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Evaluation of taste compounds of Swiss cheese (Emmentaler)

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Abstract The following substances were evaluated as potent taste compounds: acetic acid, propionic acid, lactic acid, succinic acid and glutamic acid, each in free form and/or as ammonium, sodium, potassium, magnesium and calcium salts, as well as the corresponding chlorides and phosphates. Magnesium and calcium propionate mainly caused the sweetish note in the taste profile of Emmentaler. Although bitter tasting amino acids and peptides occurred in the cheese sample, they were not detected in the taste profile.

Key words Emmentaler cheese · Taste compounds · Chemical analysis of taste · Sensorial analysis of taste

Introduction

The flavour of the Swiss cheese Emmentaler (Em), which is caused by a balance of odorants and taste compounds, is characterised by the attributes sweetish, mildly aromatic and nutty [1]. To our knowledge no systematic study of the taste compounds of Em has been reported in the literature. Only some compounds have been proven to be important, e.g. acetic acid and propionic acid [2–4]. Furthermore, there are indications [2] that calcium propionate is involved in the sweet taste, whereas high levels of butyric acid may lead to an off-flavour [3, 5].

Mixtures containing proline, glycine, serine, threonine, aspartic acid, glutamic acid, cysteine, tryptophan, histidine, lysine, combinations of amino acids found in cheddar cheese and also proline alone [3, 6–8] are regarded to be involved in the sweet taste of cheese. In addition, the magnesium and calcium salts of dipeptides have been suggested [9] as contributors to the sweet taste. However, free amino acids and peptides which have been extracted from Em tasted bouillon-like [10].

The objective of our work was to reveal the odour and taste compounds causing the flavour of Em. To approach this goal, at the beginning of the study [11], the potent odorants appearing in the neutral volatile fraction of Em were identified and quantified in an Em sample (sample A in [11]) of high flavour quality. Then, as reported in the present paper, the taste compounds including the volatile acids were evaluated in Em sample A by the combined application of chemical and sensorial analytical methods. Finally, a model study was performed [12] to verify whether the compounds evaluated are actually responsible for the flavour of Em. The model was prepared by using an unripened cheese (UC) as base. After addition of the odour and taste compounds to UC, the flavour profile of the model obtained was compared with that of the original Em sample [12]. The compounds revealed by this procedure are suitable as indicator substances to objectively characterise the flavour development during the ripening of Em.

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Materials and methods

Materials

The sample of Em was identical to sample A reported previously [11]; the rind, 2 cm in width, was removed. The following chemical

substances were purchased from commercial suppliers: acetic acid, caproic acid, caprylic acid, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, tryptophan, sodium chloride, potassium chloride, sodium-dihydrogen phosphate, calcium hydroxide, magnesium hydroxide, succinic acid, ammonia, sodium hydroxide, calcium chloride, magnesium chloride, hydrochloric acid (Merck, Darmstadt, Germany); propionic acid, 3-methylbutyric acid, proline, tyramine (Fluka, Neu Ulm, Germany); butyric acid, capric acid (Sigma, Deisenhofen, Germany); lysine, histamine, lactic acid (Serva, Heidelberg, Germany); Sephadex G-15 (Pharmacia, Freiburg, Germany).

Chemical analysis

Extraction. Cheese (100 g), cut into pieces, was suspended in distilled water (400 ml) and then homogenized for 6 min in a Waring blender. The pH of the homogenate was adjusted to 4.6 by addition of aqueous HCl (5 mol/l). After centrifugation, the supernatant was freeze-dried (extract), and the casein precipitate was discarded.

Ultrafiltration. The freeze–dried extract (5 g), dissolved in distilled water (50 ml), was filtered through the Diaflo membrane YM-10 (Amicon, Witten, Germany). The permeate (PI, 70 ml) was collected and freeze-dried. The retentate (RI, 5 ml) was diluted with water (5 ml) and then concentrated by ultrafiltration to a volume of 5 ml. After repeating the dilution and concentration steps for 7 times, RI was freeze-dried. PI (5 g), dissolved in distilled water (50 ml), was filtered through the Diaflo membrane YM-1. The permeate (PII, 70 ml) was pooled and freeze-dried. The retentate (RII, 5 ml) was diluted with water (5 ml) and then concentrated to a volume of 5 ml. The dilution and concentration steps were repeated 7 times and finally RII was freeze-dried.

