Lait (1997) 77, 359-369
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Original article

Free amino acids and water-soluble nitrogen as ripening indices in Montasio cheese*

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Summary — Traditionally, in semi-hard and hard cheeses the development of proteolysis is followed by the increase of water-soluble nitrogen (SN) as a percentage of total nitrogen (TN), but the characterisation of proteolysis for each particular cheese must consider all the processes that lead to the formation of peptides, free amino acids and products of their catabolism. In Montasio cheese, a considerable variability in the SN values and the amino acid profiles of cheeses at the same stage of ripening was recorded. This variability was probably due to the different traditional technologies adopted. Therefore, no chemometric model could be set up to evaluate the typical nature of Montasio cheese. On the other hand, a relationship between the total free amino acid content and the SN/TN ratio was demonstrated and it was found that certain amino acids could be used as an index for determining the level of proteolysis reached by the Montasio cheese.

proteolysis / free amino acid / total nitrogen / water-soluble nitrogen / Montasio cheese

Résumé — Les acides aminés libres et l'azote soluble dans l'eau pour la caractérisation du fromage Montasio. Traditionnellement, dans les fromages à pâte dure et semi-dure, le développement de la protéolyse est suivie d' une augmentation du rapport en pour cent entre l'azote soluble dans l'eau et l'azote total. Toutefois, pour caractériser la protéolyse dans chaque fromage particulier, il faut considérer l'ensemble des processus qui mène à la formation de peptides, acides aminés libres et produits de leur catabolisme. Dans le fromage Montasio, on a remarqué au même stade de maturation une forte variabilité des valeurs d'azote soluble dans l'eau, ainsi que des profils d'acides aminés. Cette variabilité est probablement due aux différentes technologies traditionnelles. Ainsi, aucun modèle chémométrique ne peut être créé pour déterminer la nature caractéristique du fromage Montasio. D'un autre côté, on peut établir une relation entre le contenu total d'acides aminés et le rapport azote soluble dans l'eau/azote total. Certains acides aminés peuvent être aussi utilisés comme indice pour déterminer le niveau de protéolyse dans le fromage Montasio.

protéolyse / acide aminé libre / nitrogène total / azote soluble dans l'eau / fromage Montasio

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* Oral communication at the IDF Symposium 'Ripening and Quality of Cheeses', Besançon, France, February 26–28, 1996.

INTRODUCTION

Many Italian cheeses have particular features related to the milk employed, the particular technology applied and the geographical environment. These typical characteristics allow such cheeses to be legally designated as 'DOC' (controlled denomination of origin) if they conform with the local milk production regulations, the traditional cheese manufacturing techniques and the cheese ripening process. Nevertheless, to ensure that these regulations and transformations are effective in practice, it is necessary to define methods for evaluating the qualitative characteristics of typical cheeses (Corradini and Innocente, 1995). The organoleptic qualities of cheese are generally determined by the physical and chemical changes that occur during ripening. In fact, the basic composition and structure of cheese are determined by the curd-producing operations, but it is during ripening that the individuality and unique characteristics of each cheese variety develop, and these are influenced by the composition of the curd and other factors, such as the microflora established during manufacture (Fox et al, 1993). The biochemical events involved in cheese ripening are traditionally assessed from a sensory evaluation of flavour, body, texture and typical eyes, finish and overall quality by experienced judges or trained consumer panels (Lavanchy et al, 1994). However, sensory analyses are subjective, and although at present the best index of consumer acceptability, they provide data that are difficult to evaluate scientifically and compare between laboratories or studies (Bourne et al, 1975). Therefore, chemical and physical analyses of cheese are used to objectively monitor ripening and to assess quality, usually to complement sensory evaluation. Although it may never be possible to assess cheese quality by chemical criteria alone, they can provide valuable indices of quality. For most hard and semi-hard cheeses, proteolysis is the most commonly used index for maturity. Proteolysis occurs in all cheese varieties and is considered to be a prerequisite for good flavour development, so that attention has been focussed on the parameters that quantify and characterise proteolysis (McSweeney and Fox, 1993). Traditionally, proteolysis is monitored by the increase in water-soluble nitrogen (SN) as a percentage of total nitrogen (TN), but the characterisation of proteolysis for each particular cheese must consider all the processes that lead to the formation of peptides, free amino acids and the products of their catabolism (Creamer, 1970). In fact, the first level of proteolysis results from enzymatic action on curdled milk and the consequent release of amino acids, but later these amino acids are further broken down by enzymes involved in deamination, decarboxylation, transamination and/or demethiolation (Hemme et al, 1982; Bech, 1992). The amino acids that are subjected to a more rapid degradation are methionine (Strecker degradation) and tyrosine (degraded to tyramine by tyrosine decarboxylase, produced by *Streptococcus faecalis* and only inactivated by the pasteurisation of the milk), while catabolic products are α-aminobutyric acid and γ-aminobutyric acid, formed by decarboxylation of glutamic acid, and ornithine, which is derived from the enzymatic degradation of arginine (Hemme et al, 1982). These reactions may be introductory to the development of important and specific flavours (Griffith and Hammond, 1989). Thus, the typical nature of cheese can be evaluated by means of the relative content of certain amino acids, and their increase and/or degradation or formation as products of amino acid catabolism (Reiter et al, 1969; Weaver et al, 1978; Aston et al, 1983; Amantea et al, 1986). This is particularly important in cheeses with characteristic eyes, such as Montasio. Montasio is a semi-hard cheese cooked at 44–46 °C, produced from unpasteurised (raw or thermised) milk in Friuli Venezia-Giulia and the Veneto

