TECHNOLOGICAL FACTOR INFLUENCING THE BIOGENIC AMINE CONTENT IN SHEEP CHEESES AND SENSORIAL ANALYSIS (TIME OF REPENING, TEMPERATURE, FORM SIZE, ANTI-MOULD)

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Abstract

Sheep cheese samples have been dosed with the following amines: histamine, tyramine, putrescine, cadaverine, β-phenylethylamine, tryptamine and their precursory aminoacids.

Their evolution has been studied as a function of the following parameters: the product size (1, 2 and 5 kg), the maturation temperature (5, 10 and 15 °C), the maturation time (1, 2, 3, 4 and 6 months), the surface treatment with or without anti-mould. Moreover the cheese samples have been submitted to sensorial analysis through a Panel Test with the purpose of correlating taster judgment with amine formation. In the present research only the results related to the 1 kg form size, that is the most commercialised form, are reported.

The results have shown that amine formation increases as a function of maturation time and, among the parameters considered, temperature has the greatest influence, because amine formation increases when temperature is increased. Discriminant analysis has shown that the amine that has the most positive effect on the sample discrimination is cadaverine, followed by tyramine and histamine.

The overall classification success is equal to 87.5%.

The elaboration data related to the sensorial analysis makes it possible to discriminate the cheese samples as a function of the different temperatures. It was

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possible to discriminate the ripened samples at temperature of 5 and 15 °C with the 100% of cases correctly classified and with the variable "spicy flavor" that mostly influenced the judgment of experienced tasters.

Riassunto

In campioni di formaggio ovino sono state dosate le seguenti ammine: istamina, tiramina, putrescina, cadaverina, β-fenilalanina, triptamina e gli aminoacidi precursori di esse. La loro evoluzione è stata studiata in funzione dei seguenti parametri: la pezzatura del prodotto (1, 2 e 5 kg), la temperatura di maturazione (5, 10 e 15 °C), il tempo di maturazione (1, 2, 3, 4, 6 mesi), un trattamento di superficie con o senza antimuffa. Inoltre i campioni di formaggio sono stati sottoposti ad analisi sensoriale mediante Panel test al fine di correlare il giudizio degli assaggiatori con la formazione di ammine. Nella presente nota vengono riportati soltanto i risultati relativi alla pezzatura da 1 kg, che è quella maggiormente commercializzata.

I risultati hanno mostrato che la formazione di ammine ha un andamento crescente in funzione del tempo di maturazione e, fra i parametri presi in considerazione, quello che ha una maggiore influenza è la temperatura, in quanto la formazione di ammine aumenta al crescere di essa. L'analisi discriminante ha mostrato che l'ammina che incide in maniera positiva sulla discriminazione dei campioni è la cadaverina, seguita dalla tiramina ed istamina. La bontà del test statistico è pari all'87.5%.

L'elaborazione dei dati relativi all'analisi sensoriale ha consentito di discriminare i campioni di formaggio in funzione delle diverse temperature di maturazione. E' stato possibile discriminare i campioni maturati alle temperature di 5 e 15 °C, con il 100% di casi correttamente classificati e con la variabile "piccante" che risulta maggiormente influenzare il giudizio degli esperti assaggiatori.

Keywords: Biogenic amines, Pecorino cheese, time and temperature of ripening, sensorial analysis.

Introduction

Biogenic amines are typical substances that are formed from aminoacids in all foodstuffs that have a fermentative process. They are formed by the metabolic activity of different microorganisms that have been specifically detected. Since the literature is very extensive, only some of the more important reviews are reported here; they cited hundreds of works (Shalaby, 1996 (165 references); Silla Santos, 1996 (128 references). In particular with regards to the cheese industry there is an extensive literature (Stratton et al., 1991 (101 references); Vicentini, Giaccio, 2006 (64 references).

