F. DI GIACOMO,
A. DEL SIGNORE,
M. GIACCIO*

Pig rennet in making Farindola ewe cheese

PROGRESS IN NUTRITION
VOL. 15, N. 4, 226-238, 2013

TITOLO
Il pecorino di Farindola

KEY WORDS
Pig rennet, calf rennet, ewe cheese, Farindola ewe cheese

PAROLE CHIAVE
Caglio di maiale, caglio di vitello, formaggio pecorino, pecorino di Farindola

Dip SFPEQ-Sezione di Metodi Quantitativi e Teoria Economica, Università "G. d'Annunzio" Chieti-Pescara
* Online University Torrevecchia Teatina (CH), Italy

Address for correspondence:
Prof. Antonella Del Signore,
Dip. SFPEQ-Sezione di Metodi Quantitativi e Teoria Economica,
Università "G. d'Annunzio" Chieti-Pescara,
Viale Pindaro, 42 - 65127 Pescara, Italy
Tel. +39-085-4537505
Fax +39-085-4537545
E-mail: signore@unich.it

Summary

Samples of ewe cheese produced with pig rennet in the Farindola area (Abruzzo, Italy) were compared with samples of ewe cheese produced with calf rennet using the same methods of processing and ripening as the Farindola cheese. To distinguish the samples chemical parameters (18 amino acids and 12 fatty acids) were chosen. In addition a taste analysis was carried out by a Panel of tasting experts. All of the data thereby obtained was analysed statistically through discriminate analysis. Both the analytical data and the taste expert Panel results show a net differentiation between the cheeses produced with the two different types of rennet. The Panel Test found that the ewe cheese made with calf rennet is systematically more spicy ("piccante" flavour) and bitter than the Farindola cheese made with pig rennet, whereas this latter is always sweeter and never bitter.

Riassunto

Sono stati presi in esame campioni di formaggio pecorino prodotti con caglio di maiale nell'area tipica di Farindola (Abruzzo, Italia) in confronto con campioni prodotti con caglio di vitello nelle stesse condizioni di processo e stagionatura. Su questi campioni sono stati dosati 18 amino acidi e 12 acidi grassi, in funzione della maturazione; in aggiunta è stata condotta un'analisi sensoriale attraverso un Panel di esperti assaggiatori. Tutti i dati ottenuti sono stati analizzati statisticamente mediante l'analisi discriminante. Sia il confronto dei dati analitici che quello del Panel test hanno mostrato una netta differenziazione fra i formaggi prodotti con due cagli diversi. Il Panel Test ha anche evidenziato che il pecorino realizzato con caglio di vitello è sistematicamente più piccante ed amaro di quello con caglio di maiale, mentre quest'ultimo è sempre più dolce e mai amaro.

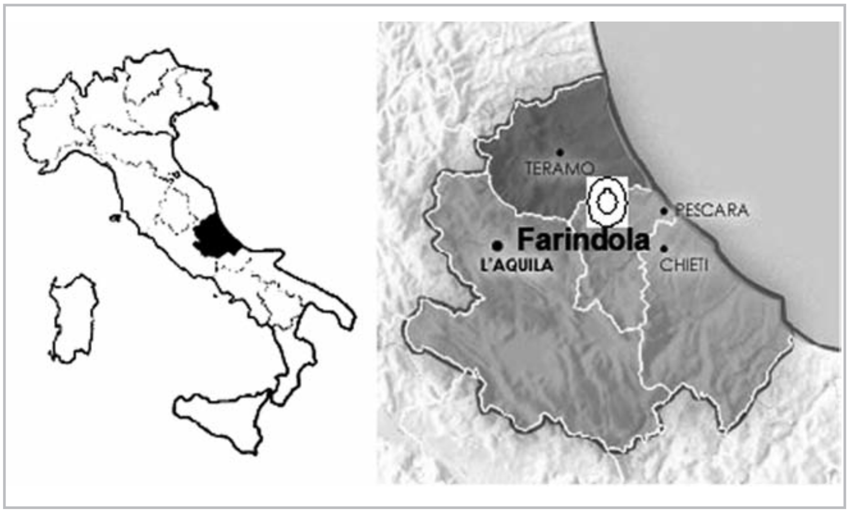
Introduction

"Pecorino di Farindola" is an artisanal ewe cheese manufactured on a small scale by farmers following traditional practices in a well-defined area of Abruzzo (Italy). It is produced from raw ewes' milk with the addition of pig rennet.