(north-east Italy) and is consumed as a table cheese after at least 2 months of ripening, whereas 8–9-month-old cheese is used for grating. One of the characteristics of Montasio cheese is the typical eyes, which depend on the use of natural milk culture in which the heterofermentative microorganisms are not excessively dominant over the homofermentative microbes. However, at present, to limit the development of defects commercial starters can also be used sometimes combined with thermal treatments of the milk. Consequently, it is possible that the characteristic eyes and the typical taste and flavour could be excessively changed.

To evaluate the typical nature of Montasio cheese by chemical parameters, proteolysis during ripening and the free amino acid composition in ripened cheese were determined. A high correlation between the intensity of flavour and the concentration of free amino acids had already been demonstrated in some Italian cheeses (Resmini et al, 1993; Pellegrino et al, 1995).

MATERIALS AND METHODS

Materials

Cheese samples

The first phase of the experimental work involved the analysis of cheeses from four different cheesemakers located in the typical production zone. At the time of sampling, a sensory evaluation form was filled out, which included a graphic representation of the arrangement of the typical holes. Later, the cheeses from a single producer were considered. These were manufactured by the genuine traditional method, the only variable being the different storage temperature for maturation, thereby demonstrating the influence of this processing parameter on the proteolytic trend during maturation. In particular, several cheeses were stored in the traditional manner in warehouses at 11 °C, while others were stored for 15, 30 or 60 days in warehouses at a controlled temperature of 5 °C and only later taken into traditional maturation cells.

Reagents

The amino acid standards used were from Sigma (L-amino acid kit). The calibration standard solution contained alanine, α-aminobutyric acid (Aaba) and γ-aminobutyric acid (Gaba), alanine (Ala), glycine (Gly), valine (Val), threonine (Thr), leucine (Leu), isoleucine (Ile), serine (Ser), proline (Pro), methionine (Met), aspartic acid + asparagine (Asx), phenylalanine (Phe), glutamic acid + glutamine (Glx), tyrosine (Tyr), ornithine (Orn), lysine (Lys) and homoserine (Hom) (internal standard) in 7% ammonia. The final amino acid concentration was 1 mg/mL. Other reagents of analytical grade were from Carlo Erba (ethanol, 28% ammonia solution, isobutanol, sulphuric acid, sodium hydroxide pellets), Fluka (heptafluorobutyryl anhydride, > 99% purity, a gas chromatography reagent) Aldrich-Chemie (Dowex 50W × 8 100–200 mesh) and Janssen (methylene chloride).

Methods

Determination of free amino acid

The quantitative analysis of amino acids, as their *N*-heptafluorobutyryl iso-butyl ester derivatives, was achieved by high resolution gas chromatography using a Varian Model 3700 instrument equipped with a FID detector, a Hewlett-Packard 3396 A integrator and a 25 m × 0.32 mm id fused silica capillary column coated with a 0.2-μm thick OV1701 film. The carrier gas was helium with a column flow rate of 1.6 mL/min at 80 °C. Both injector and detector temperature were 280 °C. The column temperature ranged from 80 to 280 °C, with an 8 °C/min rate. The cheese samples were prepared, purified and derivatised as described by Bertacco et al (1992).