The technological factors influencing the formation of biogenic amines mostly in cheeses are:

1) Raw substance treatment: an elevated microbial charge of milk generally produces a greater quantity of biogenic amines;
2) Maturation temperature: generally higher temperatures correspond with greater amines formation;
3) Maturation time: generally the amines formation is a function of maturation time;
4) Yeast strains: microorganisms have a different kind of amine production; the use of selected strains can positively or negatively influences their presence;
5) Other factors influencing the formation of amines are pH, sodium chloride, oxygen, the activity of water and the relative humidity.

In the present research samples of the same sheep cheese in different conditions have been considered: a) ripened in an industrial cheese factory of Abruzzo that produces typical sheep cheese in typical standard industrial conditions; b) ripened in laboratory conditions with different standards in comparison with those present in an industrial setting.

The comparison regarded amine evolution and, given the importance that foodstuff appreciation has for consumers, also the sensorial analysis of the product.

Materials and Methods

Samples and Parameters: 1) 1 kg forms have been used that are generally introduced into commerce after 2 months; 2) temperature: 5, 10 and 15 °C; the temperature of 10 °C is the industrial standard; 3) time: time zero, 1, 2 and 3 months; 4) treatment with anti-mould and without anti-mould; 5) relative humidity 90% ± 5.

Using the parameters mentioned above, the time evolution of 6 biogenic amines (histamine, tyramine, putrescine, cadaverine, phenylethylamine, tryptamine) and 6 precursory aminoacids (histidine, tyrosine, ornithine, lysine, phenylalanine, tryptophane) have been analysed. Such amines were selected because they are the most frequently found in a wide variety of cheese types.

Apparatus: HPLC Dionex with electrochemical Detector (AA direct gold electrode) (5) for aminoacids and with fluorimetric detector rf 2000 for biogenic amines (6-7).

Thermostatic refrigerator Frigolab 700/2RS-2TS Angelantoni Industrie S.p.A..

Sample preparation: for the amino acids analysis the samples have been homogenized in methane sulfonic acid 4 M, evaporated in nitrogen current and taken back with bi-distillated water; for the biogenic amine analysis sample has been homogenized in trichloroacetic acid at 5% and extracted with a mixture of chloroform/n-butanol (v/v 1:1). Derivatization has been carried out with Dansil-chloride (5-(dimethylamino)naphthalene-1-sulfonyl chloride) followed by extraction with ethylic ether.

Sensorial analysis: the Panel Test was constituted by 5 experienced tasters that have taken into consideration the following parameters: odour intensity, aroma intensity, sweetness, acidity, saltiness, bitterness, astringency, spiciness, elasticity, hardness, friability, stickiness, solubility and humidity. The evaluation scheme is that suggested by the National Cheese Taster organization (ONAF) in Cuneo.

Statistical Analysis: Linear Discriminant Analysis (LDA) was applied to the separation of the analysed cheese samples according to different ripening temperatures and to Panel Test judgement. As the group-membership of each sample was already known, LDA was applied to this variable set in order to evaluate the sample differentiation and classification of the data expressed as discriminant scores. LDA has been extensively discussed by several authors (8-10).

Results and Discussion

The biogenic amine evolution (mg/kg) in the industrial production conditions (ripening temperature at 10 °C), in the laboratory conditions (ripening temperature at 5 and 15 °C) is reported in tables 1, 2 and 3, respectively.

An example of general development of aminoacids and biogenic amines has been reported in Figure 1. It has been obtained in 1 kg cheese samples ripened in industrial conditions (temperature at 10 °C).

An example of sensorial analysis is reported in Figure 2; it shows a graph related to a sample matured at 5 °C and in Figure 3 a graph of a sample matured at 15 °C.

