, an enzymatic and a microbial fermentation are combined.

3) Microbial Processes

An interesting type of flavour concentration is accelerated maturation of cheeses. By adding *Lactobacillus casei-casei* as live cells and cell homogenates, Trepanier, Simard and Lee[29] succeeded in developing a Cheddar cheese with 40% increase in flavour intensity compared to normal cheeses.

Kasik and Luksas[30] describe a cheese base production by an unaerobic five-day fermentation of skim milk with *Bacillus ssp.* and *Lactococcus lactis subsp. diacetylactis*, which has to be followed by another fermentation, lasting five days with old cheddar cheese added. The authors describe also a similar process with the addition of 33% Cheddar. Kasik[31] describes an aerobic fermentation for 24 hours with reconstituted milk by *Acetobacter aceti and Lactococcus lactis subsp. lactis* leading to a cheese base medium.

Most microbial fermentations have to be combined with enzyme treatment. Johnson and Southworth[32] fermented cheese and butter with esterase, followed by a bacterial lactic acid fermentation. Yokoyama and Iwanaga[33] produced a Cheddar flavour concentrate by fermentation of 15% fat (tributyrin and palmoil) and 15% acid casein with added rennet, carboxypeptidase and pregastric esterase together with *Streptococcus cremoris*. A Parmesan flavour could be obtained by adding caproic and caprylic acids to the medium.

III. Roquefort cheese

Roquefort is a French cheese and belongs to the Blue (Veined) cheeses. These are characterized by an internal growth of moulds, which shows in a network of blue veins and is responsable for the flavour of the cheeses. There are large varieties of Blue Cheeses in many countries all over the world (Gorgonzola in Italy, Stilton in Great Britain, Danablue in Denmark, Edelpilzkäse in Germany).

A slight hetero fermentative lactic acid formation after clotting the ewe's milk leads to the formation of small cavities in the curd, inside which the (strictly aerobic) fungus *Penicillium rogueforti* develops. The second curing period is anaerobic and characterized by proteolysis and lipolysis within the loaves[34].

1. Roquefort cheese Flavour

According to Mair-Waldburg[34], Roquefort flavour was described as "strongly aromatic, fatty acid-and methyl ketone-like, piquant". Kinsella and Hwang[35] gave the following classes of non-volatile flavour compounds: organic acids, long-chain fatty acids, fatty acid soaps, alcohols, amino acids, secondary amines, and peptides. Table 2 shows the volatiles that have been found in Blue cheeses. The main classes of flavour components are fatty acids, methyl ketones, and acetaldehyde[36].

1) Flavour Impact Components: Methyl Ketones

The most striking flavour components of Blue cheese are the 2-alkanones. The intensity of the taste sensation "Roquefort" is linearly correlated with the methyl ketones concentration[37]. They are present in the cheese in about 10 to 100 mg per 100 g dry weight, usually showing decreasing amounts in the series 2-heptanone > 2-nonanone > 2-pentanone > 2-undecanone.

Table 2. Volatiles found in Cheddar[4], Blue, and Camembert[48] cheeses.

Substancd class	Cheddar			Blue			Camembert		
	Total	Aliphatic	Aromatic	Total	Aliphatic	Aromatic	Total	Aliphatic	Aromatic
Hydrocarbons	33	14	15	3	1	2	-	-	-
Alcohols	12(4)a	12	-	13(3)	12	1	14	13	1
Aldehydes	12(4)	11	1	7	6	1	5	5	-
Ketons	14(11)	14	-	12(6)	12		11	11	-
Acids	27(15)	27	-	30(14)	30	-	-	-	-
Esters	20(2)	19	1	17(2)	17	-	6	5	1
Lactones	9	9	-	7	7	-	3	3	-
Amines	14	12	2	10	9	1	5	4	1
Sulphur Compounds	6(3)	5	-	2(3)	1	-	9	9	-
Amides	6	6	-	6	6	-	1	1	-
Phenols	1	-	1	1	-	1	5	-	5
Miscellaneous	7	6	1	1	1	-	1	-	1

a The numbers in brackets indicate the number of substances for which quantitative data have been given.

