

Isolation and comparative characterization of components that contribute to the flavour of different types of cheese

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Summary

Water-soluble fractions (WSFs) of various types of cheese were comparatively investigated to characterize components that may contribute to cheese flavour. WSF of seven types (Cheddar, Edam, Gouda, Gruyère, Maasdam, Parmesan and Proosdij cheese) was prepared by homogenizing grated cheese with water in a stomacher and by removing remaining solids with a centrifuge. WSF was fractionated by serial ultrafiltration (with membranes of different molecular weight cut-off), followed by gel filtration and Sep-Pak C₁₈ chromatography. The fractions were analysed by reversed-phase HPLC, gas chromatography, amino acid analysis and sensorically. Low-molecular-weight (<500 Da) compounds were responsible for flavour in WSF. They might be small peptides, amino acids, free fatty acids or breakdown products of such compounds.

Keywords: cheese flavour; ultrafiltration; amino acids; peptides; fatty acids

1 Introduction

The maturation of cheese and the resulting development of flavour is a complex process. Despite extensive investigation, the compounds responsible for flavour are still largely unknown.

During cheese ripening, the main biochemical events yielding compounds that contribute to flavour are proteolysis, lipolysis and carbohydrate breakdown.

Proteolysis is governed by a variety of enzymes from different sources (1). Chymosin is used for curd formation and in several types of cheese it contributes significantly to ripening as well. The indigenous milk proteinase plasmin is also thought to play some role in ripening (2). The major source of proteolytic enzymes involved in cheese ripening, however, is considered to be the starter culture lactic acid bacteria (1, 3, 4). Milk proteins (caseins) are hydrolysed by the action of these proteolytic enzymes, yielding peptides of different chain length and amino acids. These peptides and amino acids are thought to be

associated directly with development of desirable (5) or undesirable (6) taste and aroma, or to act as precursors in subsequent reactions (7, 8).

The water-soluble fraction (WSF) of ripened cheese contains components that make a major contribution to the intensity of cheese flavour and it has therefore been studied extensively. Cheddar cheese has been most studied and various methods have been described to obtain WSF from this type of cheese (9, 10). Some workers used gel filtration and HPLC to fractionate WSF of Cheddar cheese (11, 12, 13). Aston & Creamer (14) extensively analysed components of WSF and assessed flavour. Others have also studied water-soluble fractions from Provolone cheese (15), Vacherin Mont d'Or cheese (16), Appenzeller cheese (17) and a Gouda-type cheese (18).

In the present study, water-soluble fractions of seven types of cheese were compared to find the contribution of their constituents to cheese flavour and flavour differentiation.

2 Materials and Methods

2.1 Cheese samples

Data about the cheeses used are summarized in Table 1. A cheese of each type was selected of normal age for consumption.

Cheeses were tasted by five experienced cheese graders before further handling and were found to be of good quality. A sector of each of the cheeses was grated (Hobart model 4812, Ohio, US) and the grated cheese was stored at -18°C until further investigation.

2.2 Extraction

The grated cheeses were extracted as outlined in Figure 1. WSF, designated Supernatant I, was acidified to pH 4.6 to precipitate protein and other high-molecular-weight material. The fraction soluble at pH 4.6, obtained after re-centrifugation, was designated Supernatant II. Acetic acid was removed by freeze-drying and the residue was redissolved in the original volume of distilled water. The cheese-flavour intensity remained essentially unaffected by this mild treatment. The upper fat layer, Protein Pellet I, Protein Pellet II and part of the Supernatant I were stored at -18°C until further analysis.

2.3 Fractionation

2.3.1 Ultrafiltration (UF). Freeze-dried and redissolved Supernatant II was further fractionated by ultrafiltration (Figure 1) at 4°C in a stirred-cell type ultrafiltration module (Amicon Corporation, MA, US), operated under a nitrogen pressure of 300 kPa with Amicon Diaflo membranes YM5 (5000 Da molecular-weight cut-off, MWCO) and YC05 (500 Da MWCO) in succession. The reten-

Table 1. Relevant data of the various types of cheese. CH, Switzerland; IE, Ireland; IT, Italy; NL, Netherlands.

| Type | Origin | Age | Starter |
|-----------------------|--------|------------|---|
| Cheddar | IE | 6–8 months | Q-starter |
| Edam | NL | 4 months | L-starter |
| Gouda | NL | 6 months | DL-starter (code Bos) |
| Gruyère | CH | 4–5 months | thermophilic lactobacilli thermophilic lactococci |
| Maasdam | NL | 6 weeks | DL-starter (code Bos) propionic acid bacteria |
| Parmesan | IT | 2 years | thermophilic lactobacilli thermophilic lactococci |
| Proesdij | NL | 6 months | DL-starter (code Bos) non-acidifying thermophilic lactobacilli and other thermophilic organisms |
| Gouda 20 ⁺ | NL | 6–12 weeks | DL-starter (code Bos) |

tates were repeatedly washed with distilled water and refiltered to free them from the lower-molecular-weight components.