TABLE 1

**BIOGENIC AMINES EVOLUTION (mg/kg)
AND OF PRECURSORY AMINOACIDS (mg/Kg)
IN THE INDUSTRIAL CONDITION PRODUCTION
(RIPENING TEMPERATURE 10 °C, WITHOUT ANTI-MOULD)**

	Time zero	1 month	2 months	3 months	4 months
1 kg FORM SIZES					
Histidine	5,150	5,310	5,518	5,378	5,390
Histamine	0.1	3.50	10.10	19.40	23.00
Tyrosine	9,750	10,520	10,600	10,960	11,310
Tyramine	0.05	10.60	48.80	67.80	43.70
Ornithine	1,560	1,731	1,680	1,794	1,850
Putrescine	0.80	3.50	7.50	7.60	8.20
Lysine	12,280	12,870	13,218	13,810	13,250
Cadaverine	0.80	2.70	6.90	5.60	6.90
Phenylalanine	10,120	10,730	11,060	11,030	11,620
β-phenylalanine	0.05	2.40	13.10	10.50	17.00
Tryptophane	1,960	2,321	2,410	2,370	2,635
Tryptamine	0.20	3.20	9.10	10.70	13.20

TABLE 2

BIOGENIC AMINES EVOLUTION (mg/kg) AND OF PRECURSORY AMINOACIDS (mg/Kg) IN LABORATORY CONDITIONS (RIPENING TEMPERATURE 5 °C)

	Time zero	1 month	2 months	3 months	4 months
1 kg FORM SIZES with anti-mould					
Histidine	5,150	5,200	5,230	5,250	5,263
Histamine	0.1	0.05	2.20	7.80	7.70
Tyrosine	9,750	10,160	11,010	10,980	11,060
Tyramine	0.05	3.10	12.10	26.60	28.70
Ornithine	1,560	1,628	1,780	1,750	1,650
Putrescine	0.80	0.30	1.50	5.30	2.00
Lysine	12,280	12,580	13,200	13,360	13,120
Cadaverine	0.80	1.50	1.80	4.80	2.20
Phenylalanine	10,120	10,150	11,130	11,150	11,380
β-phenylalanine	0.05	0.05	0.30	2.70	1.00
Tryptophane	1,960	2,151	2,451	2,320	2,691
Tryptamine	0.20	0.90	1.00	3.30	3.10
1 kg FORM SIZES without anti-mould					
Histidine	5,150	6,080	4,960	5,647	5,150
Histamine	0.1	0.05	2.50	9.40	14.20
Tyrosine	9,750	11,128	9,815	11,200	10,930
Tyramine	0.05	1.70	24.40	28.80	51.70
Ornithine	1,560	1,980	1,620	1,840	1,610
Putrescine	0.80	0.30	2.50	5.10	5.80
Lysine	12,280	13,810	12,130	13,800	12,210
Cadaverine	0.80	1.40	2.30	5.00	4.20
Phenylalanine	10,120	11,871	9,860	11,520	10,560
β-phenylalanine	0.05	0.05	1.60	2.60	18.70
Tryptophane	1,960	2,230	2,110	2,410	2,380
Tryptamine	0.20	0.30	3.50	4.80	23.50

TABLE 3

BIOGENIC AMINES EVOLUTION (mg/kg) AND OF PRECURSORY AMINOACIDS (mg/Kg) IN LABORATORY CONDITION (RIPENING TEMPERATURE 15 °C)

