Original article

Development of free amino acids in Appenzeller, Emmentaler, Gruyère, Raclette, Sbrinz and Tilsiter cheese

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Summary — The free amino acid development of Appenzeller, Emmentaler, Gruyère, Raclette, Sbrinz and Tilsiter cheese is described. During the first five months of cheese ripening, the total amount of the free amino acids increases by 140–290 mg kg⁻¹ per day. Gruyère shows the highest increase for 15 amino acids, Appenzeller for valine, methionine, phenylalanine and ornithine, Raclette for α - and γ -amino butyric acid, Emmentaler for alanine and Sbrinz for arginine. High quality cheese shows higher relative amounts of aspartic acid, asparagine and arginine and lower relative amounts of glycine, alanine, valine, methionine and isoleucine. The significant differences for Emmentaler were reversed for asparagine, alanine and methionine.

cheese / Appenzeller / Emmentaler / Gruyère / Raclette / Sbrinz / Tilsiter / free amino acid

Résumé — Développement des acides aminés libres dans les fromages appenzell, emmental, gruyère, raclette, sbrinz et tilsit. Le présent travail traite de la formation des acides aminés libres dans des fromages de type Appenzell, emmental, gruyère, raclette, sbrinz et tilsit. Durant les 5 premiers mois d'affinage, la quantité totale des acides aminés libres croît à raison d'environ 140 à 290 mg par kg et par jour. Le gruyère montre la plus forte augmentation pour 15 acides aminés, l'appenzell pour la valine, la méthionine, la phénylalanine et l'ornithine, la raclette pour les acides α - et γ -amino butyriques, l'emmental pour l'alanine et le sbrinz pour l'arginine. Les fromages de bonne qualité contiennent des concentrations relatives élevées d'acide aspartique, d'asparagine et d'arginine et des concentrations plus basses de glycine, d'alanine, de valine, de méthionine et d'isoleucine. L'effet est inverse dans l'emmental pour l'asparagine, l'alanine et la méthionine.

fromage / appenzell / emmental / gruyère / raclette / sbrinz / tilsit / acide aminé libre

INTRODUCTION

Protein breakdown of caseins during cheese ripening is very important in the development of the texture and background flavour of most cheese varieties (Desmazeaud and Gripon, 1977; Rank et al, 1985; Fox, 1989). Due to the action of rennet, peptidases from starter bacteria, secondary microflora and indigenous milk enzymes, peptides and amino acids are liberated (Langsrud et al, 1977; Klostermeyer and Krause, 1992; Barcina et al, 1995; Exterkate and Alting, 1995). The released amino acids are precursors for several amino acids not present in caseins (glutamic acid for α - and γ -amino butyric acid, arginine for ornithine and citrulline), biogenic amines (Leuthardt, 1977) and for volatile flavour compounds (eg, aldehydes, ketones, short-chain fatty acids and alcohols (Urbach, 1993).

For determination of free amino acids, different analytical methods have been developed. Moore and Stein (1951) first published a report on ion exchange chromatographic separation of amino acids. In the last 20 years reversed phase high-performance liquid chromatography (HPLC) methods with precolumn derivatisation became popular (Einarsson, 1985; Schuster, 1988; Fürst et al, 1990). Quantitative determination of free amino acids in cheese has been reported in different articles (Polo and Ramos, 1985; Resmini et al, 1985; Griffith and Hammond, 1989; de Llano et al, 1991; Lavanchy and Sieber, 1993; Resmini et al, 1993; Engels and Visser, 1994; Bütikofer, 1996).

The present work describes the content of the individual free amino acids during ripening of six hard and semi-hard cheese varieties. Linear regression was used to describe the relationship between free amino acid content and cheese age.