2.3.2 Gel filtration. The UF<500 fraction (Figure 1) from Gouda cheese was separated by gel filtration on a glass column (15 mm × 820 mm) with Sephadex G-10 (Pharmacia LKB Biotechnology AB, Uppsala, SE) at 4 °C, with NaCl 0.01 M as an eluent (flow rate 20 mL/h, volume of fraction 5 mL). Portions of 140 mg of freeze-dried UF<500 material in 1.5 mL of water were applied to the column. Absorbance was measured with an LKB 2138 Uvicord S at wavelength 280 nm (Pharmacia LKB, Uppsala, SE) and the elution position of salts was determined by measuring electrical conductivity (WTW LF Digi 550, Weilheim, DE). Appropriate fractions were pooled and freeze-dried.

2.3.3 Sep-Pak C₁₈ chromatography. Pre-packed Sep-Pak Plus C₁₈ Environmental cartridges (Waters-Millipore, MA, US) were used to fractionate the UF<500 material from the various cheeses. Two cartridges were linked and loaded with 40 mg of freeze-dried product in 1 mL of water. The cartridges were eluted with water–ethanol by increasing the volume fraction of ethanol in four steps from 0 to 0.2. Final elution was with pure ethanol. This procedure resulted in five fractions, C₁₈-1 to C₁₈-5. The eluent was removed by evaporation under reduced pressure (Büchi Rotavapor-R, Flawil, CH).

2.4 Analysis

2.4.1 High-Performance Liquid Chromatography (HPLC). The HPLC system used consisted of an ISS-100 Perkin Elmer automatic sample injector, two Waters M6000 A pumps, an AGC Waters type 680 gradient controller, a Kratos 783 UV detector operating at wavelength 220 nm and a Waters model 450 UV

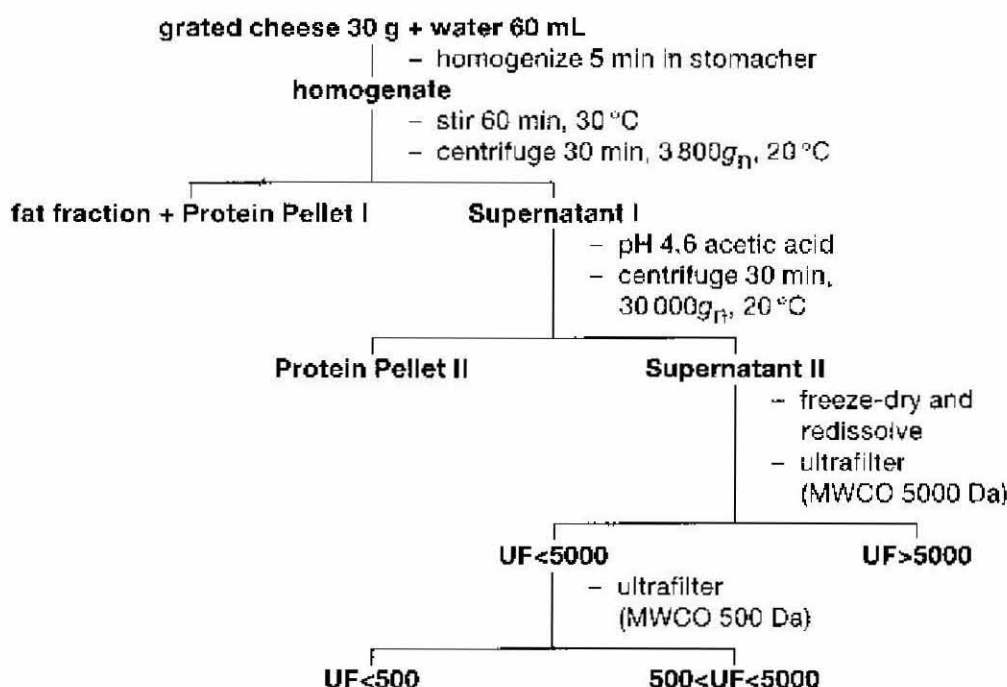


Figure 1. Extraction of grated cheese and fractionation of the extract.

detector operating at wavelength 280 nm. The equipment was linked to a Waters Maxima 820 data acquisition and processing system. Samples were chromatographed at 30 °C on a Bio-Rad HiPore RP-318 reversed-phase column (4.6 mm × 250 mm) preceded by a Bio-Rad C₁₈ cartridge guard column.

HPLC Solvent A consisted of acetonitrile/water/trifluoroacetic acid, 50/950/1, by volume. The components were separated by a 63 min stepwise linear gradient of Solvent B (acetonitrile/water/trifluoroacetic acid, 950/50/0.7, by volume) in Solvent A from 0 to 2 % Solvent B over 10 min, isocratic at 2 % Solvent B for 5 min, from 2 to 5 % Solvent B over 5 min, from 5 to 40 % Solvent B over 33 min, from 40 to 60 % Solvent B over 5 min and isocratic at 60 % Solvent B for 5 min (volume percentages throughout). The flow rate was 0.8 mL/min.