Their way of formation can be seen in fig. 1. During the reaction called thioclastic cleavage, which is a step of β-oxidation, β-hydroxy-acyl-CoA is normally broken down by thiolase to acetyl-CoA and acyl-CoA. Thiolase, however, is inhibited by high concentrations of fatty acids and of acyl-CoA. For reasons of detoxification, the FFA have to be degraded in another way. This way is the hydrolysis of β-hydroxy-acyl-CoA by thiohydrolase giving a β-ketoacid, which is rapidly decarboxylated to give a methyl ketone[38].

2) Fatty Acids

The free fatty acids are the precursors for the formation of methyl ketones, but are important for Blue cheese flavour by themselves, too, as they give a sharp flavour. The lipases from *Penicillium roqueforti* have a high specifity for setting low-chain fatty acids free from milk fat, perhaps because of the higher toxicity of the long-chain fatty acids[39]. However, the latter are removed from the medium by the thiolase reaction, and thus long-chain fatty acids are formed in spite of their toxicity. Another reason for the long-chain fatty acids not being as toxic as expected is their enrichment in the lipid phase and thus separation from the mycelium.

Woo et al[40]. observed concentrations of 32,000 mg FFAs per kg Blue cheese (wet weight). They attribute the strong musty flavour of Roquefort to octanoic acid, which is found in relatively high concentrations in ewe's milk fat, and assign the soapy flavours of Blue cheeses to free decanoic and lauric acid.

According to Haslbeck et al.,[41] free fatty acids have flavour threshold values between 15 and 160 ppm and impart the following flavour qualities; C4 rancid, C_6 rancid and goaty, C_8 musty, rancid, and soapy, C_{10} and C_{12} soapy.

3) Carbonyls and Alcohols

P. roqueforti is able to reduce the methyl ketones to the corresponding 2-alkanols, which give a flavour note comparable to that of the methyl ketones.

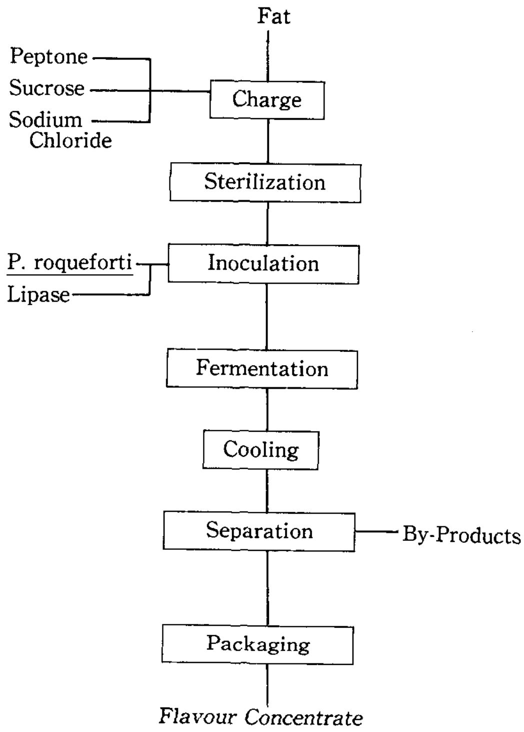

Fig. 1. Microbial process of roquefort flavour concentrate[41]

Primary alcohols can be formed from free amino acids via the aldehydes and give fruity notes. 1-Octen-3-ol, the most important flavour substance in Camembert, is formed by oxidation of linoleic acid and imparts a mushroomy flavour to the cheese[42].

4) Amino acids and Peptides

These non-volatile products of protein breakdown are responsible for the important background flavour of the Blue cheese and serve as precursors for volatile and non-volatile flavour substances like amines, aldehydes, alcohols, acids, and sulphur compounds[42].

5) Other Components

Organic acids like lactic and succinic acid are responsible for the background flavour, and esters and lactones import a fruity flavour to the Blue cheese[42].

2. Formation of the Flavour Components

1) Carbohydrate Degradation

Lactose is important for the heterofermentative formation of lactate and CO_2 in the first phase of Blue cheese curing, as it creates the conditions for Penicillium roqueforti to develop. Lactic acid ensures proper curd consistency and thus facilitates whey drainage.