This traditional product is unique among ewe cheeses in that all other ewe cheeses produced in Italy use calf rennet, while Farindola ewe cheese uses pig rennet in its curdling process. Pig rennet has been used in the eastern area of the Gran Sasso mountain range since the time of the ancient Romans: there are historical records that refer to a "cheese of the Vestini" (an ancient Italic tribe that occupied modern day Abruzzo) that utilized pig rennet in the curdling process.

The denomination "Farindola ewe cheese" appears in some texts on Italian cheese in the early 1900's. It is important to note that in the production of pig rennet the stomach of an animal of at least one year of age is used, while in the production of other rennets, younger animals (calves, lambs, kids) are used[1].

Figure 1 - Production area for Farindola ewe cheese



The territory in which this traditional production method is used includes nine towns located within the provinces of Pescara and Teramo (Figure 1).

Standards of production have been established which require, among other things, that the sheep must be raised for the entire year in the traditional production area; they may only be fed with forage which has been produced locally; the milk is made into cheese immediately after milking (or it must be refrigerated at 10-12°C) and it is curdled at from 31 to 33°C; the ripening of the cheese can extend to 12 months, etc.. Each wheel of cheese weighs between 1 to 2 kilos.

Traditionally the natural animal "rennet", obtained from the stomach of a mammal, was used to make cheese, including pig rennet. For example, Merker, in a conference held in 1918 observed that "it has been known for a number of years that pepsin, a substance produced from the mucous membrane of the pig's stomach, would give excellent results in curdling milk for the manufacture of cheese" (1). In the modern cheese manufacturing industry, however, the use of enzymes, industrially obtained, is frequently the preferred method of obtaining curd. The industrial rennet is more stable and the enzymatic power is standardized.

As a result of the preference for industrial rennet, cheeses produced with the traditional method directly from pig rennet have not been the object of a systematic study. In fact, a consultation of the major international data banks will show that there are no scien-

[1] Liquid pig rennet is made by working the mucous membrane of pig stomachs, first washing it carefully and then cutting it into narrow strips which are then covered with salt for 2 or 3 days (dry salting). It is then left to marinate in a dark glass container in a mixture of white vinegar, white wine, hot peppers and, sometimes, pepper corns. A five litre container requires two or three stomachs. The mucous membrane is left in the marinade for 3 to 4 months. The rennet is then filtered through a linen cloth at least 5 or 6 days before use and it is stored in a dark place.

tific works on cheeses obtained from pig rennet. This sentence is confirmed by the Scopus data bank, which reviews more than 18,000 journals. In fact in this data bank only one paper has been cited on this product regarding the biogenic amine content (2), whereas the other researches carried out for religious purpose.
Works are to be found, instead, regarding the characteristics of the enzymes (pepsin and chymosin) extracted from the stomach mucous of various mammals. In the case of "pig rennet" the enzyme which is extracted is pepsin. The literature on the subject reports that, of the available enzymes, pig pepsin is the only one which is potentially less proteolytic than chymosin, since it is readly denaturated in the cheesemaking process. Pig pepsin is unstable above pH 6.0 compared to chymosin, stable until to pH 6.7 (3). Clotting activity of pig pepsin was extremely pH-dependent around pH 6.6 and coagulation did not occur above pH 6.68 (4). Other works deal with the activity of enzymes and their coagulatory and proteolytic effect on curd (3-6). In one work published in 1972 pig pepsin was used in the experimentation on cheese but only "as rennet substitutes" (7). In order to differentiate between animal rennet and other enzymes, the characteristics of the chymosin gene, isolated from tissue extracts of buffalo, camel, cow and pig, were studied through SDS-PAGE Polyacrylamide gel electrophoresis and PCR amplification techniques. Two prominent proteins were found in cow and buffalo rennet, while only one protein was observed in camel and pig (8).
It is known that the type of rennet used affects the characteristics of ewe cheese and consequently, flavour development in cheese (9-13).
The ripening of cheese is a complex process in which three major catabolic pathways are involved: glycolysis, lipolysis and proteolysis (14). In particular, lipolysis is correlated with the concentration of the free fatty acids C_{10}, C_{14} e C_{16}, while proteolysis is correlated with the concentration of the total amino acids (15).
In light of these findings, it was deemed that further research was called for into the differences in composition and organoleptic qualities of two samples of ewe cheese obtained under the same conditions, but using different rennets: one sample was produced with pig rennet and the other with calf rennet. In order to identify any possible effects from the treatment of the surface of the cheese with oil and vinegar, as required by Production Regulations, two of each sample were produced, one treated and one untreated. It should be noted here that the presence of volatile substances in cheese obtained from two different rennets (pig and calf) has been discussed previously (16).