MATERIALS AND METHODS

Five cheese varieties (Appenzeller, Gruyère, Raclette, Sbrinz and Tilsiter) were investigated in the course of their ripening. The sample selec-

tion was described in the following publications: Sbrinz (Sollberger et al, 1992), Gruyère (Steffen et al, 1980), 'Walliser Raclette' (Schär et al, 1992), Appenzeller (Steffen et al, 1993a) and Tilsiter made from raw milk (Steffen et al, 1993b). Additional results were obtained in several comparisons of good quality cheese and cheese with late fermentation: Appenzeller (Steffen et al, 1981b), Emmentaler (Lavanchy et al, 1979), Gruyère (Steffen et al, 1992), Sbrinz (Steffen et al, 1981a) and Tilsiter made from raw milk (Steffen et al, 1982).

The determination of free amino acids was performed on an ion exchange amino acid analyser with postcolumn ninhydrin derivatisation and detection at 570 and 440 nm for primary and secondary amino acids according to the procedure of Lavanchy and Bühlmann (1983).

Statistical analysis

Linear regression, Kruskal-Wallis one-way analysis of variance and full factorial analysis of variance (MANOVA) calculations were carried out using the SYSTAT statistical software package (1992).

RESULTS AND DISCUSSION

In total, 22 different free amino acids could be quantified in more than one thousand cheese samples of these six cheese varieties.

Development of individual free amino acids in good quality cheese

The increase of individual free amino acids of good quality cheese during the first five ripening months can be fitted using a linear regression. The amount of each free amino acid can be calculated by the formula:

Amount of free amino acid = cheese age $\times$ slope + intercept

Gruyère shows the highest slope for aspartic acid, threonine, serine, asparagine, glutamic

Table I. Free amino acids in six cheese varieties: linear regression slope (mg kg⁻¹ day⁻¹); one to 150 days.
Acides aminés libres dans six sortes de fromages : pente de la régression linéaire.

<i>Cheese variety</i>	<i>Asp</i>	<i>Thr</i>	<i>Ser</i>	<i>Asn</i>	<i>Glu</i>	<i>Gln</i>	<i>Pro</i>	<i>Gly</i>	<i>Ala</i>	<i>Cit</i>	<i>α-Aba</i>	<i>Val</i>	<i>Met</i>	<i>Ile</i>	<i>Leu</i>	<i>Tyr</i>	<i>Phe</i>	<i>γ-Aba</i>	<i>Orn</i>	<i>Lys</i>	<i>His</i>	<i>Arg</i>
Appenzeller	5.4	5.8	2.1	11.9	45.1	4.7	21.6	4.8	5.5	2.3	0.4	19.8	6.0	12.9	27.5	5.8	16.0	1.0	6.8	29.8	7.0	-0.2
Emmentaler	1.0	5.3	4.1	1.9	33.7	5.1	15.4	3.2	5.9	3.3	0.2	12.6	4.2	6.3	21.9	3.7	14.6	0.0	6.2	20.0	4.4	0.3
Gruyère	6.2	7.5	6.6	12.1	57.6	6.8	27.2	5.2	4.9	5.6	0.0	15.9	5.6	13.4	29.3	7.5	14.5	1.1	5.7	29.9	9.7	0.6
Raclette	3.9	2.8	0.8	5.4	28.5	2.1	15.5	3.2	4.1	2.8	0.6	11.4	4.2	8.2	22.9	1.0	12.0	3.7	4.7	21.7	5.3	0.2
Sbrinz	2.9	4.3	3.2	6.0	22.8	3.1	12.0	2.7	2.6	3.4	0.0	7.6	3.3	5.2	17.3	3.5	8.6	1.3	4.5	14.4	3.6	0.9
Tilsiter	5.0	5.1	1.2	10.9	39.8	3.9	21.7	4.7	4.7	2.4	0.4	17.5	5.7	10.7	25.1	4.1	14.9	1.7	6.6	26.6	5.9	-0.2

acid, glutamine, proline, glycine, citrulline, isoleucine, leucine, tyrosine, lysine and histidine, Appenzeller for valine, methionine, phenylalanine and ornithine, Raclette for α - and γ -amino butyric acid, Emmentaler for alanine and Sbrinz for arginine (table I).