2) Fat Degradation

In mould metabolism, triglycerides are hydrolyzed to fatty acids, which in turn give the methyl ketones by the action of thiohydrolase and decarboxylase (Fig. 2). As milk lipase cannot sufficiently hydrolyze the fats if the milk is not homogenized before, a lipase preparation may be added. In Roquefort, however, only the lipases of *p. roqueforti* cause the release of the fatty acids with the intracellular lipase showing a greater activity than the extracellular one. The lipases show maximum activity at pH 6.0 to 7.8 and 30°C to 35°C, but remain quite active at the low curing temperatures of below 10°C[35].

Although 10% of the triglycerides are usually hydrolyzed, a rancid taste does not occur, probably because of the neutralization of the fatty acids at higher pH.

3) Protein Degradation

Proteolysis begins already during the first (aerobic) curing phase of the Blue cheese, and in the end of the second maturation phase 20 to 30% of the proteins are broken down. The main effectors are the mould proteases, an extracellular and an intracellular one, which are rather nonspecific and show an optimum pH between 5 and 6, and 3 and 6, respectively. Their optimum temperature being between 35°C and 43°C, they show decreased activity below 10°C. This is important in order not to lead to the formation of bitter tasting substances during the curing phases at low temperatures. Too highly, a degree of proteolysis is also prevented by the addition of salt and by the high levels of FFA[35]. Further breakdown is affected by extracellular peptidases of *p. roquefori*.

3. Process of Roquefort Flavour Concentrates

1) Synthetic Processes

It seems not to be sufficient just to mix methyl ketones and fatty acids, and perhaps some esters or phenolic compounds to give Roquefort flavour. A comparison of the different mixtures shows the significance of the background flavour imparted by products of protein breakdown.

2) Enzymatic Processes

Without added moulds, a Blue cheese flavour concentrate could be produced by the action of enzymes on cheese[43].

3) Microbial Processes

The majority of these processes take milk, milk fat and/or milk proteins as substrates for a fermentation with *P. roqueforti*[44]. Often homogenization of the milk and added lipase are necessary in order to get a high concentrations of methyl ketones. The quality of the emulsion is of major importance, as the lipase only works at the water-oil-interphase[45]. By a good choice of the fermentation conditions, a flavour concentrate could be produced in a short time, one of the main problems being reproducibility[37]. Tests with different strains of *P. roqueforti* showed a positive relationship between lipolytic activity and flavour intensity and the importance of strain selection[45]. There are other fungi, too, which are able to convert short-chain fatty acids to methyl ketones, i.e. *Monascus purpureus*[47]. Deger et al.[48] produced a Blue cheese flvouring by combinated fermentation of milk with Torulopsis sp. and *P. roqueforti*.

Fig. 2 shows a scheme for Roquefort flavour concentrate production[42] 20 to 30% butterfat are added to a culture medium of casein peptone, sucrose, NaCl and other minerals. After sterilization and cooling, the fermenter is inoculated with a spore suspenison of *roqueforti* produced by floating an emerse culture.

Linolic Acid (C_{182}, $\Delta^{9,12}$)
↓ O_2, Lipoxygenase
10-Hydroperoxy-8(E), 12(Z)-Octadecadienoic acid
↓ Hydroperoxidelyase
1-Octen-3-ol and 10-Oxo-8(E)-decenoic acid

Fig. 2. Formation of 1-octen-3-ol in mushrooms[37]

Lipase may be added. Fermentation takes place at 30 °C with intensive aeration and agitation by rotary pumping. After 48 h, the fermentation process is terminated by pasteurization (30 min at 60°C to 80°C). Still hot, the fat phase containing the flavour substances is separated from the water phase and the mycelium.

For an optimum relation between growth and metabolism of *P. roqueforti* on the one hand and flavour production on the other hand, the fat proportion in the culture medium must not exceed 30%. The process is sensitive to infections and has to be conducted sterilely until the separation.

IV. Camenbert cheese

Camembert is a soft cheese with white surface mould growth, which is responsable for the flavour of the cheese. The origin of this cheese lies in Northern France. Another well-known cheese of this type is Brie.

The first production phase after clotting by rennet and lactic acid bacteria is characterized by the dominance of lactococci. In the second phase, lactic acid streptococci and lactobacilli (*L. plantarum* and *L. casei*) grow and reach their maximum. On the cheese surface and, later, inside the cheese, there is development of different types of yeasts and moulds leading to a rise of pH because of lactate degradation.