Materials and methods

Cheese making: Freshly milked ewe milk, coming from ewes that produce less than 1 litre/day in about 100 milking days, was kept cool (10-12°C) but not refrigerated. The milk was curdled at 31-33°C with pig rennet obtained using the method described in footnote 1 above. The setting time varied from 40 to 60 minutes; after the curdle was broken into granules of 0.5 to 2 cm, it was placed into straw forms to harden; it was then dry salted with coarse salt on both sides – one side of the cheese was salted one day and the other side, the next. The salt was then washed off. The surface of the cheeses were periodically treated with extra virgin olive oil and vinegar (or tomato juice)[2]. The period of ripening varied from a minimum of three months to a maximum of a year and each whole cheese weighed between 1 and 2 kg.

[2] From this point on, for purposes of simplicity, the words "treated" or "non-treated" will be used.

Samples and Parameters: 1) 2 kg forms have been used. They are generally introduced into commerce after 3-6 months, but even up to 1 year, ripened to a temperature between 10 and 14°C; 2) time: time 3, 6 , 9 and 12 months; 3) treatment: with oil and vinegar and without.
Using the parameters mentioned above, the time evolution of 18 aminoacids (Asparagine, Glutamine, Serine, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Cystine, Isoleucine, Leucine, Phenylalanine, Lisine, Tryptophan), and of 12 fatty acids ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) have been analysed.

Apparatus: HPLC Dionex with electrochemical Detector (AA direct gold electrode) (17) for aminoacids (18-19).
GasChromatograph Agilent Technologies 6890N with mass spectrometry Agilent 5973 inert, column HP 5MS for fatty acids.

Sample preparation: The moisture content of the chesses was determined by keeping the samples in contact with currents of dry air at 102°C for sixteen hours.
For the amino acids analysis, the samples have been homogenized in methanesulfonic acid 4 M, evaporated in nitrogen current and taken back with bi-distillated water.
For the fatty acids analysis a cold trans-esterification with methanolic solution of potassium hydroxide has been carried out.

Sensory analysis: Experimental cheeses were sampled after the four periods of ripening (after 3, 6, 9 and 12 months of ripening). Sensory analysis was performed by a trained analytical Panel Test of 5 members that have taken into consideration the following parameters: sweetness, acidity, saltiness, bitterness, spiciness, humidity, solubility and friability. The intensity of the sensation is distributed on a scale that goes from 0 to 7. For sensory analysis, the recommendations and vocabulary reported in Bárcenas et al. were considered (20). 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 (21-23).

Results and discussion

The free amino acid evolution during the ripening is reported in Table 1. The values are expressed in mg 100 g^{-1} of cheese dry matter. Figure 2 shows the development of the total free amino acids in function of the ripening of the cheeses made with pig and calf rennet, treated and untreated.
The free fatty acid evolution during the ripening is reported in Table 2. The values are expressed in mg 100 g^{-1} of cheese dry matter. Figure 3 shows the development of the total free amino acids in function of the ripening of the cheeses made with pig and calf rennet, treated and untreated.
Results of the Panel Test for all samples are reported in Table 3. The effects of the free amino acids on taste and on flavor are reported by Kubícková & Grosch (24), Yvon & Rijnen (14). The effects of the free fatty acids on taste and flavor are reported by Woo & Lindsay (25), Freitas & Malcata (26), House & Acree (27), Collins, McSweeney, Wilkinson (28).
An example of the results of sensorial analysis is reported in Figure 4; it shows a graph related to a sample obtained with treated pig

Table 1 - Evolution of free amino acids (mg 100 g^{-1} of cheese dry matter) during the ripening (1 = pig rennet treated; 2 = pig rennet untreated; 3 = calf rennet treated; 4 = calf rennet untreated)[a].