2.4.2 Amino acid analysis. Amino acid compositions were determined on a 4151 Alpha Plus amino acid analyser (Pharmacia LKB, Uppsala, SE). Total amino acids were determined after hydrolysis of the samples with HCl 6 M in evacuated tubes at 110 °C for 24 h.

2.4.3 Estimation of free fatty acids. UF<500 fractions (10 mL) were treated as described by de Jong and Badings (19) to estimate free fatty acids.

2.4.4 Sensory analysis. The various fractions were evaluated by a panel of five or six experienced tasters. During round-table discussions, the panel members tasted 400-µL portions placed directly on the tongue, after two-fold dilution

with distilled water of each liquid sample shown in Figure 1. The overall flavour quality and the cheese-flavour intensity were scored. Overall flavour quality meant general appreciation. During each session, no more than six samples were assessed. General appreciation was scored on a scale from 3 (very poor) to 8 (very good) and cheese-flavour intensity on a scale from 0 (none) to 4 (very strong).

The tests were 'blind' and the taste of the cheese fractions was assessed taking the de-acidified Supernatant II of the corresponding cheese as reference (Figure 1). The averages of the individual scores are presented.

Since the salt was completely transferred to the permeates during ultrafiltration, NaCl was added to the retentates in order to restore its concentration to that originally present in Supernatant II (NaCl determined by Mohr titration). Fat and protein pellets were freeze-dried and resuspended in distilled water.

Gel filtration fractions (Section 2.3.2) were tasted after removal of water by freeze-drying and subsequent dissolution of the dry material in 2 mL of distilled water. The same procedure was followed with Sep-Pak C₁₈ fractions (Section 2.3.3) after evaporation of the eluent.

3 Results

3.1 Fractionation and taste evaluation

Extraction of water-soluble components from cheese and subsequent UF steps resulted in various fractions suitable for flavour assessment. Lest we influence the flavour, we avoided the use of buffers and organic solvents during this fractionation. However we had to add acetic acid to precipitate proteins and to freeze-dry to remove the acid (Section 2.2). So tests were arranged to evaluate the effect of these treatments. Taste trials revealed that once-only acidification with acetic acid and subsequent freeze-drying (Section 2.2) or rotary evaporation (Section 2.3.3) of the fractions had only a minor effect.

The fat fractions and Protein Pellets I and II (Figure 1), after resuspension in distilled water, were organoleptically assessed. The protein pellets were always almost tasteless and odourless, whereas the fat fractions possessed a buttery aroma. The flavour of the water-soluble Supernatant II fractions of all the cheeses was described as cheese-like. Moreover, the taste panel members recognized some of the Supernatant II fractions as originating from the corresponding cheeses, Gruyère, Gouda and Parmesan.

The highest average values among water-soluble fractions for overall flavour quality and cheese-flavour intensity (Table 2) were for the UF permeates, except for the fraction originating from Edam cheese.

The UF<500 fraction from Gouda cheese was further fractionated by Sephadex G-10 gel filtration. Because the eluent fractions were to be organoleptically tested, use of salt was minimized in the eluent. As a consequence, some ionic interaction between sample components, e.g. tryptophan, and col-

Table 2. Average flavour scores of water-soluble fractions. For definition of scales see Section 2.4.4.

| Cheese type | Fraction | | | | | | | | | |
|-------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|
| | Supernatant II | | UF > 5000 | | UF < 5000 | | 500 < UF < 5000 | | UF < 500 | |
| | overall flavour | cheese flavour | overall flavour | cheese flavour | overall flavour | cheese flavour | overall flavour | cheese flavour | overall flavour | cheese flavour |
| Cheddar | 6.8 | 1.2 | 5.2 | 0.2 | 6.3 | 1.0 | 5.5 | 0.1 | 6.5 | 1.0 |
| Edam | 6.1 | 0.5 | 5.3 | 0 | 6.1 | 0.6 | 5.6 | 0.2 | 5.7 | 0.3 |
| Gouda | 6.5 | 1.0 | 5.1 | 0 | 6.6 | 0.9 | 5.3 | 0.2 | 6.2 | 0.8 |
| Gruyère | 7.0 | 1.5 | 4.8 | 0.2 | 6.5 | 1.5 | 5.3 | 0.3 | 6.5 | 1.3 |
| Maasdam | 6.7 | 1.4 | 5.3 | 0.3 | 6.5 | 1.4 | 5.5 | 0.4 | 6.2 | 1.1 |
| Parmesan | 7.0 | 1.5 | 5.6 | 0.2 | 6.9 | 1.4 | 5.3 | 0 | 6.6 | 1.3 |
| Proesdij | 6.5 | 1.5 | 5.1 | 0.4 | 6.5 | 1.6 | 5.2 | 0.3 | 6.4 | 1.4 |

umn material remained, so that separation was not strictly on the basis of molecular size. Figure 2 shows a gel filtration pattern of the UF<500 fraction of Gouda cheese and electrical conductivities, indicative of the elution of salts. Sensory analysis followed solution of the freeze-dried material in distilled water (Table 3) and showed the cheese-like flavour mainly in one pool, comprising Column Fractions 9 to 13. These fractions contained about a quarter of the salt in the UF<500 fraction loaded onto the column (total salt content in the UF<500 fraction from Gouda cheese 7 g/L) (Figure 2). The pooled Fractions 1 to 8 lacked cheese flavour, even after addition of NaCl to 7 g/L.