The third phase begins with the first visible growth of a white thin mycelium of *P. camemberti*, followed by the development of a dense and white felt on the cheese surface. *P. camemberti* does not grow inside the cheese like the other moulds and yeasts. The enzymes of the fungus diffuse into the cheese and lead thus to a ripening from the outside to the inner parts, which includes lactic acid degradation leading to a pH-rise.

1. Camembert cheese Flavour

According to Woo et al.,[40] the flavour of Camembert is "cooked mushroom-like, musty, sulphury, mildly nutty". Mair-Waldburg[34] describes the flavour as follows:

In the beginning a weadk alcoholic whey odour, then mushroom-like and later weakly or strongly ammonia-like, dependent on ripening stage and packaging. The flavour is in the beginning in general mild, in the outer layer mushroom-like, in the inner parts aromatic and weakly salted, in the overripe status a raw milk Camembert may taste soapy.

Adda[49] gave the overview of volatiles isolated from Camembert, which is shown in Table 2.

1) Flavour Impact Components: 1-Octen-3-ol and Related Substances

1-Octen-3-ol seems to be the impact component of Camembert flavour. Because of its very low flavour threshold value of 0.01 ppm, it makes the cheese taste mushroom-like even in low concentrations[50]. Octenol is known as flavour active substance in all mould cheeses with Camembert containing 5 to 10 times more octenol than Roquefort.

There is not much knowledge about the formation of octenol in cheese. Following Kaminski et al.,[51] various species of *Penicillium* are able to from octenol by the enzymatic oxidation of linoleic acid. Whereas the pathway in fungi is not yet fully understood, octenol formation in mushrooms follows the reaction shown in Fig. 2.

In the headspace of *Penicillium*-cultures other compounds with eight carbon atoms were found, which participate in a musty-earthy odour, but which are present in much lower conentrations than

Table 3. Synthetic Camembert Flavour[53]

Ingredients	%(W/W)
2-Heptanone	0.8
2-Nonanone	17.6
2-Heptanol	9.3
2-Nonanol	5.1
Phenol	1.0
Butyric acid	61.9
1-Octen-3-ol	3.9
Methyl cinnamic acid	0.4

1-octen-3-ol: 3-octanone, 3-octanol, 1-octanol and 2-octen-1-ol[52]. The autoxidative enzymatic formation of these components is known in plants and mushrooms and may be similar in *Penicillium.*

2) Fatty Acids

In traditionally produced Camembert, 6 to 10% of the total fatty acids are present in free form, whereas after modern manufacture there are only 3~5% FFAs. Most significant is the free oleic acid: milk fat contains 34% unsaturated C18 fatty acids (mostly oleic acid), and the FFA of Camembert consist of 45% of C_{18} fatty acids[53].

5% of the FFA are not the result of lipolysis, but are formed by degradation of carbohydrates and amino acids. They are of short chain length and represent only 5% of the total FFAs.

The amount of FFAs is about 700 ppm in German Camembert and 2,700 ppm in French Brie (Roquefort: 32,000 ppm)[40] The FFAs participate in basic cheese flavour and are important flavour precursors.

3) Carbonyls and Alcohols

Methyl ketones and secondary alcohols are present and contribute to Camembert flavour. Their concentration is only about from 15 to 25% of the one found in Blue cheese. While heptanone is the most important methyl ketone in Blue cheese, nonanone is the most significant one in Camembert. During ripening, the concentrations of nonanone and undecanone rise, whereas those of the short chain methyl ketones decrease.

One reason for the relatively low concentration of methyl ketones in Camembert compared to Blue Cheeses is the lower rate of hydrolytic formation of free fatty acids. Another reason is the missing inhibition of the thiolase by a high FFA concentration (Fig. 1). The fatty acids formed are converted to octenol, which has a much lower flavour threshold than the methyl ketones[38]. The most significant secondary alcohols are 2-hepatanol and 2-nonanol, whereas 3-methyl-1-butanol is the highest concentrated one of all alcohols.

4) Sulphur Compounds

Degradation products of sulphur-containing amino acids are not only formed by the metabolism of coryneform bacteria (*Brevibacterium linens*), but also by *P. camemberti* and *Geotrichum candidum.* Whereas methyl sulfide, methyl disulfide and 3-methyl-thiopro-panol participate in basic cheese flavour and are present inother cheeses, too, 2, 4-dithiapentane, 2, 4, 5-trithiahexane and 3-methylthio-2, 4-dithiapentane are specific for traditionally manufactured Camembert, to which they give a garlic flavour[53].