Determination of nitrogen content

The water-soluble extract was prepared by grinding 4 g grated cheese in a mortar with water at 50 °C until a homogeneous sample was obtained, and the paste then diluted to 100 mL (Stadhouders, 1960). The homogenate was filtered and analysed for nitrogen by the Kjeldahl method (AOAC, 1980). The TN was determined by placing 0.5 g grated cheese directly in a Kjeldahl digestion flask. The protein content was obtained by multiplying the percentage of TN by 6.38 and

Table I. Free amino acid content (mg/100 g dry matter) in 60-day-old cheeses from four different cheese producers located in the typical production zone for Montasio cheese.
Teneur en acides aminés libres (mg/100 g de matière sèche) dans des fromages de 60 j provenant de quatre producteurs différents localisés dans la zone typique de production du Montasio.

Amino acids	*Dairy 1 (samples)*				*Dairy 2 (samples)*				*Dairy 3 (samples)*				*Dairy 4 (samples)*		
	1	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*
Ala	117.5	223.1	120.8	56	23.3	90.1	95.6	9.3	69.5	23.8	51	27.6	12.2	141.4	89
Aaba	0	0	0	0	0	11.2	5.7	1.9	0	0	0	1.4	0	0	0
Gly	258.8	393.8	276.7	87.9	49.3	182.9	208.3	12.8	95.8	68.4	57.3	39.8	32.8	207.3	204.7
Val	83.8	110.6	110	48.4	21.9	94.3	91.4	33	50.3	41	41.4	25.7	15.8	78.8	89.2
Thr	79.1	89.6	109.9	39.4	25.4	79.8	110.3	12.7	19.3	43.6	20	15	19.5	43.1	82.8
Ile	134.5	176.7	159.7	39.2	25.3	120.9	158.8	16.4	47.1	41.4	23.9	18.4	19.7	81.1	118.7
Leu	419.8	569.6	579	240.5	115.8	416.7	474.9	102.3	183	223.3	153.2	104.9	110.7	323	492.1
Ser	98.6	105.4	148.1	81.1	37.8	111.4	173.2	7.3	38	73.5	43.51	16.2	29.8	68	127.2
Pro	302.8	338.3	362.6	112.4	71.1	300.3	426.5	39.8	161.6	140.5	72.3	57.3	53.4	194.3	312.7
Gaba	6.6	0	3.3	23.3	45.6	124.4	187.1	22	21.4	112.9	9.5	7.8	1	19.4	7.6
Met	37	99	12.8	29.9	77.4	39.4	40.9	6.8	30.6	4.6	17.9	15.7	12.8	29.9	26.1
Asx	222.7	274.2	351.3	156.3	85.5	291	319.6	89.2	117.4	210.2	115.4	77.4	99.7	159.1	314.8
Phe	183.9	259.1	287	125.8	63.7	231.3	303.4	64.5	97.7	146.7	81.2	57.5	70.5	140.4	282.9
Glx	524.5	564	839.9	235.9	123.4	465.7	537.6	153	224.4	221.1	199.8	138.1	237.6	308.9	769.3
Orn	74.1	49.4	163.9	65.9	71.5	264.1	262.2	34.2	29.1	272.8	97.4	91.7	64.1	86.6	282.6
Tyr	58.69	109	159.5	61.87	27.27	50.04	133.6	29.95	44.43	43.57	19.74	20.67	39.37	59.16	108.6
Lys	347.4	178.7	520	124.6	119.4	419.9	519.3	80.06	120.5	226.3	70.49	79.24	104.5	114.9	353.2
Total	2956	3541	4210	1530	983.6	3309	4048	723.2	1354	1895	1076	795.4	924.7	2058	3670

the ratio of SN/TN was used as an index of proteolysis.

Statistics

Calculation of mean and median values, interquartile range, Kolmogorov–Smirnov normality test, standard deviation (SD), coefficient of variation (CV), linear and second-order polynomial curve fitting, coefficient of regression (r) and coefficient of determination (r^2) was performed using the statistical package SPSS for Windows, release 6.0.

RESULTS

Fifteen samples of 60-day-old Montasio cheese from four different cheese makers were analysed first. The working conditions adopted (Bertacco et al, 1992) allowed the identification of the following amino acids: Aaba, Ala, Gly, Val, Thr, Leu, Ile, Ser, Pro, Gaba, Met, Asx, Phe, Glx, Tyr, Orn and Lys, while the internal standard was homoserine (Hom). Table I reports the free amino acid composition of the samples under examination expressed in mg/100 g cheese dry matter. It can be seen that the free amino acid profile was very variable. Before the statistical analysis, the verification of the distribution of the data for the individual amino acids was performed by the Kolmogorov–Smirnov normality test: the comparison between the experimental and theoretical distribution based on the assumption of normality was highly significant, with a high P level (table II). In table III the statistical parameters of the individual amino acids determined in cheese of the same age but from four different producers are reported. It can clearly be seen that, even without considering the amino acids that are easily degraded during the course of ripening (methionine and tyrosine) or that are released following catabolic processes at the expense of other amino acids (ornithine, α-aminobutyric acid and γ-aminobutyric acid), the variation remained very high. The considerable variability recorded in the amino acid profiles of the 60-day-old cheeses prevented the setting up of a chemometric model for Montasio, as has been done using the same techniques for other typical Italian cheeses (Resmini et al, 1988).