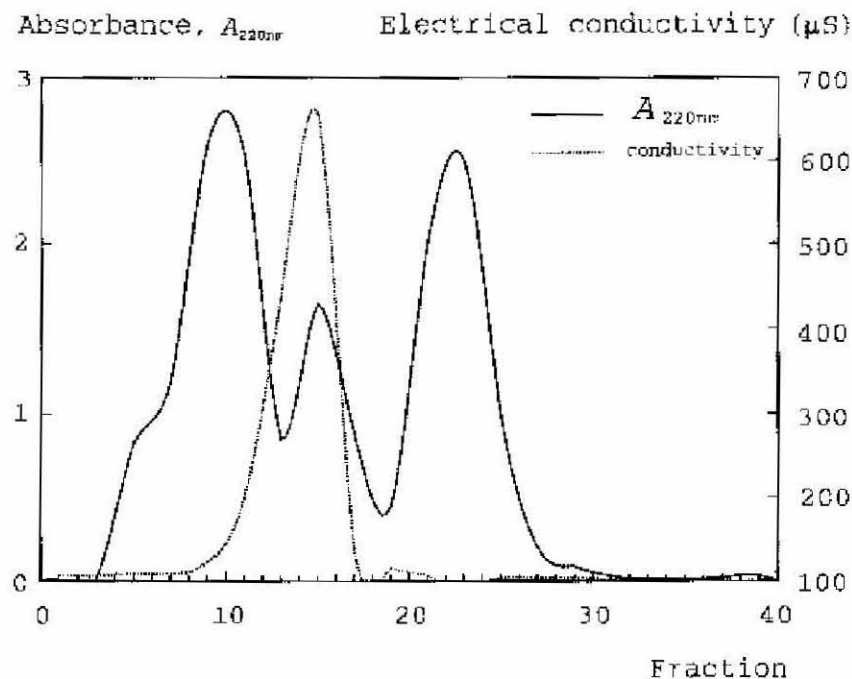


Figure 2. Sephadex G-10 gel filtration chromatogram of the UF<500 fraction from Gouda cheese.

Table 3. Results of sensory analysis on Sephadex G-10 gel filtration pools originating from Gouda cheese. For definition of scales see Section 2.4.4.

| Combined fractions | Overall flavour quality | Cheese flavour intensity |
|--------------------|-------------------------|--------------------------|
| UF<500 (reference) | 6.5 | 1.0 |
| G-10 1 to 8 | 4.8 | 0.2 |
| G-10 9 to 13 | 6.8 | 1.5 |
| G-10 14 to 19 | 4.2 | 0.2 |
| G-10 20 to 24 | 5.8 | 0.2 |
| G-10 25 to 30 | 4.8 | 0 |

Table 4. Results of sensory analysis on Sep-Pak C₁₈ fractions originating from Gouda cheese. For definition of scales see Section 2.4.4.

| Fractions | Overall flavour quality | Cheese flavour intensity |
|--------------------|-------------------------|--------------------------|
| UF<500 (reference) | 6.5 | 1.0 |
| C ₁₈ -1 | 7.0 | 2.0 |
| C ₁₈ -2 | 4.5 | 0 |
| C ₁₈ -3 | 5.0 | 0 |
| C ₁₈ -4 | 5.0 | 0 |
| C ₁₈ -5 | 5.0 | 0 |

Besides gel filtration (only on the Gouda cheese UF<500 fraction), Sep-Pak C₁₈ chromatography was used to fractionate UF<500 material from various types of cheese. For Gouda cheese (Table 4), only the first eluted fraction possessed a cheese flavour. The same results were obtained for Cheddar, Parmesan, Gruyère, Proosdij and Maasdam (results not shown). UF<500 from Edam cheese was not further fractionated.

3.2 HPLC and analysis for amino acids

HPLC patterns for UF<500 fractions were similar for all the cheeses (Figure 3). The composition of the main peaks was determined by analysis for amino acids after collecting the peak material at the outlet of the UV detector. The peaks at 6.7, 11.7 and 25.2 min were due to tyrosine, phenylalanine and tryptophan, respectively.