5) Aromatic Substances

2-Phenylethanol shows a pleasant rosy odour and is formed during phenylalanine degradation by yeasts in the beginning of the ripening phase. Other derivatives found in traditionally produced Camembert are 2-phenylethylacetate and 2-phenylethylpropionate[53]. 1, 3-Dimethoxybenzene can be detected in all mould-fermented soft cheeses and has a nutty flavour, but nothing is known about its formation.

6) Other Components

Methyl cinnamic acid might be another impact component[54]. N-Isobutylacetamide was found in mould-fermented cheese and might participate in a bitter taste[55]. A musty-earthy flavour is got in white mould-ripened cheeses by 2-methyl-isoborneol from the metabolism of terpenes[52].

2. Formation of the Flavour Components

1) Carbohydrate Degradation

During the first two ripening phases, the lactose is totally degraded to lactic acid by homofermentative lactococci. There is thus no more lactose present after a 20~30 days maturation period of traditionally ripened Camembert.

In the beginning of the second maturation phase, the pH lies at 4.6. The develping yeasts and fungi degrade the lactic acid, and the pH increases to 6.0 in the inner parts and to 7.0 in the rind, this strong lactate degradation being due to the surface growth of *Penicillium*[53].

The neutralization of the cheese mass has various efffects. On the one hand, the higher pH makes the surface growth of aerophilic bacteria possible, which participate in flavour forming. On the other hand, different ripening enzymes are activated at a neutral pH. Other effects are the softening of the cheese mass and the migration of ionic calcium and phosphate into the outer layers of the loaf.

2) Fat Degradation

In mould fermented cheese an extensive lipolysis can proceed without leading to rancid off-flavours. One reason might be the neutralization of the FFAs in the course of the increase of pH. As the milk lipase in only of lower significance in cheese production, the milk fat is degraded by microbial lipases. *P. camemberti* possesses an extracellular lipase with a maximum activity at pH 9 and 35°C, which is still very active at pH 6.0 and at from 0°C and 20°C and which is activated by calcium ions. The first signs of activity of this lipase show with the first mycelium growth of *Penicillium.* It is maximal after 16 days before decreasing again. The lipolysis is maximal in the rind. A selective lipase of *Geotrichum candidum* is responsible for the high oleic acid content of Camambert[53].

3) Protein Degradation

During coagulation, the rennet attacks the α_{s1}-casein and leads to the formation of the curd. At the end of the maturation, the milk proteinase participates in the formation of γ-ca-seins. Of much more importance, however, is the proteolysis by enzymes from *Penicillium.* The fungus possesses exo-and endoproteases with different pH-optima.

The most important proteolytic enzymes from *Penicillium* are an aspartate proteinase and a metalloproteinase with optima activity at from pH 5.5 to 6.0 and stability over a large pH range. They are excreted from the fungus and diffuse into the inner parts of the cheese, their activity thus decreasing from without to within. The acid carboxypeptidase from *P. camemberti* degrades peptiodes and forms amino acids. It is of special significance for the degradation of bitter peptides[53].

The other microorganisms, also, participate in proteolysis. Enzymes from the lactococci form small peptides and amino acids. Geotrichum candidum shows extra- and intracellular protease activities, while the yeasts show only an intracellular activity. Protein degrading enzymes have also been found in *Brevibacterium* linens, which is important for flavour formation in traditionally produced Camembert[56].

The amino acid degradation by decarboxylation, deamination, and transamination finally leads to volatiles like aldehydes, acids, alcohols, amines and sulphur compunds. Very important is the ammonia formation by deamination, which can represent up to 9% of the total nitrogen in traditionally manufactured Camembert.

3. Process of Camembert Flavour concentrates

The available patent literature describes the methods of fermentative production of Cheddar and Blue Cheese flavour concentrates but not of fermentative Camembert flavour concentrates. Enzymatic methods for camembert are not described, either.

A synthetic Camembert flavour can be made by adding the mixture shown in table 3 in the concentration of 250 ppm to process cheese.

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