In Appenzeller and Tilsiter cheese the concentration of arginine decreases during ripening, which indicates that the liberation of arginine is slower than its conversion to ornithine. The concentration of asparagine and glutamine decreases in Gruyère cheese after 200 days of ripening (fig 1) due to the fact that the decarboxylation reaction to aspartic and glutamic acid is faster than their release from peptides.

Emmentaler cheese shows quite a different amino acid pattern. By using propionic acid bacteria, the enzyme aspartase is also present. Free asparagine and aspartic acid are deaminated in a first step to fumaric acid (Brendehaug and Langsrud, 1985; Crow, 1986), which explains the slow increase of these two amino acids in Emmentaler cheese.

The regression coefficients are shown in table II. Aspartic acid, threonine, proline, glycine, methionine, isoleucine, leucine, phenylalanine and histidine showed regression coefficients that were higher than 0.7; arginine, α - and γ -amino butyric acid showed the lowest regression coefficients.

For several amino acids, the linear regression intercept was significantly different from zero (table III). This can be related to the accelerated release or conversion of free amino acids during the first day of ripening. During manufacture of these six cheese varieties, rennet and starter cultures are added and the temperature is raised to 40–57 °C for more than 30 min. Under these conditions, free amino acids are formed three to five times faster. After one day of ripening, the sum of free amino acids in Appenzeller and Tilsiter was on average 1500–1600 mg kg⁻¹, in Raclette and Sbrinz 1100–1200 mg kg⁻¹ (the free amino acids in Emmentaler and Gruyère cheese were not determined after one day). Even ornithine and γ -amino butyric acid, which are not present in casein, could be found at levels of 20–80 mg kg⁻¹ in one-day-old cheese.

The increase in the sum of free amino acids in good quality cheese can be fitted to a linear regression within the first 150 ripening days (table IV). The standard deviation of the slope is below 8 mg kg⁻¹ day⁻¹, except for Raclette cheese with a standard deviation of 14 mg kg⁻¹ day⁻¹. The sum of free amino acids in Gruyère cheese increases by more than 290 mg kg⁻¹ day⁻¹ followed by Appenzeller, Tilsiter, Emmentaler and Raclette cheese. The extra-hard Sbrinz cheese shows the slowest increase of the free amino acids, with only 139 mg kg⁻¹ day⁻¹.

Fig 1. Development of glutamine and asparagine in Gruyère cheese.
Développement de la glutamine et de l'asparagine dans le gruyère.

Table II. Free amino acids in six cheese varieties: regression coefficient.
Acides aminés libres dans six sortes de fromages : coefficient de régression.

<i>Cheese variety</i>	<i>Asp</i>	<i>Thr</i>	<i>Ser</i>	<i>Asn</i>	<i>Glu</i>	<i>Gln</i>	<i>Pro</i>	<i>Gly</i>	<i>Ala</i>	<i>Cit</i>	α - <i>Aba</i>	<i>Val</i>	<i>Met</i>	<i>Ile</i>	<i>Leu</i>	<i>Tyr</i>	<i>Phe</i>	γ - <i>Aba</i>	<i>Orn</i>	<i>Lys</i>	<i>His</i>	<i>Arg</i>
Appenzeller	0.87	0.89	0.56	0.79	0.94	0.57	0.93	0.90	0.79	0.72	0.60	0.93	0.94	0.90	0.95	0.87	0.94	0.15	0.82	0.92	0.89	0.19
Emmentaler	0.86	0.92	0.95	0.37	0.93	0.87	0.91	0.91	0.96	0.81	0.16	0.94	0.95	0.88	0.97	0.83	0.96		0.95	0.94	0.83	0.26
Gruyère	0.78	0.76	0.70	0.86	0.88	0.67	0.87	0.91	0.67	0.92		0.77	0.78	0.83	0.80	0.76	0.85	0.06	0.61	0.70	0.85	0.02
Raclette	0.82	0.86	0.40	0.90	0.66	0.55	0.84	0.83	0.84	0.60	0.41	0.79	0.83	0.78	0.91	0.24	0.91	0.47	0.57	0.81	0.71	0.19
Sbrinz	0.63	0.83	0.77	0.89	0.80	0.39	0.73	0.83	0.47	0.83	0.03	0.68	0.83	0.73	0.89	0.74	0.89	0.48	0.70	0.62	0.74	0.52
Tilsiter	0.86	0.91	0.18	0.80	0.92	0.50	0.91	0.90	0.76	0.68	0.52	0.91	0.94	0.89	0.93	0.79	0.93	0.61	0.82	0.94	0.86	0.10