Table II. Normality of distribution of free amino acids: values assessed by the Kolmogorov–Smirnov test.
Estimation de la normalité de la répartition des valeurs en acides aminés libres avec le test de Kolmogorov–Smirnov.

Amino acids	*Kolmogorov–Smirnov test*	P-*level*
Ala	0.5137	0.9545
Aaba	1.5510	0.0160
Gly	0.7899	0.5606
Val	0.6820	0.7410
Thr	0.7756	0.5843
Ile	0.9120	0.3764
Leu	0.6439	0.8014
Ser	0.5898	0.8775
Pro	0.7156	0.6851
Gaba	1.3418	0.0546
Met	0.0899	0.3933
Asx	0.6964	0.7173
Phe	0.6137	0.8457
Glx	0.9634	0.3113
Tyr	0.9933	0.2773
Orn	1.1249	0.1591
Lys	1.0275	0.2416
Total	0.5592	0.9133

The amino acid profile expressed in terms of relative percentage (g amino acid/g total free amino acids) showed less variability. In this case, the coefficient of variation was on average lower (see table IV) if those amino acids subject to degradation and those of catabolic origin were excluded. The relative content of the individual amino acids does not reflect the intensity of proteolysis

Table III. Statistical results of the free amino acid content (mg/100 g dry matter), determined in cheeses of the same age but produced in four different dairies ($n = 15$).
Résultats statistiques de la teneur en acides aminés libres (mg/100 g de matière sèche) de fromages du même âge mais provenant de quatre laiteries différentes.

Amino acids	*Min*	*Max*	*Median*	*Mean*	*IQR*	*SD*	*CV*
Ala	9.35	223.1	69.50	76.68	93.65	56.45	73.62
Aaba	0.00	11.18	0.00	1.34	1.40	3.01	223.61
Gly	12.78	393.79	95.77	145.09	159.00	107.90	74.36
Val	15.82	110.62	50.34	62.38	58.43	31.79	50.97
Thr	12.75	110.33	43.12	52.64	63.30	34.34	65.24
Ile	16.39	176.74	47.13	78.80	110.61	57.66	73.17
Leu	102.31	579.01	240.52	300.59	359.12	170.90	56.85
Ser	7.32	173.21	73.48	77.28	73.59	47.86	61.93
Pro	39.84	426.45	161.59	196.39	241.61	127.33	64.83
Gaba	187.11	38.93	19.41	39.47	38.93	54.21	137.34
Met	4.55	98.96	29.87	32.00	26.57	24.99	78.08
Asx	351.28	191.29	159.10	192.25	191.29	93.78	48.78
Phe	57.52	303.37	140.40	159.71	188.61	87.93	55.05
Glx	123.44	839.89	237.60	369.56	337.86	223.94	60.60
Tyr	19.74	159.52	50.04	64.37	78.65	41.62	64.66
Orn	29.13	282.60	86.58	127.30	198.08	91.45	71.83
Lys	70.49	520.04	124.63	225.24	248.70	157.08	69.74
Total	723.20	4210	1976.50	2204.89	2604.35	1238.70	56.18

IQR: interquartile range; SD: standard deviation; CV: coefficient of variation.

Table IV. Statistical results of the free amino acid content, expressed as the relative percentage of total amino acid content determined in cheeses of the same age but produced in four different dairies.
Résultats statistiques exprimés en pourcentage relatif de teneur totale en acides aminés, de la teneur en acides aminés libres de fromages de même âge mais provenant de quatre laiteries différentes.