Gel chromatography. A glass column (59 cm × 2.6 cm) was filled with Sephadex G-15 suspended in aqueous acetic acid (0.5 mol/l). This solvent was also used as eluent (35 ml/min flow rate). The effluent was monitored at 254 nm. Between applications, eluent (300 ml) was pumped through the column (35 ml/h flow rate). The lyophilised permeate PI (600 mg), dissolved in aqueous acetic acid (0.5 mol/l, 4 ml), was separated on the column into four fractions (G I to G IV, Fig. 1) which were pooled and then freeze-dried.

Amino acids and ammonia. PII (0.74 mg/ml), dissolved in the starting buffer system for amino acid analysis [13], was applied to the amino acid analyser LC 3000 (Biotronic, Maintal, Germany). The free amino acids were quantified as reported in [14].

Free fatty acids [15, 16]. Cheese (1.000 g) was extracted with diethyl ether/heptane (1 + 1, by vol). After isolation by solid phase extraction on Bond-Elut-Aminopropyl (ICT, Bad Homburg, Germany), the free fatty acids were determined with a Carlo Erba gas chromatograph, type 4200 (Carlo Erba, Hofheim, Germany) by using a fused silica capillary FFAP (15 m × 0.53 mm, film thickness 1.0 μm; J and W Scientific, Folsom, USA). The capillary was coupled with a flame ionisation detector (FID), and helium was used as carrier gas (9 ml/min flow rate). The samples were applied by the on-column injection technique at 65°C. After 2 min the temperature of the oven was raised by 10°C/min to 240°C, which was held for 6 min, and then raised by 2.5°C/min to 250°C which was held for 15 min.

Chloride. After homogenisation of the cheese sample (4 g) in aqueous HNO_3 (0.4 mol/l, 33 ml), chloride was determined by potentiometric titration [17].

Biogenic amines. These were converted into *N*-dansyl derivatives and then determined in the cheese sample by high performance liquid chromatography [18].

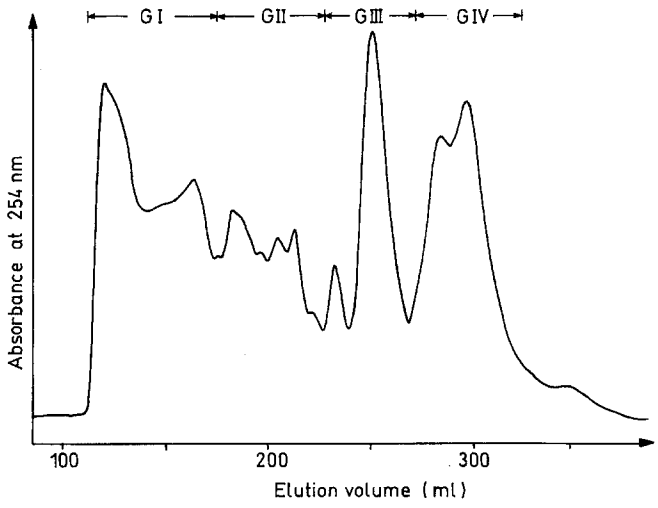

Fig. 1 Gel chromatogram of the permeate PI on Sephadex G-15

Sodium, potassium, magnesium and calcium. These were determined in PII by atomic absorption spectroscopy [19].

Lactic acid and succinic acid. These were enzymatically determined in PII [20, 21].

Phosphate. Phosphate in PII was converted into molybdenum blue and then determined by photometry [22].

Sensory analysis

Taste profile analysis [23, 24]. The taste profiles of the samples were evaluated in an isolated sensory panel room at 21 ± 1°C. The panel consisted of four to seven experienced assessors, who were trained to assess aqueous solutions of the reference taste compounds reported in [12]. The stimuli were the fractions obtained from Em (Tables 2 and 3) and the mixtures of compounds presented in Tables 6 and 7. The stimuli were dissolved in tap water at the concentration levels given in the tables. The pH of each sample was adjusted to 5.6 by the addition of some drops of diluted aqueous NaOH or HCl. In each session four samples (15 ml each) were presented in covered glass beakers (diameter 40 mm, capacity 45 ml). After removing the cover, the panellist pipetted 1 ml of the solution onto their the tongue. Between successive samples the mouth was rinsed with tap water of 30 to 35°C. The intensity of the taste attributes sweet, sour, salty, bitter, nutty, monosodium glutamate (MSG) and soapy were determined as a point on the scale: 0 (none), 0.5, 1, 1.5 ··· 3 (extremely strong). The results obtained by the panellists were averaged and rounded to the nearest 0.5 points.