Table III. Free amino acids in six cheese varieties: linear regression intercept (mg kg^{-1}) (only if significantly different from 0).*Acides aminés libres dans six sortes de fromages : ordonnée à l'origine de la régression linéaire.*

Cheese variety	Asp	Thr	Ser	Asn	Glu	Gln	Pro	Gly	Ala	Cit	α -Aba	Val	Met	Ile	Leu	Tyr	Phe	γ -Aba	Orn	Lys	His	Arg
Appenzeller						121			82									66	134			26
Gruyère		65	56	57		191			181			92	61		315	86	102	108	139	458		54
Raclette						93										53		130	101			22
Sbrinz						63			82			87				52		89	184			
Tilsiter			55			158			89									114				51

Table IV. Linear regression of the sum of 22 amino acids of six different cheese varieties aged one to 150 days.*Régression linéaire de la somme des acides aminés libres des six variétés de fromage de un à 150 jours.*

<i>Cheese variety</i>	<i>n</i>	<i>Slope</i>	<i>SD_{slope}</i>	<i>r</i>
Appenzeller	85	245	7	0.969
Emmentaler	58	173	6	0.971
Gruyère	184	293	7	0.950
Raclette	29	169	14	0.919
Sbrinz	85	139	6	0.920
Tilsiter	94	222	6	0.964

Slope: calculated linear regression slope (mg kg⁻¹ day⁻¹); SD_{slope}: standard deviation of slope; *n*: number of determinations; *r*: regression coefficient.

Slope : pente de la régression linéaire (mg kg⁻¹ jour⁻¹); *SD_{slope}* : écart type de la pente de la régression linéaire; *n* : nombre des déterminations; *r* : coefficient de régression.

Comparison of good quality cheese and cheese with late fermentation

The increase of free amino acids in cheese is normally higher in cheese with late fermentation than in good quality cheese (table V). Steffen et al (1979) defined cheese with late fermentation (due to CO₂ production) by bad storage properties, increase in loaf height and asymmetrical eye formation during ripening at 10–13 °C. Statistically significant differences can be found for Emmentaler, Gruyère and Tilsiter cheeses with late fermentation, where the sum of free amino acids is 38, 19 and 18% higher.

To compare the free amino acid patterns of different cheese varieties, the original results were normalised by the following formula:

$$\text{rel } x_i = \frac{x_i}{\sum x_i} \times 100\%$$

where: x_i = absolute amount of an individual amino acid (mg kg⁻¹); rel x_i = relative amount of individual amino acids (%); $\sum x_i$ = sum of the absolute amount of free amino acids (mg kg⁻¹).

With the Kruskal–Wallis one-way analysis of variance, the following significant differences could be found: at least three good quality cheese varieties had higher relative amounts of aspartic acid, asparagine and arginine and lower of glycine, alanine, valine, methionine and isoleucine (table VI); the significant differences for Emmentaler cheese were reversed for asparagine, alanine and methionine.