Samples	Asp	Glu	Ser	Gly	Hys	Arg	Thr	Ala	Pro
3 months									
1	15.53	88.35	10.67	10.22	17.51	51.42	16.35	25.76	36.41
2	16.07	111.6	12.98	11.92	20.23	62.22	18.00	28.75	38.57
3	15.84	116.25	12.90	10.12	19.85	63.75	19.76	26.45	27.55
4	18.64	93.48	11.22	10.65	18.38	67.44	15.2	22.89	29.14
6 months									
1	37.68	302.82	42.24	13.50	41.25	62.4	32.9	78.22	128.36
2	41.03	355.35	49.27	14.30	38.25	82.46	36.44	82.82	143.24
3	42.43	370.80	48.60	12.50	43.56	92.96	33.00	80.50	108.22
4	47.68	309.00	44.18	13.84	40.26	95.05	33.12	83.30	124.60
9 months									
1	38.28	317.19	64.71	29.3	75.32	45.80	42.55	92.11	139.62
2	42.10	359.70	71.50	28.25	82.46	42.50	42.90	90.24	157.30
3	43.95	391.55	69.14	31.50	81.55	56.77	46.20	84.00	129.36
4	48.93	327.69	65.85	29.14	86.15	65.00	43.57	86.50	148.46
12 months									
1	31.66	485.67	17.79	23.62	158.17	15.18	66.66	47.66	248.33
2	32.30	508.95	19.89	19.60	180.40	15.58	66.12	47.49	283.59
3	33.85	585.00	18.56	21.94	182.25	27.50	73.14	46.00	233.90
4	37.50	587.89	18.22	22.42	194.61	25.57	76.78	44.98	266.92

Samples	Tyr	Val	Met	Cys	Ile	Leu	Phe	Lys	Trp	Total
3 months										
1	29.24	112.52	16.46	5.13	44.16	178.48	58.36	52.77	20.61	791
2	30.42	123.51	17.39	6.41	50.60	196.88	61.47	55.13	21.87	886
3	27.62	113.68	18.58	5.75	45.08	180.32	60.24	55.68	23.55	846
4	28.88	116.52	18.31	5.40	46.25	184.50	57.30	53.23	19.54	821
6 months										
1	52.68	350.46	28.63	4.89	111.00	333.04	82.32	139.05	45.32	1,892
2	53.68	363.64	30.22	7.02	125.11	362.34	81.06	144.15	43.85	2,060
3	50.40	356.92	31.82	5.70	120.19	340.40	86.72	126.70	53.05	2,011
4	55.93	354.02	34.78	6.15	115.8	337.50	90.94	140.25	51.25	1,986
9 months										
1	51.04	378.78	38.13	5.34	166.75	336.14	113.10	203.65	89.25	2,236
2	49.81	388.41	40.00	5.80	172.50	340.22	120.51	225.95	103.86	2,374
3	52.28	385.86	39.10	5.64	174.42	350.88	116.36	210.07	108.98	2,389
4	52.33	382.31	41.85	6.16	173.85	343.80	118.38	223.86	82.10	2,338
12 months										
1	40.18	424.02	39.90	4.00	220.78	470.45	146.90	253.75	97.28	2,805
2	43.35	427.84	44.00	4.64	223.62	470.22	165.12	292.50	114.33	2,974
3	45.00	432.66	44.85	5.08	229.78	480.20	162.94	279.34	122.04	3,039
4	43.54	425.23	46.13	4.82	230.77	481.74	165.38	303.08	92.55	3,084

[a] The results obtained from 10 observations, for each free amino acid, as far as intralaboratory repeability is concerned, are expressed as mean (mg 100 g^{-1}) and standard deviation. The % standard deviation ranged from 1.61 to 10.06%

Figure 2 - Evolution of total free amino acid content in cheese made with pig rennet and calf rennet, with and without oil and vinegar

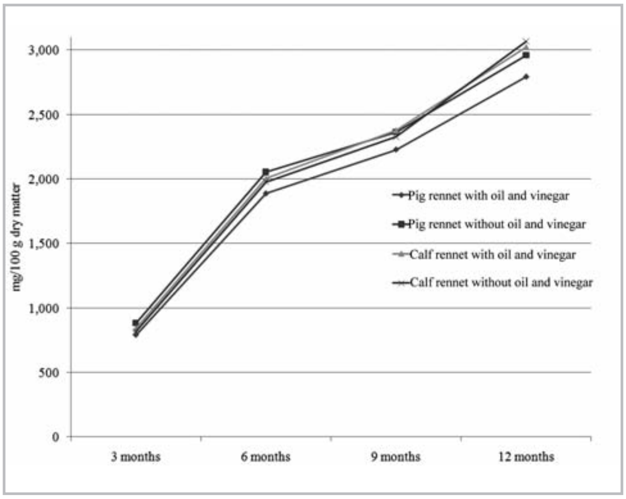


Figure 3 - Evolution of total free fatty acid content in cheese made with pig rennet and calf rennet, with and without oil and vinegar