Because most amino acids hardly absorb radiation of wavelength 220 nm, UF<500 fractions from the various cheeses were analysed for amino acids. The concentrations of free and total amino acids were measured (Figure 4). In all the UF<500 fractions, free amino acids were the main components. The individual amino acid profiles (not shown) were similar for all the cheeses. Glutamic acid, leucine and phenylalanine were the major amino acids in both free and total amino acid fractions. Valine, proline and lysine were also quite abundant. Notable was the presence of non-casein amino acids like γ -amino butyric acid and ornithine in the UF<500 fractions. The concentration of total amino acids was

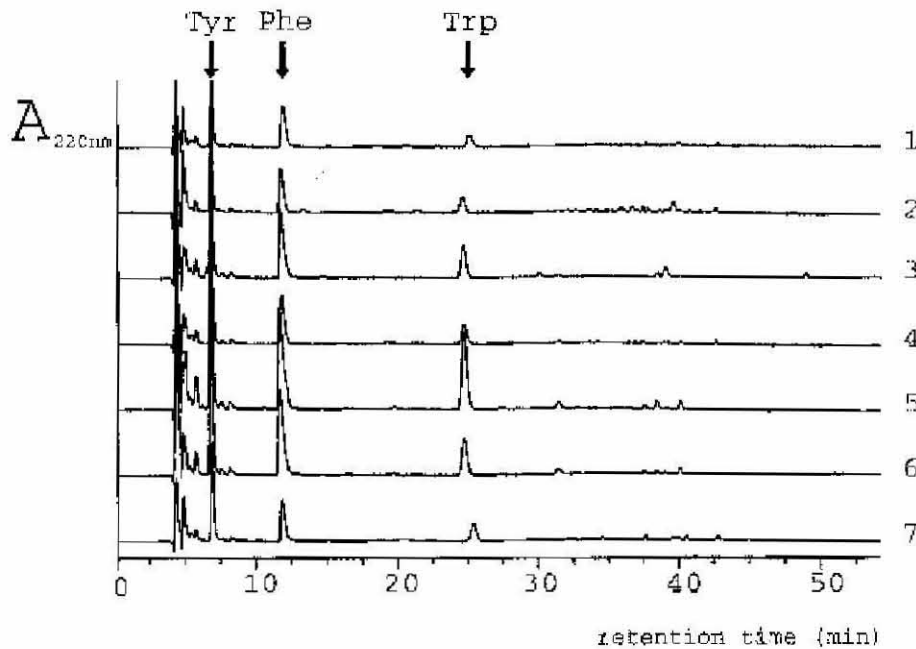


Figure 3. Reversed-phase high-performance liquid chromatograms of UF<500 fractions from cheeses. Types of cheese: 1. Cheddar; 2. Edam; 3. Gruyère; 4. Maasdam; 5. Parmesan; 6. Proosdij; 7. Gouda.

higher than of total free amino acids in all the samples, indicating the presence of peptides (Figure 4). The small peaks between 30 and 45 min in the chromatograms of Figure 3 were probably due to these peptides.

Maasdam, Cheddar, Gouda and Edam cheese contained about the same mass concentrations (g L^{-1}) of free amino acids in the UF<500 fraction (Figure 4). For Gouda and Edam cheese, the concentration of total amino acids in the UF<500 fraction was somewhat higher than for Maasdam and Cheddar cheese. UF<500 fractions from Gruyère, Proosdij and especially Parmesan cheese contained the highest concentrations of total amino acids. The proportion of total free amino acids in the UF<500 fractions of these cheeses was, however, slightly smaller (Figure 4), indicating the presence of somewhat higher concentrations of small peptides.

Reversed-phase HPLC profiles of UF-fractions from Gouda and Proosdij cheese (both about 6 months old) were compared (Figure 5). The absorbance profile at wavelength 220 nm of the 500<UF<5000 fraction from Proosdij cheese (made with lactococci and lactobacilli) (Trace 2 in Figure 5) differed markedly from that of the corresponding fraction from Gouda cheese (made with lactococci only) (Trace 5), particularly in the region 30–45 min, where larger peptides eluted. These larger peptides broke down faster in Proosdij cheese than in Gouda cheese.

The combined Sephadex G-10 Fractions 9 to 13 from Gouda cheese (Figure 2) contained about half the peptide material of the UF<500 fraction (Figure 6), as calculated from the ratio of free to total amino acids. The combined frac-

Mass concentration of amino acids (g/L)