Application of multivariate ANOVA

Resmini et al (1985, 1993) identified genuine Parmigiano–Reggiano and Grana Padano cheese by their free amino acid composition via a chemometric model. In the present work, the possibility of classification was tested on the

Table V. Comparison of the sum of free amino acids of good quality cheese and cheese with late fermentation.*Comparaison de la somme des acides aminés libres des fromages de bonne et mauvaise qualités.*

Cheese variety	Cheese quality						Kruskal–Wallis one-way ANOVA
	Bad (late fermentation)			Good			
	n	Slope	r ²	n	Slope	r ²	
Sbrinz	52	132	0.955	49	125	0.939	
Emmentaler	57	238	0.939	59	173	0.943	*
Gruyère	49	237	0.980	43	201	0.954	*
Appenzeller	61	255	0.959	59	252	0.958	
Tilsiter	54	266	0.940	54	226	0.919	*

Legend: see table IV; ANOVA: analysis of variance; * significantly different, $P < 0.01$.* Différence significative, $p < 0,01$.**Table VI.** Comparison of the individual free amino acids of good quality cheese and cheese with late fermentation.*Comparaison des acides aminés libres des fromages de bonne et mauvaise qualité.*

Amino acid	Tilsiter 125–165 ^a 108 ^b	Appenzeller 125–165 ^a 120 ^b	Gruyère 165–235 ^a 92 ^b	Sbrinz 285–375 ^a 101 ^b	Emmentaler 105–165 ^a 120 ^b
Aspartic acid	++	++	++	+	
Threonine	++			++	
Serine					--
Asparagine	++	++	++	+	--
Glutaminic acid			—		
Glutamine		++	+		
Proline			--		--
Glycine	--	--	--	--	--
Alanine	--		--	--	++
Citrulline		—			
α-Amino butyric acid	--	--			
Valine	--		--	--	
Methionine	—	--	—		+
Isoleucine	--	--	--		--
Leucine			+		++
Tyrosine		++			
Phenylalanine					++
γ-Amino butyric acid	--	--			
Ornithine	++			--	++
Lysine		--	--		+
Histidine	++	++			—
Arginine	+		++	++	

Relative amino acid concentration in good quality cheese: ++: high $P < 0.01$; +: high $P < 0.05$; --: low $P < 0.01$; —: low $P < 0.05$. ^a cheese age (days); ^b n value.Concentration relative des acides aminés dans les bons fromages: ++: élevée, $p < 0,01$; +: élevée, $p < 0,05$; --: faible, $p < 0,01$; —: faible, $p < 0,05$. ^a Âge du fromage (jours); ^b valeur de n .

Table VII. Classification of different cheese varieties with multivariate analysis of variance of free amino acids.

Classification des différentes variétés de fromage selon l'analyse de variance multivariée.

Cheese variety	n	Correct classification (%)
Emmentaler	116	97
Gruyère	320	93
Raclette	30	80
Sbrinz	254	93
Appenzeller	169	75
Tilsiter	158	70

relative amounts of free amino acid by multivariate ANOVA. The cheese varieties Emmentaler, Gruyère and Sbrinz could be correctly classified to more than 93% (table VII). The two cheese varieties Appenzeller and Tilsiter are very similar in texture and flavour, as manufacturing and ripening conditions are comparable. Therefore it is not surprising that they could only be correctly classified to 70–75%. Because the production of Raclette cheese was artisanal and the production parameters were very different, the cheese could only be correctly classified to 80% by its amino acid pattern.

FUTURE TRENDS

Depending on the origin of the milk, cheese manufacturing parameters, indigenous microorganisms, added starter cultures and ripening conditions, specific free amino acid patterns may be used to characterise different cheese varieties. The production of cheese specialties with traditional manufacturing and informative labelling (AOP, or Appellation d'Origine Protégée) is expensive, and therefore requires protection against cheaper industrial imitation products (Linden and Chamba, 1994). Chemometric models (Resmini et al, 1985, 1993) and multivariate statistics help to define models for detecting falsification of cheese labelling.

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