	Time zero	1 month	2 months	3 months	4 months
1 kg FORM SIZES with anti-mould					
Histidine	5,150	5,610	5,750	5,820	5,621
Histamine	0.1	15.30	9.10	9.40	20.20
Tyrosine	9,750	10,830	11,080	11,520	11,950
Tyramine	0.05	26.80	54.70	32.10	29.10
Ornithine	1,560	1,760	1,831	1,895	1,730
Putrescine	0.80	4.60	7.40	6.70	6.00
Lysine	12,280	13,015	13,700	13,850	13,390
Cadaverine	0.80	3.90	7.10	6.80	6.80
Phenylalanine	10,120	11,658	11,510	11,560	11,860
β-phenylalanine	0.05	8.00	17.00	25.10	23.20
Tryptophane	1,960	2,690	2,638	2,560	2,574
Tryptamine	0.20	5.50	10.40	18.00	19.20
1 kg FORM SIZES without anti-mould					
Histidine	5,150	5,480	5,910	5,710	5,790
Hstamine	0.1	13.50	10.50	11.10	25.30
Tyrosine	9,750	10,920	10,890	11,360	11,720
Tyramine	0.05	27.10	49.90	45.10	33.10
Ornithine	1,560	1,850	1,986	1,860	1,926
Putrescine	0.80	0.90	7.60	6.40	6.20
Lysine	12,280	12,930	13,920	13,720	14,180
Cadaverine	0.80	4.10	6.90	6.80	7.10
Phenylalanine	10,120	11,923	11,980	11,510	12,050
β-phenylalanine	0.05	5.90	14.10	24.50	27.50
Tryptophane	1,960	2,715	2,820	2,490	2,592
Tryptamine	0.20	4.60	9.40	16.10	13.50

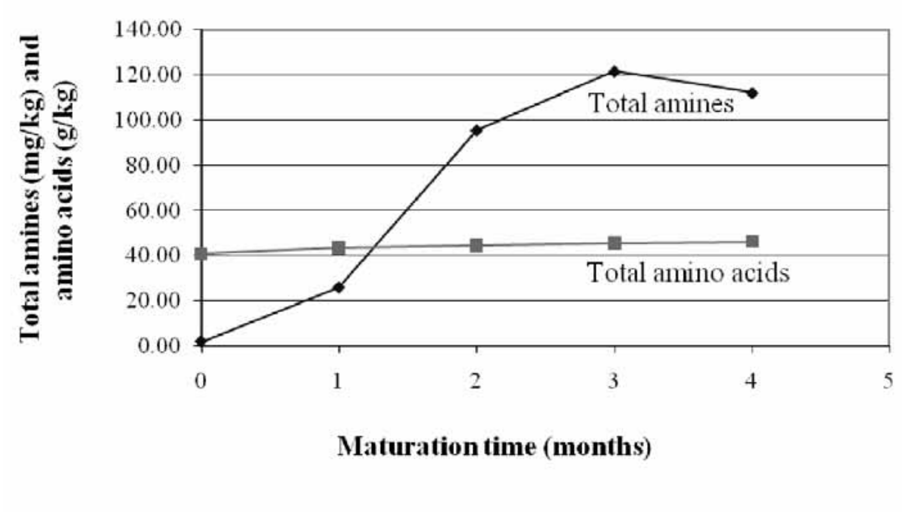

Fig. 1 – Total amines and aminoacids development in the industrial conditions in 1 kg sheep cheese form sizes ripened at 10 °C