Amino acids	*Min*	*Max*	*Median*	*Mean*	*IQR*	*SD*	*CV*
Ala	1.26	6.30	3.17	3.40	2.18	1.690	49.936
Aaba	0.00	0.34	0.00	0.06	0.17	0.109	178.039
Gly	1.77	11.12	5.42	5.99	1.94	2.383	39.784
Val	2.16	4.56	2.98	2.97	1.25	0.751	25.295
Thr	1.42	2.73	2.47	2.25	0.74	0.375	16.633
Ile	2.19	4.99	3.02	3.19	1.60	0.906	28.411
Leu	11.73	16.09	13.63	13.59	2.24	1.419	10.441
Ser	1.01	5.3	3.44	3.36	1.15	0.944	28.096
Pro	5.51	11.93	8.01	8.34	2.86	1.757	21.068
Gaba	0.00	5.96	1.55	1.90	4.02	1.914	100.557
Met	0.24	7.86	1.45	1.79	1.30	1.759	98.016
Asx	7.53	12.34	8.75	9.26	2.59	1.416	15.299
Phe	6.22	8.92	7.27	7.36	0.83	0.653	8.878
Glx	11.67	21.16	16.25	17.06	4.88	3.641	21.341
Tyr	1.51	4.14	2.92	2.98	1.60	0.820	27.503
Orn	1.39	14.39	5.60	6.30	5.93	3.453	54.801
Lys	5.05	12.89	11.41	9.99	3.96	2.527	25.285

For abbreviations see table III.

Table V. Statistical results concerning the SN/TN % ratio, calculated from the analyses performed on 98 samples of Montasio cheese from four different cheese producers.
Résultats statistiques du rapport SN/TN calculé à partir d'analyses effectuées sur 98 échantillons de Montasio provenant de quatre producteurs différents.

Dairy	n	*Min*	*Max*	*Mean*	*Median*	*IQR*	*SD*	*CV*
1	26	18.07	43.28	26.55	24.73	11.23	7.32	27.57
2	21	14.49	40.91	25.07	23.38	9.01	7.28	29.06
3	23	13.93	34.71	21.36	17.99	10.44	5.96	27.90
4	28	14.07	24.82	18.65	18.00	3.29	2.53	13.56
Total	Σ	13.93	43.28	22.73	20.81	8.54	6.68	29.41

For abbreviations see table III

as much as it indicates several qualitative aspects and can thus supply very useful information on the characterisation of the cheese, particularly if the amino acids used are those with the lowest interquartile ranges such as Phe, Leu, Thr and Asx.

In contrast, the ratio of SN/TN was chosen as a parameter to indicate the progress of ripening. Table V reports the maximum and minimum values, means, medians, interquartile ranges, standard deviations and coefficients of variation of the SN/TN ratio calculated from the analyses of cheeses (a total of 98 samples) produced by the four cheese makers considered in this study, representative of about 25% of the entire annual Montasio production. Table VI presents an assessment of the normal distribution of the above-mentioned ratio. A high coefficient of variation can be seen both within and between cheese producers. The variability in the SN value and the amino acid profile of cheeses at the same stage of ripening thus appeared to be related to a different evolution of the proteolytic processes, probably due to the different processing conditions adopted by the various cheese producers (temperature of ripening cells, type of starter culture, thermal treatment of the milk) or due to unexpectedly abnormal conditions in the biological ripening agents that are the basis of the proteolytic process.

On the basis of these considerations, we decided to evaluate the relationship between the total free amino acid content/TN ratio to the SN/TN ratio, and to extend analysis to cheeses at different stages of ripening. In particular, it is known that storage at low temperatures for the first few months of ripening, although an ideal means of obtaining good control over swelling, generally slows down the biochemical ripening phenomena, which become more marked the longer the cheese is stored at low temperature (Aston et al, 1985). This was exploited to obtain cheeses with different degrees of ripening, in which the SN/TN ratio and the amino acid profile were determined after

Table VI. Normality of distribution of the SN/TN ratio assessed by the Kolmogorov–Smirnov test.
Estimation de la normalité de répartition du rapport SN/TN avec le test de Kolmogorov–Smirnov.

Dairy	n	*Kolmogorov–Smirnov test*	P-*level*
1	26	0.8565	0.4455
2	21	0.8688	0.5965
3	23	1.2844	0.7338
4	28	0.9850	0.2864
Total	Σ	1.4550	0.0290

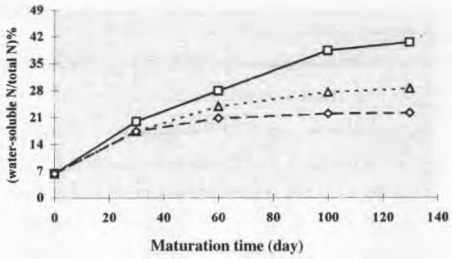


Fig 1. Trend regarding the SN/TN ratio for the cheeses matured at three different temperatures during the early stages of maturation; -□- normal maturation; -△- conditioned at 5 °C for 30 days; -◇- conditioned at 5 °C for 60 days.
Évolution du rapport SN/TN pour des fromages affinés à trois températures différentes dans les premiers stades de la maturation.