Recognition threshold of taste compounds [24]. The stimuli were fatty acids, salts, biogenic amines as well as the miscellaneous substances listed in Tables 4 and 5. Apart from caprylic and capric acid, the stimuli (10–100 mmol/l) were dissolved in tap water. The pH of these stock solutions was adjusted to 5.6 by the addition of diluted aqueous NaOH or HCl. Caprylic and capric acid were emulsified with sucrose palmitate stearate [25] and then the pH was adjusted to 5.6. Aliquots of each stock solution were diluted with tap water (pH 5.6) to prepare seven to nine serial dilutions. The taste threshold value approximated in pre-testing lay in the centre of the dilution series. In triangle tests, the recognition taste thresholds of each stimulus were determined by starting with the strongest concentration and then proceeding to progressively lower concentrations. In each triad, one glass beaker contained 15 ml of

the sample; each of the other two beakers contained 15 ml of tap water (pH 5.6). The test was performed by four to seven experienced assessors who tasted samples of 1 ml at room temperature. The mouth was rinsed with tap water (30–35°C) between sample pairs. The most dilute concentration in which the taste quality of the substances was correctly identified was taken as the recognition threshold. The results obtained by the panellists were averaged.

Results and discussion

Taste of the Em extract

Em was extracted with water, and the extract obtained was lyophilised after precipitation of casein; the yield amounted to 8.2%. An aqueous solution of the lyophilisate in a concentration equal to that in the Em extract was inedible. Therefore, it was diluted with water to one-fourth of the concentration and then tasted. The results in Table 1 show that the taste profile of the diluted extract was very similar to that of the starting material of Em regarding the sour and salty qualities. The nutty and MSG qualities were weaker in the diluted extract, and the sweet note was lacking.

Taste of fractions

The extract was separated by ultrafiltration into the retentate RI and the permeate PI (Table 2). RI containing the compounds with a molecular mass $> 10^4$ amounted to 18% of the extract, and PI (molecular mass $< 10^4$) to 82%. The soapy taste of the high molecular fraction RI (Table 2), possibly caused by protein/fatty acid complexes [26], was absent in Em.

The permeate, PI, tasting like the extract was then filtered through a membrane having a molecular mass cut-off of 1000. As shown in Table 2, the taste profile of the permeate PII, obtained in a yield of 52% (freeze-dried extract = 100%), was very similar to that of the extract with exception of the weaker nutty note. The slightly bitter taste of the retentate RII (Table 2) was neither perceived in Em nor in the extract.

The permeate PI was separated by gel chromatography on Sephadex G-15 into four fractions (G I to G IV in Fig. 1) of which the taste profiles are summarised in Table 3. Most of the taste attributes of PI were perceived in fraction G II containing the compounds with a molecular mass of < 750. However, the overall taste of PI was only restored after mixing of the four fractions. Gel chromatography of PI exposed bitter-tasting substances which appeared in the fractions G I and G IV. Further investigations [27] suggest that the bitter taste of these substances is masked by the glutamic acid occurring in fraction G II. Actually, the mixture of the fractions G I to G IV was not bitter (Table 3).

Altogether, it was concluded from the results of both the ultrafiltration experiment and the separation of PI by gel chromatography, that only low molecular mass compounds contribute significantly to the taste of Em.

Table 1 Taste profiles for grated Emmentaler (Em) and its aqueous extract

Taste quality	Taste intensity[a]	
	Grated Emmentaler	Aqueous extract[b]
Sweet	1.5	0
Sour	1.5	1.5
Salty	2	2
Bitter	0	0
Nutty	2.5	1.5
MSG[c]	2	1.5

[a] The intensity was scored at 22°C (see section "Sensory Analysis")
[b] The lyophilisate, dissolved in water, was diluted to 25% of its concentration in Em
[c] Taste impression was like that of an aqueous solution of monosodium glutamate (MSG; 50 mmol/l)

Table 2 Taste of the fractions obtained by ultrafiltration of the Em extract

Taste quality	Taste intensity[a]				
	Aqueous extract[b]	Fraction[c,d]			
		RI	PI	RII	PII
Sour	1.5	0	1	0	1.5
Salty	2	0	2	0	1.5
Bitter	0	0	0	1	0
Nutty	1.5	0	1.5	0	0.5
MSG[e]	1.5	0	1	0	1.5
Soapy	0	2	0	0	0

[a,b,e] Refer to footnotes a, b, and c in Table 1
[c] The lyophilised fraction was dissolved in tap water, diluted to 25% of its concentration in Em and tasted after adjustment of pH 5.6
[d] RI, retentate (molecular mass, $M_r > 10^4$); PI, permeate ($M_r < 10^4$); RII, retentate (M_r 10^3 to 10^4); PII, permeate ($M_r < 10^3$)