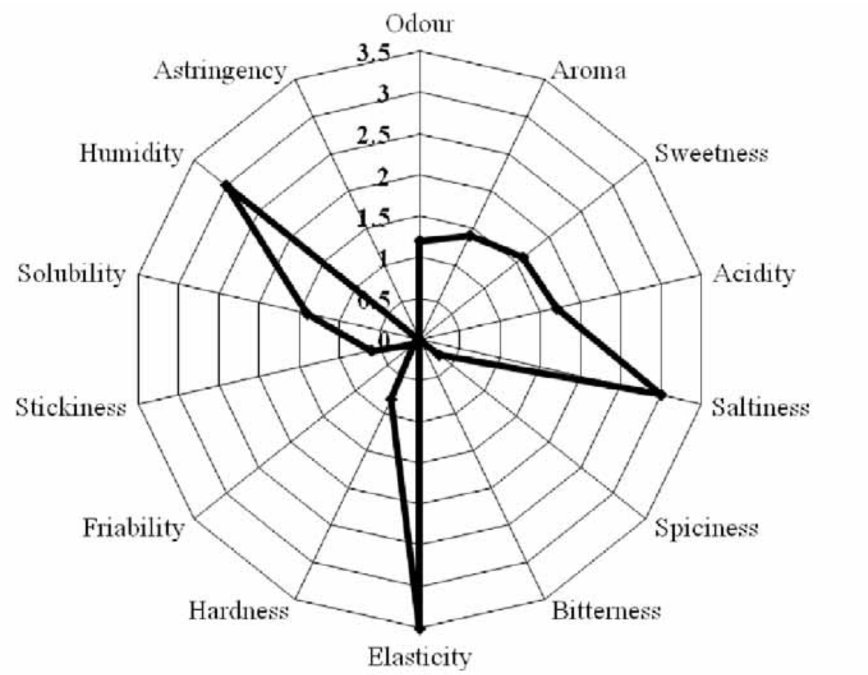

Fig. 2 – Sensorial analysis example of sample ripened at 5 °C.

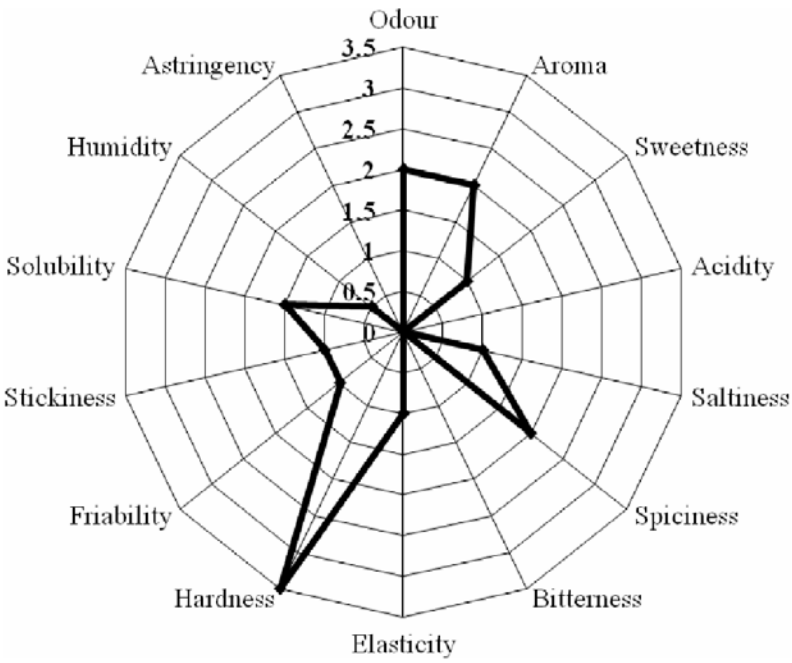

Fig. 3 – Sensorial analysis example of sample ripened at 15 °C.

All data obtained were analysed statistically using the multivariate statistical approach, in particular Linear Discriminant Analysis. This methodology was applied to separate the cheese samples based on the presence of biogenic amines, using different temperatures of ripening, as class identity. The aim of this procedure was to evaluate sample differentiation and classification of data expressed as discriminant scores.

Therefore, depending on the number of groups, one or two discriminant functions were extracted. To determine the number of linear discriminant functions to retain, Bartlett's classical test was applied,

$$b = -[N - (p + g)/2 - 1] \ln \Lambda$$

where N stands for the number of observations, p for the number of variables, g for the number of groups and Λ represents the ratio of the within-group sum of squares to the total sum of squares. Wilks' Λ value provides information pertaining to how much of the total variability is due to the differences between the group means or to the within-group variability. The value of Λ can range between 0 and 1: Λ = 1 when the two group means are equal, while Λ = 0 if they differ (11).

Once a set of q variables has been selected, the classification rule (also known as Fisher's linear Discriminant functions) can be computed using

$$b_{ij} = (n-g)\sum_{l=1}^{q} w^*_{il}\overline{X_{lj}}$$

$i = 1, 2, \ldots, q;\ j = 1, 2, \ldots, g$

for the coefficient, and

$$a_j = \log p_j - \frac{1}{2}\sum_{i=1}^{q} b_{ij}\overline{X_{ij}}$$

$j = 1, 2, \ldots, q$

for the constant, where pj is the prior probability of group j.