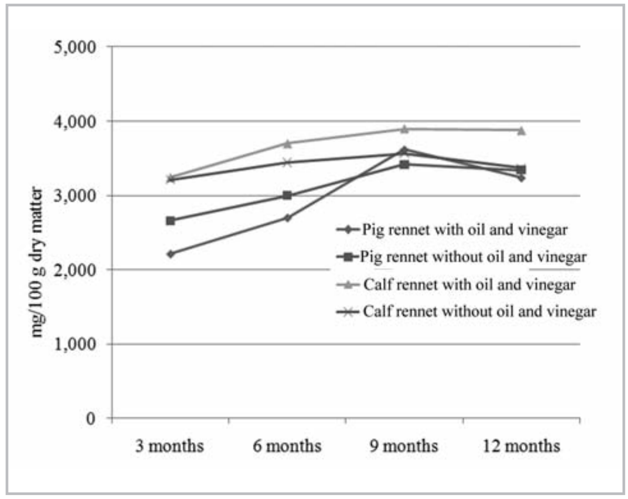


Table 2 - Evolution of free fatty acids (mg 100 g $^{-1}$ of cheese dry matter) during the ripening (1 = pig rennet treated; 2 = pig rennet untreated; 3 = calf rennet treated; 4 = calf rennet untreated)[a]

Samples	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total
3 months													
1	114	43	19	38	35	251	791	27	310	495	31	54	2,208
2	133	41	18	68	45	313	939	36	352	596	43	74	2,658
3	146	61	28	80	55	410	1,007	50	445	825	65	72	3,244
4	157	55	26	72	89	413	1,010	44	405	790	64	86	3,211
6 months													
1	143	52	21	61	74	326	901	36	361	611	52	59	2,697
2	151	54	24	78	78	389	986	43	392	669	61	73	2,998
3	202	73	39	96	86	538	1,121	58	475	853	83	75	3,699
4	168	62	32	85	95	493	1,053	49	428	824	75	81	3,445
9 months													
1	185	47	30	102	143	514	1,094	55	466	818	91	73	3,618
2	106	35	20	92	138	503	1,083	51	461	792	75	61	3,417
3	132	56	35	123	155	566	1,182	69	502	902	98	79	3,899
4	101	37	19	106	140	511	1,109	61	482	842	90	69	3,567
12 months													
1	100	41	26	95	121	477	1,023	44	421	756	73	60	3,237
2	66	30	19	87	131	508	1,045	61	443	792	95	65	3,342
3	94	50	32	115	121	561	1,180	69	560	890	126	78	3,876
4	65	33	15	118	136	470	1,166	58	435	712	90	69	3,367

[a] The results obtained from 10 observations, for each free fatty acid, as far as intralaboratory repeability is concerned, are expressed as mean (mg 100 g $^{-1}$) and standard deviation. The % standard deviation ranged from 3.5 to 7.5%

Table 3 - Panel Test for four ewe cheese samples as a function of ripening

	Sweet	Friability	Solubility	Umidity	Pungent	Bitter	Salty	Acidity
Pig rennet treated								
3 months	4.2	3	5	3	0	0	1.5	2
6 months	1.8	5	5	2	1.3	0	3	0.5
9 months	2.4	5	5	2.6	0.9	0	3	0
12 months	2.4	5.7	6	2.1	1.4	0	3	0
Mean	*2.7*	*4.675*	*5.25*	*2.425*	*0.90*	*0*	*2.625*	*0.625*
Pig rennet untreated								
3 months	3.2	3	5	3	0	0	1.7	3
6 months	2.2	4.1	4.1	1	0.5	0	2	1
9 months	2.8	4.1	5	2.5	0.7	0.4	2.5	0
12 months	2.6	6	5.6	2.1	1.4	0.8	2.7	0
Mean	*2.7*	*4.3*	*4.925*	*2.15*	*0.65*	*0.3*	*2.225*	*1*
Calf rennet treated								
3 months	3.5	4	5.5	4.5	0	0	2.5	4.2
6 months	1.4	5	4.1	2.2	1.8	0	3.5	1
9 months	2.2	5	5	2.5	2.6	1.1	3	0
12 months	2	6.5	6.5	2.8	2.2	1.1	3	0
Mean	*2.275*	*5.125*	*5.275*	*3*	*1.65*	*0.55*	*3*	*1.3*
Calf rennet untreated								
3 months	2.9	4	5	4.5	0	0	2.4	4.5
6 months	2	5	5	2.2	0.7	0	2.7	1
9 months	2.4	5	4.2	2.2	1.8	0.5	2.2	0
12 months	2.2	5.5	5.6	2.1	1.1	1.1	2.1	0
Mean	*2.375*	*4.875*	*4.95*	*2.75*	*0.9*	*0.4*	*2.35*	*1.375*

rennet with a ripening time of 9 months and a graph of a sample obtained with treated calf rennet with a ripening time of 9 months.

