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Production of Biogenic Amines in PDO Italian Cheeses during Ripening

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SUMMARY

Biogenic amines and their precursor amino acids were determined simultaneously, using an ion-pair HPLC in the Italian PDO cheeses, produced in Piedmont region. The cheeses analysed were Gorgonzola, Toma Piemontese, Castelmagno, Raschera, Bra and Murazzano.
Among free precursor amino acids, phenylalanine showed a constant and significant increase in all cheeses analysed (up to 2000 mg/Kg in Gorgonzola): this amino acid could be considered as an useful ripening marker. Changes in the concentration of four biogenic amines were determined, showing the presence of histamine and tyramine in long-ripened cheeses (up to a total concentration of 145 mg/Kg in Toma Piemontese, 313 mg/Kg in Castelmagno and 120 mg/Kg in Gorgonzola) and in Murazzano cheese (266 mg/Kg in the type produced using pure ovine milk). However, the presence of biogenic amines in good ripened cheeses was not directly correlated with high precursor amino acids concentration. Gorgonzola showed high free amino acids concentration (up to 2000 mg/Kg) probably because the very high proteolytic activity of *Penicillium roqueforti*, but the correlation of total biogenic amines was limited.

INTRODUCTION

Biogenic amines are organic bases formed because of normal metabolic activity in micro organisms. Various fermented and seasoned foods as cheese, sauerkraut, wine, beer, fish, and meat products may contain biogenic amines [1]. Biogenic amines are vasoactive and/or psychoactive compounds, these compounds are particularly referred to the so-called "cheese reaction". A content of 500 mg Kg^{-1} of total biogenic amines in food is considered critical.
The fundamental parameters directly related to the accumulation of biogenic amines in cheese are:

- general hygienic conditions during industrial/artisan cheese making;
- decarboxylating activity of some starter and non-starter bacterial strains.

For this reason, amines are considered a potential marker for the control of hygienic conditions during cheese production. The formation of biogenic amines in cheese is enhanced by temperature (mainly if >18°C), pH>5 and low salt content. Also the ripening and storage temperatures are other important parameters.
The formation of some biogenic amines (histamine, tyramine, tryptamine, and 2-phenylethylamine) and their related precursor amino acids (histidine, tyrosine, tryptophan and phenylalanine) has been studied in Italian PDO cheeses, produced in Piedmont region.
The analysed cheeses are all classified with the PDO label: Gorgonzola, Toma Piemontese, Castelmagno, Raschera, Bra and Murazzano; the principal characteristics of these cheeses are listed in Table 1.

Table 1. Principal characteristics of the Piedmont PDO cheeses analysed in this study.

Cheese	*Milk used*	*Minimum days of ripening*	*Protein (% dry matter)*	*Fat (% dry matter)*	*Ashes (% dry matter)*	*Micro organisms involved in cheese making*
Gorgonzola	Cow	50	37,7	57,0	n.d	*Penicillium roqueforti*
Castelmagno	Cow	60	37,8	50,9	5,6	Spontaneous fungal flora, unidentified
Toma Piem.	Cow	60	38,3	51,9	5,6	Lactic bacteria
Bra	Cow	45	40,2	50,3	5,7	Lactic bacteria
Raschera	Cow	30	38,6	51,4	6,4	Lactic bacteria
Murazzano	Sheep/Cow	fresh	41,6	52,3	5,5	Lactic bacteria
Murazzano*	Sheep	fresh	34,8	58,3	4,0	Lactic bacteria

EXPERIMENTAL

Cheese Sampling

Samples of cheeses were manufactured in a commercial scale in industrial plant or in cooperatives, according to the standard protocols reported in PDO regulations. These samples were kindly supplied from "Associazione Produttori Latte Piemonte". The samples were taken at different days of ripening, following the standard protocols. A total representative sample of 100 g was obtained by grating and homogenising the pieces collected from whole cheese; each sample was then analysed, avoiding any loss of moisture.

Compositional Analysis

Cheese moisture, ash and fat were determined according to the Italian Official Methods for Cheese analysis [2]. Total nitrogen value was determined by the Kjeldahl method [3] using the Kjeltec system (Tecator, Sweden); protein was calculated using the conversion factor 6.38. Total fat was determined using the Soxhlet apparatus with dichloromethane for 14 hours.

Biogenic amines (tyramine, histamine, tryptamine and 2-phenylethylamine) and their precursor amino acids were determined using an ion-pair HPLC method previously optimised for Gorgonzola PDO cheese [4]. Experiments were carried out on an Shimadzu Class VP HPLC system, equipped with a temperature controller (Column Oven CTO-10AS) and a UV-VIS detector SPD-10A, using a ODS 2 column, (4,6 I.D., 250 mm length, Waters). Each sample (10 g) was extracted with 50 mL of trichloroacetic acid (5%, w/v). All chemical reagents (reagent grade) were purchased from Fluka Chemie AG.

RESULTS AND DISCUSSION

Biogenic amines and amino acids were determined using a method optimised for cheeses [4].

Table 2. Biogenic amines and related precursor amino acids in Gorgonzola PDO during ripening (mg kg^{-1}; mean ± S.D).

Gorgonzola samples	tyrosine	histidine	phenylalanine	tryptophan	tyramine	histamine
0 days	81±2	94±2	58±1	43±2	-	-
23 days	99±8	39±0	105±2	32±1	6±0	3±0
55 days	344±3	160±1	209±2	108±9	5±1	40±4
70 days	743±13	296±1	491±3	221±2	28±6	49±3
82 days	2072±4	804±3	1190±62	640±1	26±6	52±1
100 days	3412±115	1581±3	1882±15	1464±3	53±2	53±10

Table 3. Biogenic amines and related precursor amino acids in Castelmagno PDO during ripening (mg kg^{-1}; mean ± S.D).