A significant Wilks Λ value was obtained when the cheese samples were classified as a function of the two different temperatures of ripening (5 and 15 °C). In particular, samples ripened at the 3 different temperatures, treated with or without anti-mould, have been compared. The data obtained with the ripening temperature of 10 °C have not been reported because they yielded intermediary values. In this case, one discriminant function was estimated, since the number of groups in this sample was 2, and 2–1 is the maximum allowable number of eigenvalues for the matrix $W^{-1}B$. The first discriminant eigenvalue (1.722) had a Wilks Λ value close to zero (0.367).

The distribution of data expressed as discriminant scores along the first eigenvector is presented in Figure 4. In this representation of all data, the two sample classes, corresponding to cheese samples ripened at 5 °C *(G1)* and at 15 °C *(G2)*, respectively, were clearly distinct.

Based on the values for the two linear discriminant functions for each sample, the group membership could be predicted using a classification rule. Table 4 summarises the results of the classification for the cheese samples, where the actual and predicted group membership and, on the diagonal, the number of the samples classified correctly, are shown. In this case, all cheese samples were correctly assigned to the group they belong to, with one exception regarding samples ripened at 15 °C. Furthermore the overall classification success was 87.5%.

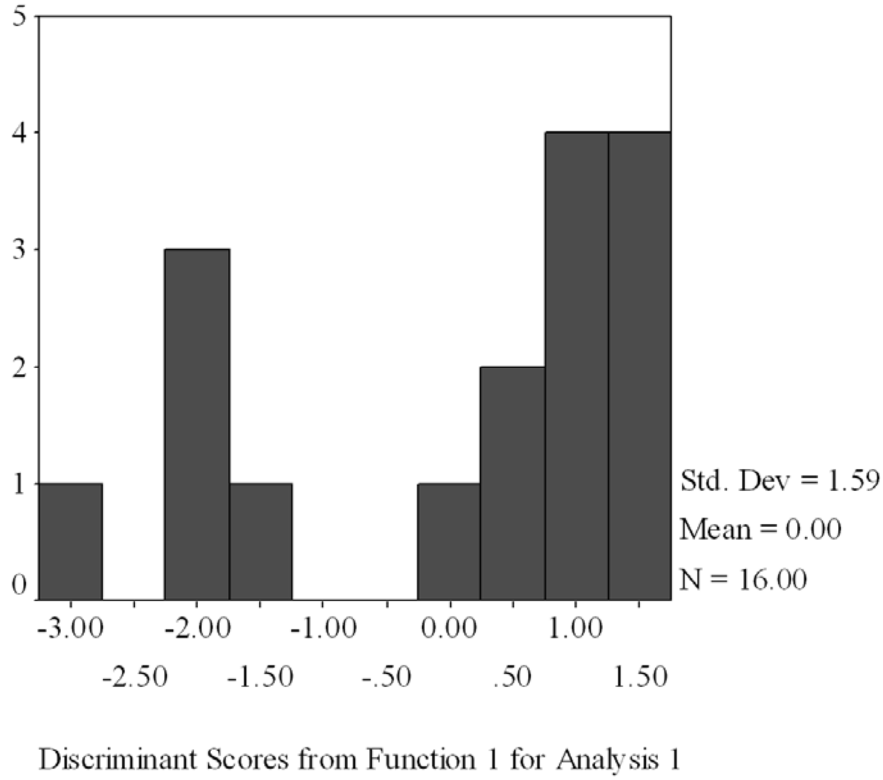

Fig. 4 – The distribution of data, related to cheese samples as function of the two different temperatures (5 and 15 °C), expressed as discriminant scores along the first eigenvector.