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 free amino acids and the free fatty acids, respectively and furthermore on the sensorial analysis. 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: $\Lambda = 1$

when the two group means are equal, while $\Lambda = 0$ if they differ.
Once a set of q variables has been selected, the classification rule (also known as Fisher's linear Discriminant functions) can be computed using

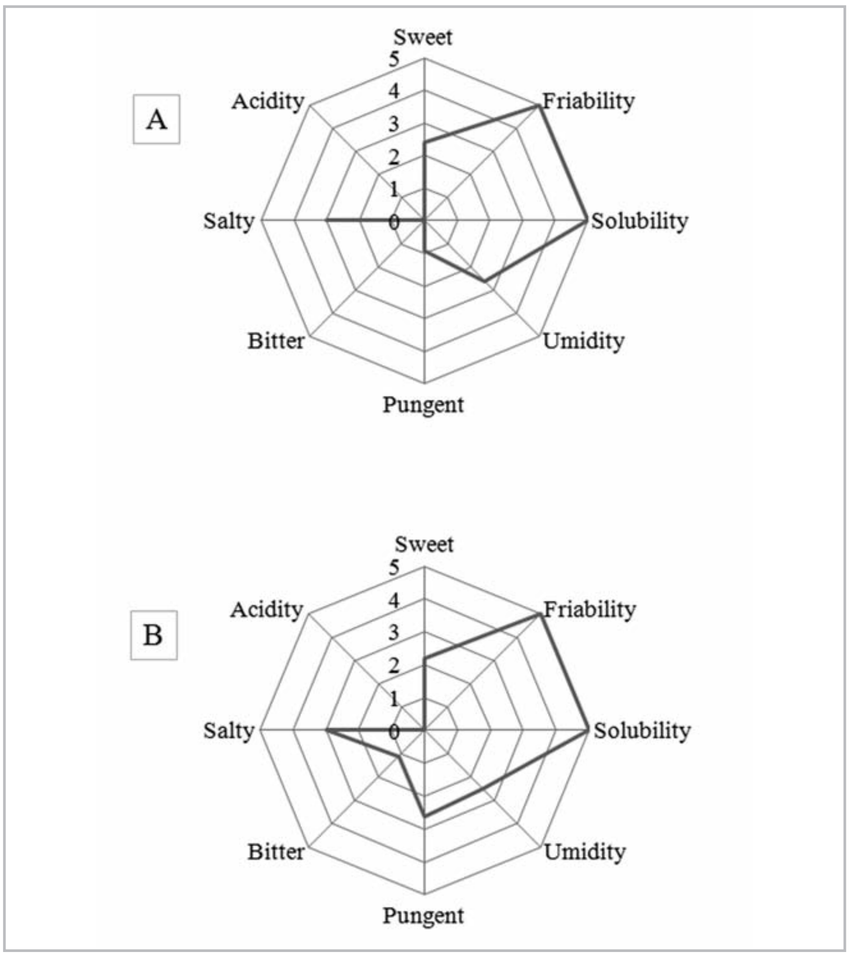
$$b_{ij} = (n-g)\sum_{l=1}^{q} w_{il}^{*}\overline{X_{lj}}$$

$i = 1, 2, \ldots, q$; $j = 1, 2, \ldots, g$
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 p_j 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 rennets, using amino acid data set. 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 (12.732) had a Wilks Λ value close to zero (0.073).
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 with pig rennet and cheese samples with calf rennet, respectively, were clearly distinct.

Figure 4 - Graph A related to a sample obtained with pig rennet with oil and vinegar with a ripening time of 9 months and graph B related to a sample obtained with calf rennet with oil and vinegar with a ripening of 9 months



Based on the values for the two linear discriminant functions for each sample, the group membership could be predicted using a classification rule. In this case, all cheese samples were correctly assigned to the group they belong to. Furthermore the overall classification success was 100.0%.

According to Wilks Λ value another distribution was quite significant. In fact, if the data set regarding the free amino acid content is analysed as a function of treatments, the results obtained are the following. 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 (7.030) had a Wilks Λ value close to zero (0.125).

The distribution of data expressed as discriminant scores along the first eigenvector is presented in Figure 6. In this representation of all data, the two sample classes, corresponding to cheese samples treated and untreated, 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. In this case, all cheese samples were correctly assigned to the group they belong to. The overall classification success was 100%.