Castelmagno samples	tyrosine	histidine	phenylalanine	tryptophan	tyramine	histamine
7 days	82±7	24±1	148±16	24±2	13±0	29±3
28 days	111±1	19±2	193±21	29±2	23±2	30±1
42 days	165±11	12±2	334±27	55±6	39±1	43±2
62 days	261±3	16±1	554±4	82±22	72±4	78±2
77 days	233±18	94±38	712±126	133±31	123±12	92±17
90 days	458±2	157±3	890±17	139±1	183±5	112±1
155 days	3612±195	1331±20	2918±64	1586±25	199±7	495±18

All cheeses showed a significant increase in free amino acids during ripening (Tables 2-7) as reported for other cheeses [5]. The concentration of each amino acid exceeded 1000 mg/Kg only in the cheese with longer ripening, in the presence of a massive fungal growth. In another PDO cheese produced in Piedmont (Robiola di Roccaverano) the amount of each amino acid was <20 mg/Kg; this is a fresh cheese, produced without ripening [6].
Among free precursor amino acids, phenylalanine is the principal amino acid in all samples of Toma Piemontese, Bra, Raschera, Murazzano and in some Castelmagno samples. Tyrosine was the amino acid prevalent in all Gorgonzola samples and in the sample of Castelmagno at 155 days of ripening, showing a possible correlation with fungal growth.
Phenylalanine showed a constant and significant increase in all cheeses analysed (up to 1880 mg/Kg in Gorgonzola and 2900 mg/Kg in Castelmagno); this amino acid could be considered as a good ripening marker. Apparently, decarboxylation of phenylalanine did not occur, in contrast to tyrosine and histidine, for the absence of phenylalanine-decarboxylating bacterial strains; the formation of 2-phenylethylamine, if present, could be related to the action of other enzymes, for example tyrosine-decarboxylase, that has shown a slight activity towards phenylalanine, amino acid structurally-related to tyrosine. Also this amino acid could be considered an useful ripening marker.

Table 4. Biogenic amines and related precursor amino acids in Toma Piemontese PDO during ripening (mg kg^{-1}; mean ± S.D of two different batches) [7].

Toma Piem. samples	tyrosine	histidine	phenylalanine	tryptophan	tyramine	histamine
4 days	85±39	24±1	98±40	9±3	tr	-
19 days	170±23	56±29	295±27	18±2	10±1	12±1
29 days	141±23	98±49	333±35	16±4	26±4	14±3
39 days	180±25	108±31	439±42	23±6	34±9	15±1
49 days	188±25	96±56	504±60	29±9	60±14	22±9
64 days	254±23	253±94	709±36	57±21	108±46	37±9

Table 5. Biogenic amines and related precursor amino acids in Bra PDO during ripening (mg kg^{-1}; mean ± S.D).

Bra samples	tyrosine	histidine	phenylalanine	tryptophan	tyramine	histamine
0 days	90±8	28±3	95±0	8±4	-	-
9 days	165±24	48±4	234±30	18±2	-	tr
20 days	340±3	244±2	543±12	37±2	-	6±0
27 days	369±15	116±14	614±21	37±5	-	7±1
57 days	533±20	456±48	643±33	46±3	-	9±2

Changes in the concentration of four biogenic amines were determined, showing the presence of histamine and tyramine in long-ripened cheeses (up to a total concentration of 145 mg/Kg in Toma Piemontese, 313 mg/Kg in Castelmagno and 120 mg/Kg in Gorgonzola) and in Murazzano cheese (266 mg/Kg in the type produced using pure ovine milk). 2-phenylethylamine and tryptamine are present only in Gorgonzola and Castelmagno, but in little concentration (<35 mg/Kg). However, the presence of biogenic amines in good ripened cheeses was not directly correlated with high precursor amino acids concentration. Gorgonzola showed high free amino acids concentration (up to 2000 mg/Kg) probably because the very high proteolytic activity of *Penicillium roqueforti*, but the correlation of total biogenic amines was limited.

Table 6. Biogenic amines and related precursor amino acids in Raschera PDO during ripening (mg kg^{-1}; mean ± S.D).

Raschera samples	tyrosine	histidine	phenylalanine	tryptophan	tyramine	histamine
5 days	27±3	12±4	35±16	6±2	-	-
12 days	37±0	18±1	75±1	7±0	-	-
26 days	39±1	18±1	114±6	6±1	-	-
33 days	41±1	13±3	137±8	6±1	-	-
54 days	56±1	15±5	249±48	5±1	-	-

Table 7. Biogenic amines and related precursor amino acids in Murazzano PDO during ripening (mg kg^{-1}; mean ± S.D).

Murazzano samples (mixed milk)	tyrosine	histidine	phenylalanine	tryptophan	tyramine	histamine
4 days	24±0	11±0	44±0	5±0	4±0	-
11 days	50±0	18±0	256±1	13±0	29±0	-
18 days	51±0	31±1	549±8	26±1	80±2	-
Murazzano* samples (sheep' milk)						
4 days	37±1	23±1	75±1	5±1	22±1	-
11 days	28±0	16±1	634±2	40±5	205±2	-
18 days	47±0	42±2	940±1	48±1	267±2	-

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