different ripening times. In particular, several cheeses were stored at 5 °C for 15, 30 or 60 days during the early stages of ripening, while others were maintained at the traditional ripening temperature (11 °C) for the entire ripening period. The evolution of the SN/TN ratio as a function of the maturation time for this cheese is shown in figure 1: it is clear that there was a net slowing down of the proteolytic process involved in the maturation of the cheeses conditioned at low temperature during the early stages of ripening. Figure 2 describes the relationship between the SN/TN ratio and the total free amino acid content, expressed as a percentage of total free amino acids in total pro-

Table VII. Regression coefficients between the individual amino acids and the SN/TN ratio. (y = individual amino acids; x = SN/TN).
Coefficients de régression entre les acides aminés individuels et le rapport SN/TN (y = *acides aminés individuels;* x = *SN/TN).*

Amino acids	y = ax + b (r)	$y = ax^2 + bx + c$ (r)
Ala	0.7205	0.8459
Gly	0.8862	0.9352
Val	0.8914	0.9370
Thr	0.8913	0.9400
Ile	0.8691	0.9289
Leu	0.9418	0.9686
Ser	0.8359	0.8444
Pro	0.8922	0.9659
Asx	0.9321	0.9642
Phe	0.9288	0.9676
Glx	0.8996	0.9408
Tyr	0.8213	0.8746
Lys	0.8938	0.9559
Met	0.8769	0.9340
Orn	0.8658	0.9224
Aaba	0.7305	0.5392
Gaba	0.5549	0.5601

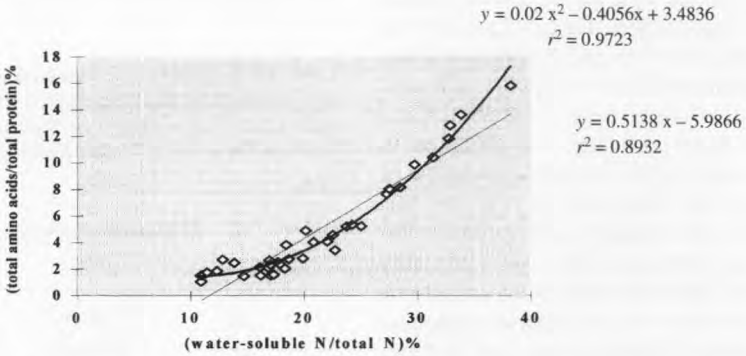


Fig 2. Graphic representation of the relationship between total free amino acid content (expressed as a percentage of total protein content) and the SN/TN % ratio.
Représentation graphique de la relation entre la teneur totale en acides aminés libres (exprimée en pourcentage de la teneur totale en protéine) et le rapport SN/TN.

tein. This relationship is well described by a second-order polynomial equation, which had the best coefficient of determination (r^2) of the various functions evaluated. The lack of a linear relationship was due to the fact that release of amino acids was initially slower than the increase in the SN/TN ratio, as during the early stages of seasoning the proteins are initially hydrolysed to peptides of differing molecular weights and only later are these peptides further degraded to amino acids. Table VII reports the coefficients of regression (r) calculated for the individual amino acids; in this case also, the r values were higher when it was a second-order equation. The coefficient of regression was > 0.95 for Leu, Pro, Phe, Asx, Lys and Leu. These amino acids can therefore be used with a good degree of certainty as an index for determining the level of proteolysis (stage of ripening) reached by Montasio cheese independently of its age (in days from the date of production). The amino acids which, in contrast, did not appear to have any type of relationship whith the degree of proteolysis (% SN/TN) were Aaba and Gaba (fig 3); these amino acids are Gly catabolic products. However, one of the principal characteristics of Montasio cheese, as previously mentioned, is the presence of holes that should have a well-defined shape, distribution and size. The preliminary data show that the characteristic openings in this cheese, rather than the extent of proteolysis, can be related to the presence of Aaba and Gaba (fig 4). Furthermore, the presence