A significant Wilks Λ value was obtained when the cheese samples were classified as a function of the rennets and of the treatment, using amino acid data set. In this case, 3 discriminant functions were estimated, since the number of groups in this sample was 4, and 4-1 is the maximum allowable number of eigenvalues for the matrix $W^{-1}B$. The first discriminant eigenvalue (27.724) had a Wilks Λ value close to zero (0.002).

The distribution of data expressed as discriminant scores along the first eigenvector is presented in Figure 7. In this representation of all data, the four sample classes, corresponding to cheese samples with treated pig rennet (1), untreated pig rennet (2), treated calf rennet (3) and untreated calf rennet (4), respectively, were clearly distinct.

Based on the values for the four linear discriminant functions for each sample, the group membership could be predicted using a classification rule. In this case, all cheese samples were correctly assigned to the group they belong to. Furthermore the overall classification success was 100.0%.

The same methods of elaboration

Figure 5 - The distribution of data regarding the free amino acid content, related to cheese samples as function of the two rennets, expressed as discriminant scores along the first eigenvector

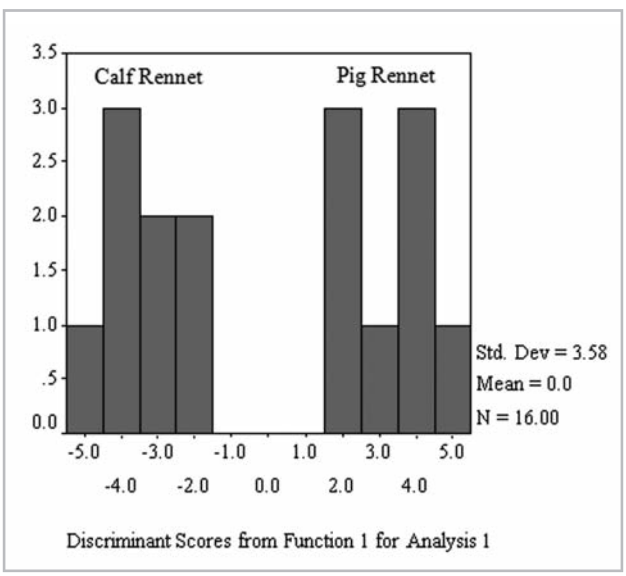


Figure 6 - The distribution of data regarding the free amino acid content, related to cheese samples as function of the treatments, expressed as discriminant scores along the first eigenvector

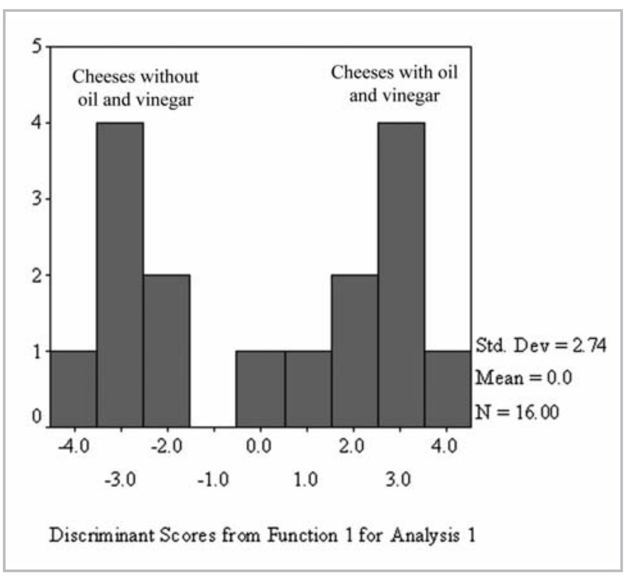
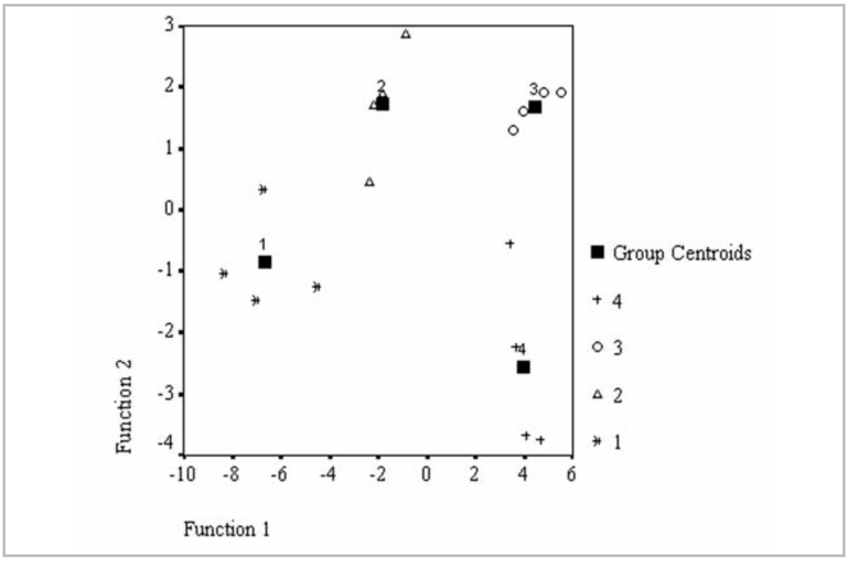