Table 3 Taste of the fractions obtained by gel chromatography of permeate PI

Taste quality	Taste intensity of fraction[a,b]				
	G I	G II	G III	G IV	Mixture of G I to G IV
Sour	0	2	0	0	1.5
Salty	0	1.5	0	0.5	1.5
Bitter	0.5	0	0	1	0
Nutty	0	1	0.5	0	1.5
MSG[c]	0	1	0	0	1.5

[a,c] Refer to footnotes a and c in Table 1
[b] Refers to footnote c in Table 2

Concentrations of taste compounds in Em

Free amino acids, ammonia, lactic and succinic acids as well as sodium, potassium, magnesium, calcium and

phosphate were determined in PII, the free fatty acids, chloride and biogenic amines in separately prepared extracts of Em. On the basis of these results (Table 4), the taste activity values (TAVs), which, in analogy to the odour activity values [28], are defined as the ratio of concentration to taste threshold, were calculated and are listed in Table 4. The definitions of TAV and the odour activity value take into account that the taste and odour thresholds respectively, do not reflect the threshold in foods [29].

The highest TAVs were found for acetic acid and propionic acid. This result confirms the strong contribution of both acids to the flavour of Em. Butyric acid and capric acid surpassed also their taste threshold values, but their TAVs of 4.5 and 1.1, respectively, were much lower than the TAVs of acetic acid and propionic acid.

Glutamic acid was the major taste compound in the fraction of free amino acids followed by leucine which has a seven fold lower TAV. The concentration levels of

Table 4 Concentrations and taste activity values (TAV) of low molecular compounds of Em

Compound	Concentration[a] (mmol/kg)	Taste threshold[b] (mmol/kg)	TAV[c]
Fatty acids:[d]			
Acetic acid (2:0)	37.3	0.9	41
Propionic acid (3:0)	56.3	0.4	140
Butyric acid (4:0)	0.94	0.2	4.5
3-Methylbutyric acid (i-5:0)	0.015	0.1	0.15
Caproic acid (6:0)	0.38	0.7	0.5
Caprylic acid (8:0)	0.21	0.7	0.3
Capric acid (10:0)	0.42	0.4	1.1
Free-amino acids:[e]			
Aspartic acid	0.74	20	0.04
Threonine	6.18	35	0.18
Serine	4.11	25	0.16
Glutamic acid	20.9	2	10.5
Proline	13.9	25	0.56
Glycine	4.04	25	0.16
Alanine	5.50	12	0.46
Valine	11.2	20	0.56
Methionine	2.89	5	0.58
Isoleucine	4.49	10	0.45
Leucine	16.0	11	1.45
Tyrosine	2.63	4	0.66
Phenylalanine	6.26	45	0.14
Histidine	1.92	45	0.04
Lysine	13.9	80	0.17
Tryptophan	0.46	4	0.12
Minerals:			
Sodium	94	5[f]	19
Potassium	19.5	10[f]	1.95
Magnesium	14.9	3[f]	5.0
Calcium	191	5[f]	38
Chloride	66.8	5[g]	13
Phosphate	20.4	5[g]	4.1
Biogenic amines:			
Tyramine	1.21	0.5	2.4
Histamine	1.85	0.6	3.1
Miscellaneous substances:			
Lactic acid	26.8	12	2.3
Succinic acid	11.7	0.4	27
Ammonia	11.4	5	2.3

[a] Mean of duplicates which varied less than the percentage given in parentheses: fatty acids (7%), free amino acids and ammonia (9%), minerals (7%), biogenic amines (12%), lactic and succinic acid (7%)
[b] Recognition threshold in water
[c] TAVs were calculated by dividing the concentrations of the compounds by their taste thresholds in water
[d] Taste thresholds of the fatty acids 8: 0 and 10: 0 emulsified with sucrose palmitate stearate [25]; the taste thresholds of the fatty acids as well as those of lactic and succinic acids and ammonia were determined at pH 5.6
[e] Taste thresholds according to [24, 31, 32]
[f] Threshold of chloride
[g] Threshold of the sodium salt

Table 5 Taste threshold values of propionates at pH 5.6

Salt	Threshold value[a] (mmol/kg)	
	Range	Mean
Magnesium propionate	2.3–4.7	3.5
Calcium propionate	4.7–9.4	7.1

[a] Recognition threshold for the sweet taste

the other free amino acids lay below their corresponding taste threshold values.