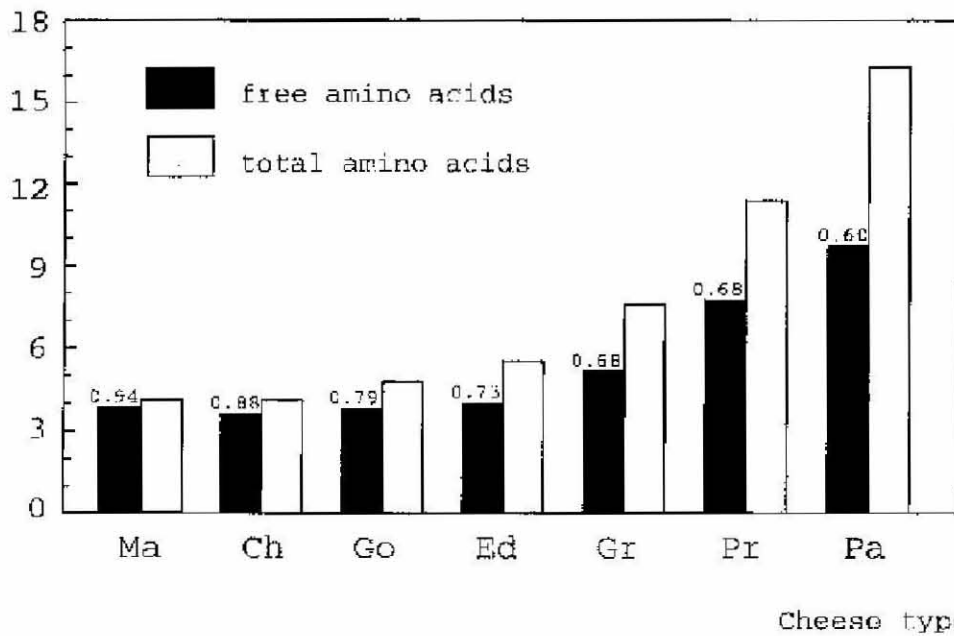


Figure 4. Total free and total (free + peptide-bound) amino acids in UF<500 fractions from cheeses. Values above columns, fraction of free amino acids in total amino acids. Types of cheese: Ma: Maasdam, Ch: Cheddar, Go: Gouda, Ed: Edam, Gr: Gruyère, Pr: Proosdij, Pa: Parmesan.

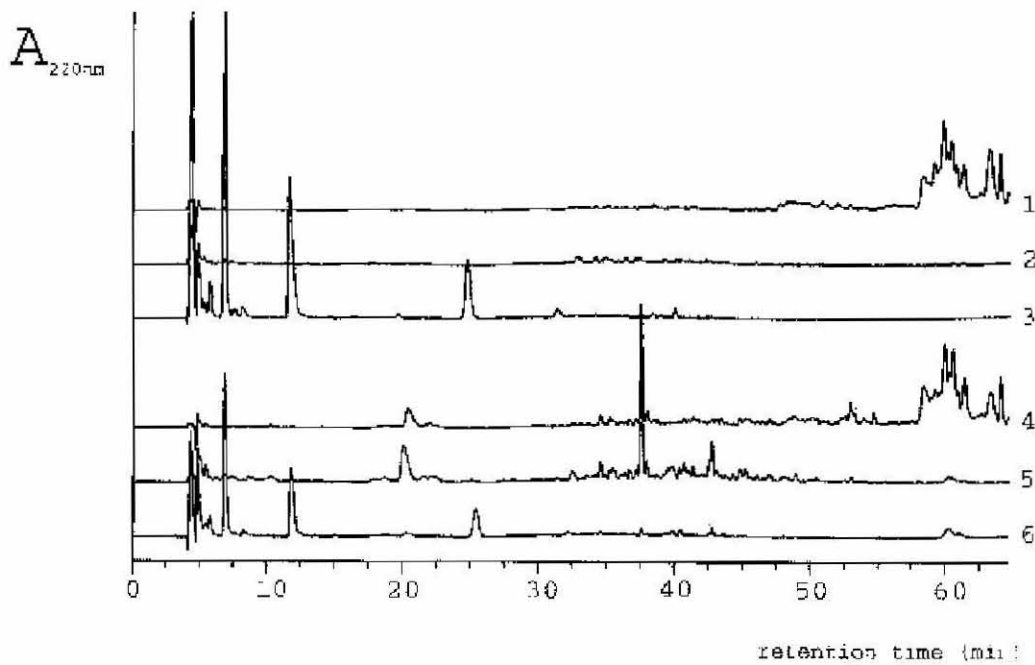


Figure 5. Reversed-phase high-performance liquid chromatograms of fractions from Gouda and Proosdij cheese. Fractions: 1, UF>5000 Proosdij; 2, 500<UF<5000 Proosdij; 3, UF<500 Proosdij; 4, UF>5000 Gouda; 5, 500<UF<5000 Gouda; 6, UF<500 Gouda.

tions 1 to 8 completely lacked cheese flavour. Furthermore, the removal of this 1-8 pool (Figure 6A) from the UF<500 fraction had no discernible effect on the cheese flavour of the remaining material represented by the 9-13 pool of Figure 6B.

Reversed-phase analysis of Sep-Pak C₁₈ fractions from UF<500 material of Gouda cheese revealed that only strongly hydrophilic peptides (retention time < 10 min) were present in the fraction with cheese flavour (C₁₈-1, Figure 7). These peptides represented about 10 % of the total amino acids in fraction C₁₈-1. More hydrophobic peptides (retention time > 10 min) were possibly present in subsequent C₁₈ fractions, which, however, lacked flavour.

3.3 Analysis for free fatty acids

Besides amino acids and peptides, volatile compounds, such as fatty acids, occur in WSF of cheeses and therefore in UF<500 fractions. The presence of volatile compounds in these fractions became especially evident during sensory tests. Almost all the samples with a cheese-like taste possessed a 'cheesy' odour as well.

Only relatively short-chain free fatty acids (<C9) were present in the UF<500 fractions (Table 5). Gruyère, Maasdam and Parmesan cheese contained the highest amounts, C3 and C4 being the major representatives. For comparison, free fatty acids were also determined in the UF<500 fraction of a low-fat cheese (Gouda, 20¹).