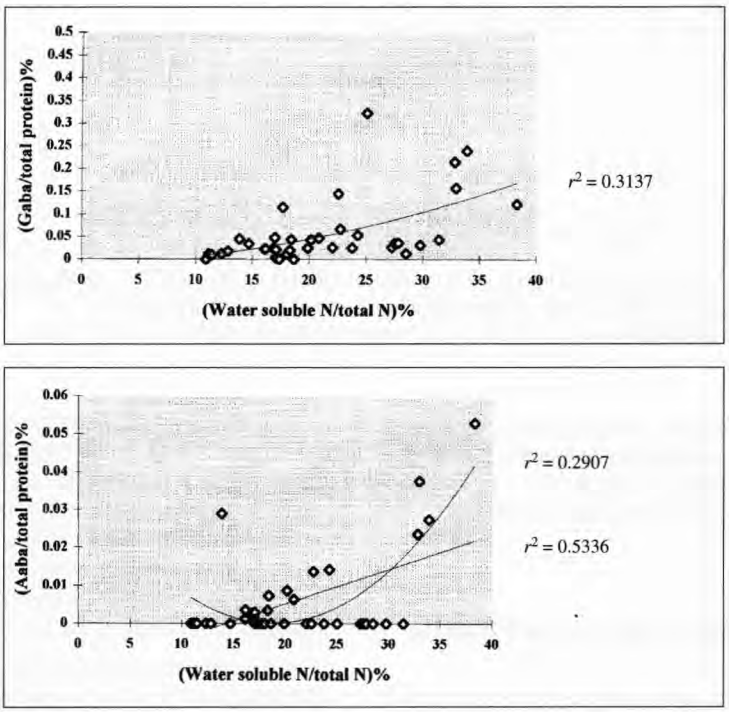


Fig 3. Correlation between the Aaba and Gaba content, expressed as a percentage of the total protein content, and the SN/TN % ratio.
Corrélation entre la teneur en Aaba et Gaba (exprimée en pourcentage de la teneur totale en protéine) et le rapport SN/TN.

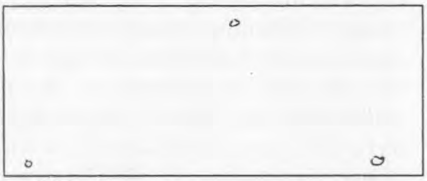
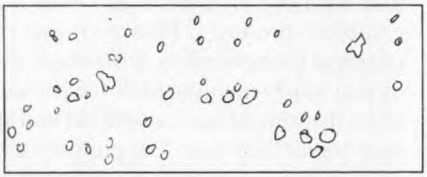
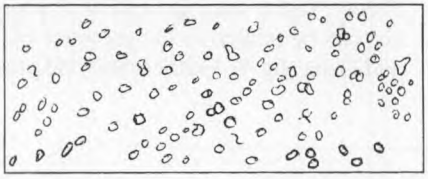
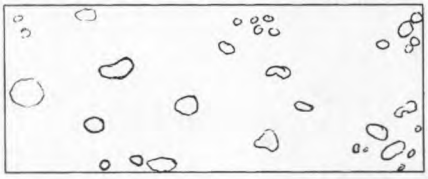
Cheeses		$\frac{Gaba}{\text{Tot FAA}}$ (%)	$\frac{Aaba}{\text{Tot FAA}}$ (%)	$\frac{\text{Tot FAA}}{\text{Tot prot}}$ (%)	$\frac{SN}{TN}$ (%)
1		0	0	8.67	26.29
2		0.11	0	2.30	17.05
3		0.94	0	4.95	19.90
4		1.84	1.20	2.44	13.86

Fig 4. Holes and proteolytic parameters in Montasio cheese after 60 days of ripening. FAA: free amino acids; prot: protein; SN: water-soluble nitrogen; TN: total nitrogen.
Ouvertures et paramètres de protéolyse de fromages Montasio après 60 j d'affinage.

of aminobutyric acids (small amounts of Aaba and a moderate amount of Gaba) has been reported for Montasio cheese with good sensorial characteristics (Bertacco et al, 1994).

DISCUSSION AND CONCLUSION

The results obtained from the analysis of data on free amino acid composition showed that, owing to the wide range of values recorded for samples with the same ripening time, it was not possible to determine the age of a Montasio cheese from its free amino acid content. On the other hand, considerable differences in the degree of proteolysis (expressed as the SN/TN ratio) in cheeses of the same age were noted owing to the variability in several processing parameters, of which the temperature of the ripening cells appeared to have the greatest influence.

In contrast, the usefulness of an analysis of free amino acids was confirmed as an indicator of the degree of proteolysis in cheese, independently of age. In fact, a relationship between the total free amino acid content and the SN/TN ratio was demon-

strated. This relationship was not valid for the aminobutyric acids, derived from secondary catabolic processes: instead, the higher or lower concentration of these amino acids appeared to be related to the formation of the characteristic eyes in Montasio cheese.

ACKNOWLEDGMENT

The author would like to thank C Corradini for his valuable suggestions and for critically reading the manuscript.