All of the ions investigated might be involved in the taste of Em, as their TAVs, based on the taste thresholds of the corresponding chlorides or potassium salts, respectively, were higher than one. This was also found for the biogenic amines, ammonia, lactic acid, and in particular for succinic acid.

To check the suggestion [2] that calcium propionate is involved in the sweetish note of Em, the sensory properties of calcium and magnesium salts of some acids were compared. At the concentration levels occurring in Em only calcium and magnesium propionate, but not the corresponding acetates and butyrates, caused a sweet taste at pH 5.6. As shown in Table 5, the taste threshold of calcium propionate was approximately twice as high as that of magnesium propionate.

The high concentration levels of propionic acid, magnesium and calcium in Em suggest that the salts of these ions contribute strongly to the sweetish note of Em. This conclusion could appear to contradict the finding (Table 1) that this taste note was lacking in the aqueous extract. However, this is not the case for the following reason: after precipitation of casein, the pH of the extract amounted to 4.6 which was lower than the pK_a value of 4.87 reported for propionic acid [30]. Consequently, the volatile undissociated form of this acid was present in the extract at pH 4.6 and it was continuously removed by the freeze-drying process from the equilibrium with the non-volatile anion.

Taste of mixtures

It has been shown in a study of the taste of bouillon [24] that amino acids occurring in concentration levels below their taste threshold values, but stimulating the same taste quality, e.g. sweet, may contribute to the overall flavour when the sum of their TAVs is greater than one. Therefore, the compounds quantified in Em were grouped according to their characteristic taste qualities as shown in Table 6.

The highest taste intensities were found for group no. 2, which, as inedible at the concentration level occurring in Em, had to be diluted to one-third of its strength

Table 6 Groups of compounds and their taste properties

No.	Group[a] Compounds/Ions	Taste quality[b] (intensity)[c]
1	Amino acids: pro, ala, gly, thr, ser	Sweet (1)
2	Acids: 2:0, 3:0, 4:0, 6:0, lactic acid, succinic acid, Na, K, Mg, Ca, Cl, phosphate, ammonia	Sour (2), salty (2), Sweet (1)
3	Amino acids: Val, Leu, Ile, Phe, Tyr, His, Lys	Bitter (3)
4	Glutamic acid	MSG-like (3)
5	Tyramine, histamine	Burning (2)

[a] Group nos. 1, 3–5: the concentrations of the compounds in 1 l of tap water were equal to those in 1 kg of Em (see Table 4); the concentration of group no. 2 was reduced to one-third of that in Em
[b] Taste quality at pH 5.6
[c] The intensity of the taste given in parentheses was scored at room temperature (see section "Sensory analysis")

Table 7 Taste profile of recombined groups nos. 1–4 at pH 5.6

Group no.	Taste profile
2	Sour, salty, sweetish
2 plus 4	Sour, salty, sweetish, MSG-like
2 plus 4 plus 1	No difference to group no. 2 plus 4
1–4	No difference to group no. 2 plus 4

at the beginning of the sensory test. This group tasted sour, salty and slightly sweet. The latter note, as discussed above, was mainly caused by the magnesium and calcium salts of propionic acid. The intensity of the sweet taste of group no. 1 containing five amino acids was equal to that of group no. 2. This means that it was weaker than the sweet note of group no. 2, as no. 1 was not diluted before the sensorial test. The mixture of amino acids in group no. 3 tasted strongly bitter, and group no. 4 containing only glutamic acid tasted bouillon-like. The two biogenic amines in group no. 5 caused a burning sensation which was absent in Em.

Starting with no. 2, the groups were recombined in a stepwise manner. The taste of each new mixture was compared, in a triangle test, with the mixture in which this group was lacking. The results in Table 7 indicate that the bouillon-like note was perceivable in the taste profile after the addition of group no. 4 to no. 2. In contrast, additions of groups nos. 1 and 3 did not change the intensity and the overall taste of the mixture containing the groups nos. 2 and 4. Consequently, the compounds occurring in nos. 1 and 3 did not contribute to the taste of Em.

Apart from the nutty note, which was caused by the retronasal effect of odorants [12], the taste profile of the mixture containing groups no. 2 and 4 agreed with that of Em. This result suggests that the following

substances are the characteristic taste compounds of Em: acetic acid, propionic acid, lactic acid, succinic acid, glutamic acid, each in free form and/or as ammonium, sodium, potasium, magnesium and calcium salts as well as the corresponding chlorides and phosphates.

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