Mass concentration of amino acids (g/L)

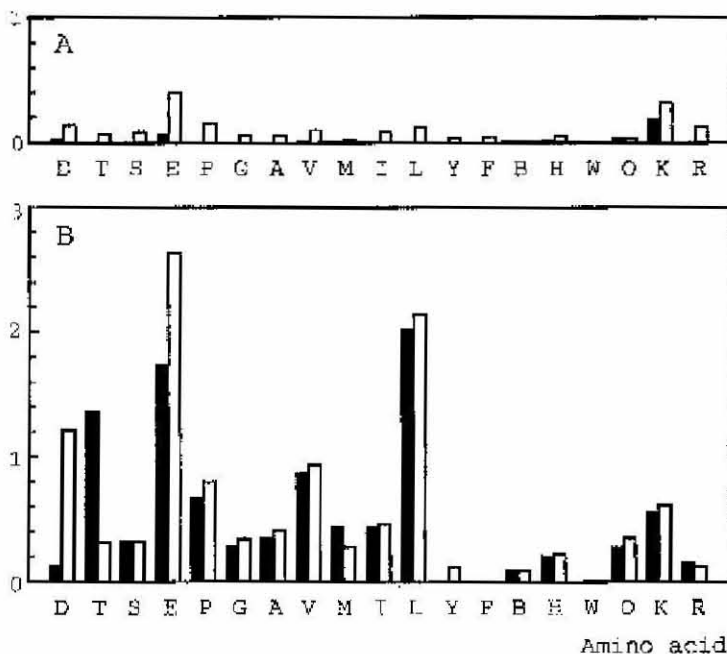


Figure 6. Mass concentrations of free (filled bars) and total (free + peptide-bound) (open bars) amino acids in Sephadex G-10 fractions 1-8 (A) and fractions 9-13 (B) from Gouda cheese.

One-letter codes (20) were used for the notation of amino acids. B, γ -aminobutyric acid; O, ornithine. Note that the concentration of free T includes that of free Q and N (co-eluting on the column) and that the respective concentrations of total E and D include total Q and N, which are converted by acid hydrolysis.

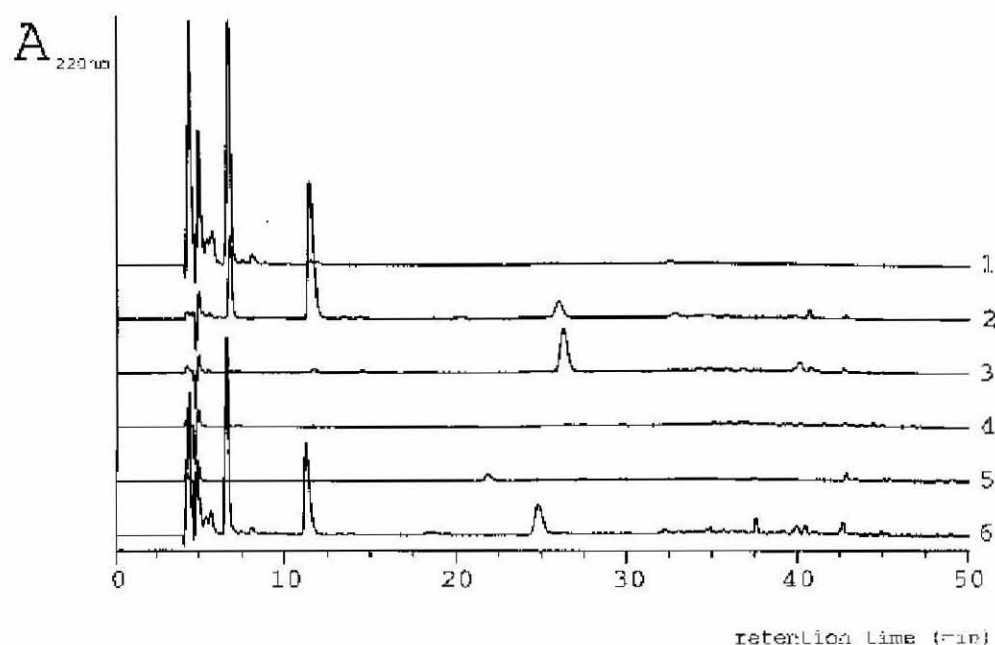


Figure 7. Reversed-phase high-performance liquid chromatograms of Sep-Pak C_{18} fractions from Gouda cheese. 1, C_{18} -1; 2, C_{18} -2; 3, C_{18} -3; 4, C_{18} -4; 5, C_{18} -5; 6, UF<500.