TABLE 4

CLASSIFICATION TABLE FOR 2 GROUPS OF CHEESE SAMPLES AS A FUNCTION OF TWO DIFFERENT TEMPERATURES (G1 = CHEESES RIPENED AT 5 °C; G2 = CHEESES RIPENED AT 15 °C).

	Predicted Group		
Actual Group	G1	G2	Total
G1	6	2	8
G2	0	8	8
Total	6	10	16
Accuracy of prediction, %	**75.0**	**100.0**	**100.0**

According to Wilks Λ value another distribution was quite significant. In fact if the whole data set is analysed as a function of the Panel Test, with a comparison between samples ripened at 5 °C and at 15 °C, the results obtained are the following. The data relating to the variables of bitterness and astringency were eliminated from the Panel test set since they were present in only two of the samples. In this case, one discriminant function was estimated, since the number of groups in this sample was 2, and 2–1 is the maximum allowable number of eigenvalues for the matrix $W^{-1}B$. The first discriminant eigenvalue (9.731) had a Wilks Λ value close to zero (0.093).

The distribution of data expressed as discriminant scores along the first eigenvector is presented in Figure 5. In this representation of all data, the two sample classes, corresponding to cheese samples ripened at 5 °C *(G1)* and at 15 °C *(G2)*, respectively, were distinct.

Based on the values for the two linear discriminant functions for each sample, the group membership could be predicted using a classification rule. Table 5 summarises the results of the classification for the cheese samples, where the actual and predicted group membership and, on the diagonal, the number of the samples classified correctly, are shown. In this case, all cheese samples were correctly assigned to the group they belong to. The overall classification success was 100%.

The total quantity of amines formed in the samples treated with anti-mould is slightly more elevated in comparison to the samples without anti-mould. Moreover this difference is not very significant because the statistical classification of the two groups have a Wilks Λ value close to the unity (0.833) and the overall classification success is equal to 68.8%. Instead this parameter influences the sensorial analysis because the Wilks Λ value is 0.221 and the overall classification success is of 93.8% (Table 6 and Figure 6).

In conclusion the statistical analysis of data regarding the content of biogenic amines in cheeses puts in evidence that amine formation is in function of temperature increase. Moreover, the distinction among the different maturation temperatures and the treatment with or without anti-mould has been demonstrated, also as a function of the taste variables.