REFERENCES

Amantea GF, Shiva BJ, Nakai S (1986) Culture effect on ripening characteristics and rheological behaviour of Cheddar cheese. *J Food Sci* 51, 912-918

AOAC (1980) *Official Methods of Analysis*. Assoc Official Analytical Chemists, Washington, DC

Aston JW, Durward IG, Dulley JR (1983) Proteolysis and flavour development in Cheddar cheese. *Aust J Dairy Technol* 38, 55-59

Aston JW, Giles JE, Durward IG, Dulley JR (1985) Effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese. *J Dairy Res* 52, 565-572

Bech AM (1992) Enzymes for the acceleration of cheese ripening. In: *Fermentation-produced enzymes and accelerated ripening in cheese-making. Bull Int Dairy Fed* 269, 24-28

Bertacco G, Boschelle O, Lercker G (1992) Gas chromatographic determination of free amino acids in cheese. *Milchwissenschaft* 47, 348-350

Bertacco G, Giomo A, Boschelle O, Corradini C (1994) Caratterizzazione del formaggio Montasio in relazione al contenuto in amminoacidi liberi. Nota II. *Sci Tecn Latt-Casearia* 45, 421-441

Bourne MC, Sandoval AMR, Villalobus MC, Buckle TS (1975) Training a sensory texture profile panel and development of standards scales in Columbia. *J Texture Stud* 4, 204-223

Corradini C, Innocente N (1995) Evaluation by characteristic parameters of the ripening in few typical cheeses. In: *Proc Congr on Cheese Ripening*, 1 June, 1995, *Holland College*, Delft, the Netherlands

Creamer LK (1970) Protein breakdown in Gouda cheese. *NZ J Dairy Sci Technol* 5, 152-154

Fox PF, Law J, Mc Sweeney PLH, Wallace J (1993) Biochemistry of cheese ripening. In: *Cheese: Chemistry, Physics and Microbiology. Vol 1: General Aspects* (Fox PF, ed) Chapman & Hall, London

Griffith R, Hammond EG (1989) Generation of Swiss cheese flavour components by the reaction of amino acids with carbonyl compounds. *J Dairy Sci* 72, 604-613

Hemme D, Bouillame C, Métro F, Desmazeand MJ (1982) Microbial catabolism of amino acids during cheese ripening. *Sci Aliments* 2, 113-123

Lavanchy P, Bérodier F, Zannoni M, Noël Y, Adamo C, Squella J, Herrero L (1994) *Guida per la valutazione sensoriale della struttura dei formaggi a pasta dura o semidura*. INRA, Paris

Mc Sweeney PLH, Fox PF (1993) Methods of chemical analyses. In: *Cheese: Chemistry, Physics and Microbiology. Vol 1: General Aspects* (Fox PF, ed) Chapman & Hall, London

Pellegrino L, Hogenboom JA, Pazzaglia C, Tedesco R (1995) Caratterizzazione analitica del formaggio Fontina sulla base della sua composizione in amminoacidi liberi. *Latte* 10, 1086-1093

Reiter B, Sorokin Y, Pickering A, Hall AJ (1969) Hydrolysis of fat and protein in small cheeses made under aseptic conditions. *J Dairy Res* 36, 75-80

Resmini P, Pellegrino L, Pazzaglia C, Hogenboom JA (1985) Gli amminoacidi liberi nella tipizzazione del formaggio Parmigiano Reggiano, ed in particolare nel prodotto grattugiato. *Sci Tecn Latt-Casearia* 36, 557-592

Resmini P, Pellegrino L, Hogenboom JA, Bertuccioli M (1988) Gli amminoacidi liberi nel formaggio Parmigiano Reggiano. In: *Ricerca triennale sulla composizione e su alcune peculiari caratteristiche del formaggio Parmigiano Reggiano*. Consorzio del Formaggio Parmigiano Reggiano, Reggio Emilia, 113-121

Resmini P, Hogenboom JA, Pazzaglia C, Pellegrino L (1993) Gli amminoacidi liberi nella caratterizzazione analitica del formaggio Grana Padano. *Sci Tecn Latt-Casearia* 1, 7-19

Stadhouders J (1960) De eiwithjtrolise tijdens de kaasrijping de enzymen die het eiwit inkaashjdroljseren. *Neth Milk Dairy J* 14, 83-90

Weaver JC, Kroger M, Thompson MP (1978) Free amino acid and rheological measurements on hydrolysed lactose Cheddar cheese during ripening. *J Food Sci* 43, 579-583