4 Discussion

WSF makes a major contribution to the intensity of the cheese flavour (9, 14). Several studies have been devoted to the fractionation and analysis of WSF from Cheddar cheese (11–14). Our study revealed that UF<500 fractions of the cheeses contained the components responsible for cheese flavour, except in Edam cheese. This finding agrees with those of other workers on the fractionation of Cheddar cheese (14) and Comté cheese (21), in which components <1000 Da and <500 Da, respectively, proved responsible for cheese flavour. The peptides and proteins in the UF>500 fractions do not contribute directly to the actual flavour of the

Table 5. Free fatty acids in UF<500 fractions from various types of cheese. Concentrations in μM . C3:0, propionic acid; C4:0, butyric acid; C4:0 iso, isobutyric acid; C5:0 iso, isovaleric acid; C6:0, caproic acid; C8:0, caprylic acid.

| Cheese type | C3:0 | C4:0 | C4:0 iso | C5:0 iso | C6:0 | C8:0 | sum C3-C8 |
|-----------------------|------|------|----------|----------|------|------|-----------|
| Cheddar | 8 | 73 | 0 | 0 | 7 | 0 | 88 |
| Edam | 19 | 64 | 35 | 93 | 10 | 0 | 221 |
| Gouda | 13 | 146 | 7 | 22 | 17 | 0 | 205 |
| Gruyère | 1345 | 1716 | 276 | 398 | 47 | 1 | 3783 |
| Maasdam | 9122 | 67 | 6 | 45 | 12 | 1 | 9253 |
| Parmesan | 46 | 1156 | 1 | 7 | 134 | 3 | 1347 |
| Proosdij | 15 | 114 | 4 | 10 | 20 | 1 | 164 |
| Gouda 20 ⁺ | 8 | 21 | 5 | 19 | 5 | 0 | 58 |

cheeses. The deviant results for Edam are not fully explicable. Though the protein pellets from this cheese were devoid of a cheese-like flavour, association of flavour components with proteins or larger peptides could have been responsible for the loss of flavour of WSF. However repeated extraction of these pellets with distilled water did not result in WSF with cheese flavour.

The UF<500 fractions of the various cheeses include low-molecular-weight peptides (probably not larger than tetrapeptides), amino acids and further break-down products. Their formation results from the action of enzymes from milk and lactic acid bacteria (1, 22). The larger amounts of peptides and free amino acids in UF<500 fractions from Gruyère, Proosdij and Parmesan cheese than those from other cheeses were probably due to the action of lactobacilli, which can produce larger amounts of amino acids than lactococci (23, 24). For Parmesan cheese, the greater age also contributes to the higher concentration of free amino acids. Lactobacilli are responsible for a higher rate of break-down of larger peptides in 500<UF<5000 fractions of Gruyère, Proosdij and Parmesan cheese. The higher production rate of free amino acids in these cheeses may, together with other properties of the lactobacilli, account for the considerable difference in flavour characteristics. Though free amino acids were present in the fractions with a cheese-like flavour (UF<500), their direct contribution to the actual cheese flavour is probably limited (25). Free amino acids more likely act as precursors for cheese flavour compounds; both enzymic and non-enzymic processes may contribute to their break-down (1, 26). So there is not necessarily a relation between cheese flavour and concentration of total free amino acids, which is further illustrated by differences in flavour between cheeses with almost the same concentration of free amino acids (Cheddar, Maasdam, Gouda, Figure 4). The presence of non-casein amino acids, such as γ -aminobutyric acid and ornithine, in the UF<500 fractions can be ascribed to enzymic conversion of glutamic acid and arginine, respectively (15, 27, 28).

The role of peptides in the UF<500 fractions in relation to flavour is not clear. The pooled Fractions 1–8 of the Sephadex G-10 separation, and the Sep-Pak fractions C₁₈-2 to C₁₈-5, containing mainly small peptides, lacked cheese flavour (typical examples in Tables 3 and 4). Several authors mention small peptides as direct flavour components in cheese (5, 16, 29). Our results with the cheesy flavoured Fractions 9–13 of the Sephadex G-10 separation and the Sep-Pak C₁₈-1 fraction of various cheeses indicate that the direct contribution of small peptides to the actual cheese flavour is probably limited.

Small peptides and amino acids must be mainly responsible for basic flavours (e.g. brothy (14), savoury (30), sweet (31, 32), bitter (6), salty), on which actual cheese flavours are superimposed after formation from amino acids by enzymic or chemical pathways, or by both.

Only shorter-chain fatty acids (<C9) were present in the UF<500 fractions. They could influence flavour more than larger fatty acids (>C8) (33). The larger fatty acids, which are certainly present in cheese, are most likely retained in the fat layer during extraction.

Parmesan and Gruyère cheese contained relatively high amounts of free fatty acids. In these cheeses, butyric acid (C4:0) probably plays an important role in flavour (26). The high concentration of propionic acid (C3:0) in Maasdam cheese develops by the action of propionic acid bacteria. UF<500 fractions from Gouda, Edam, Proosdij, Cheddar and Gouda 20+ cheese contained much less free fatty acids than those of Parmesan and Gruyère, and so the contribution of fatty acids to their flavour will be much less.

Free fatty acids are formed in cheese by lipolysis of fats (34, 35, 36) and through catabolism of lactic acid and amino acids by bacteria (mainly short-chain free fatty acids like C3 and C4) (26, 36, 37). The larger amounts of C4:0 in Parmesan and Gruyère cheese might also originate from butyric acid fermentation (late blowing).

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