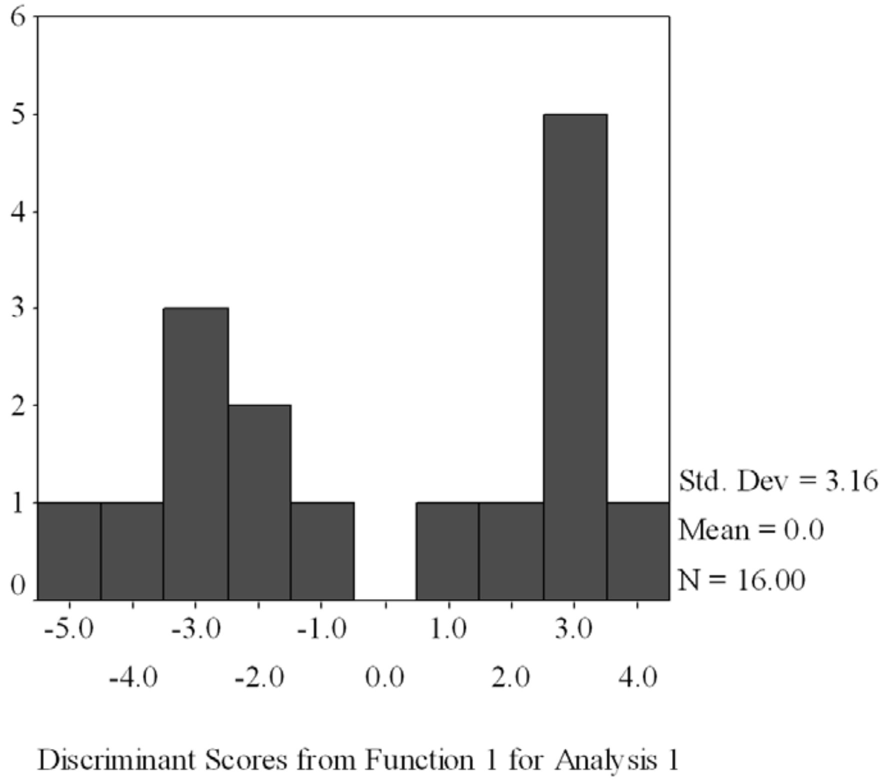

Fig. 5 – The distribution of data, related to cheese samples ripened at 5 °C (G1) and at 15 °C (G2), as function of the Panel test, expressed as discriminant scores along the first eigenvector.

TABLE 5

CLASSIFICATION TABLE FOR 2 GROUPS OF CHEESE SAMPLES AS A FUNCTION OF PANEL TEST (G1 = CHEESES RIPENED AT 5 °C; G2 = CHEESES RIPENED AT 15 °C).

	Predicted Group		
Actual Group	G1	G2	Total
G1	8	0	8
G2	0	8	8
Total	8	8	16
Accuracy of prediction, %	**100.0**	**100.0**	**100.0**

TABLE 6

CLASSIFICATION TABLE FOR 2 GROUPS OF CHEESE SAMPLES AS A FUNCTION OF PANEL TEST (G1 = CHEESES WITH MOULD TREATMENT; G2 = CHEESES WITHOUT ANTI-MOULD TREATMENT).

	Predicted Group		
Actual Group	G1	G2	Total
G1	8	0	8
G2	1	7	8
Total	9	7	16
Accuracy of prediction, %	**100.0**	**87.5**	**100.0**

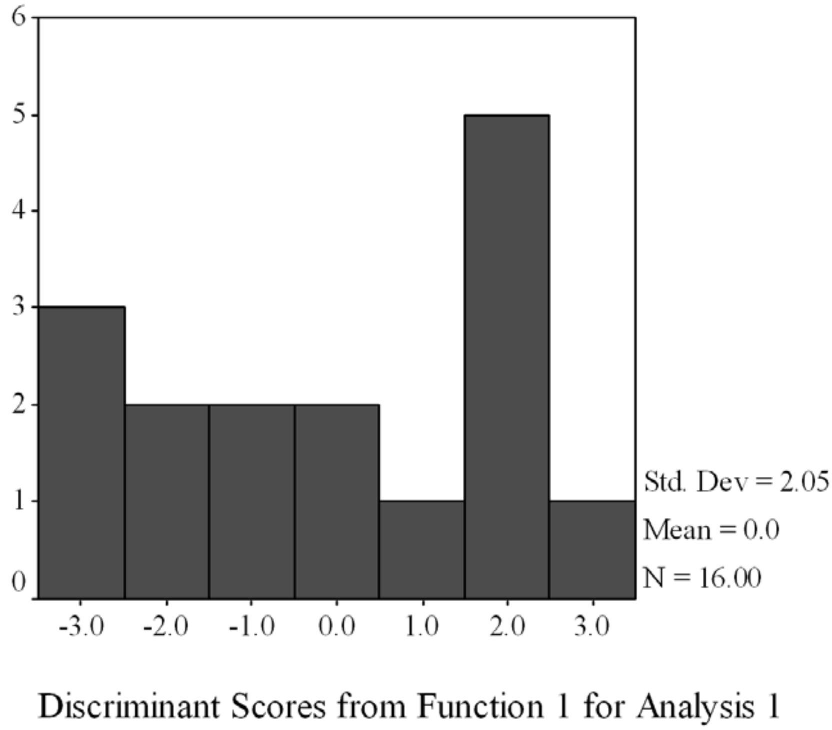

Fig. 6 - The distribution of data, related to cheese samples treated with or without anti-mould, as function of the Panel test, expressed as discriminant scores along the first eigenvector.

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