Figure 7 - The distribution of data regarding the free amino acid content, related to cheese samples as function of rennets and treatments, expressed as discriminant scores along the first two eigenvectors (1 = pig rennet with oil and vinegar; 2 = pig rennet without oil and vinegar; 3 = calf rennet with oil and vinegar; 4 = calf rennet without oil and vinegar)



were used on the data set relating to the content of free fatty acids, and the results obtained are reported in Figures 8, 9 and 10.
The cheese samples, as was to be expected, are correctly classified, showing an overall classification success of 100% in function of the ripening time, utilizing the data on both free amino acids as well as that on free fatty acids
According to Wilks Λ value another distribution was quite significant. In fact, if the whole data set is analysed as a function of Panel test, the results obtained are the following. In this case, 3 discriminant functions were estimated, since the number of groups in this sample was 4, and 4-1 is the maximum allowable number of eigenvalues for the matrix $W^{-1}B$. The first discriminant eigenvalue (3.603) had a Wilks Λ value close to zero (0.030).
The distribution of data expressed as discriminant scores along the first eigenvector is presented in Figure 11. In this representation of all data, the four sample classes, corresponding to cheese samples with treated pig rennet (1), untreated pig rennet (2), treated calf rennet (3) and untreated calf rennet (4), respectively, were distinct.
Based on the values for the four linear discriminant functions for each sample, the group membership could be predicted using a classification rule. In this case, all cheese samples were correctly assigned to the group they belong to. The overall classification success was 93.8%.

Conclusion

The production of both free amino acids and free fatty acids is consistently lower when pig rennet is used in the production of cheese. This indicates that pig rennet has less and lipolytic and proteolytic activity. These differences also have an influence on the organoleptic characteristics of cheese.
In fact, the Panel Test found that the ewe cheese made with calf rennet is consistently more spicy ("piccante" flavour) and bitter than the Farindola one made with pig rennet, whereas this latter is always sweeter and never bitter. These characteristics remain the same even when the ripening is extended (up to 12 months).
This can be explained by the well-known fact that pre-gastric esterase is responsible for the extensive lipolysis which results in the characteristic "piccante" flavour of some cheeses (29-30). This "piccante" flavour is due primarily to the release of short chain FFAs ,

Figure 8 - The distribution of data regarding the free fatty acid content, related to cheese samples as function of the two rennets, expressed as discriminant scores along the first eigenvector. 100.0% of original grouped cases correctly classified.

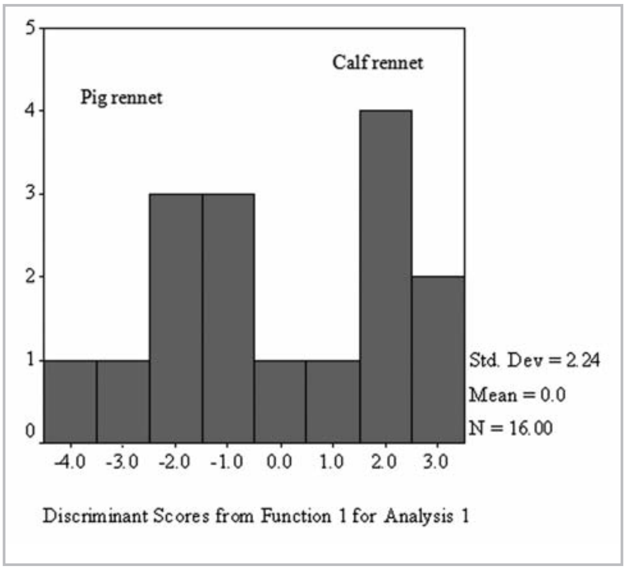


Figure 9 - The distribution of data regarding the free fatty acid content, related to cheese samples as function of the treatments, expressed as discriminant scores along the first eigenvector. 93.8% of original grouped cases correctly classified.

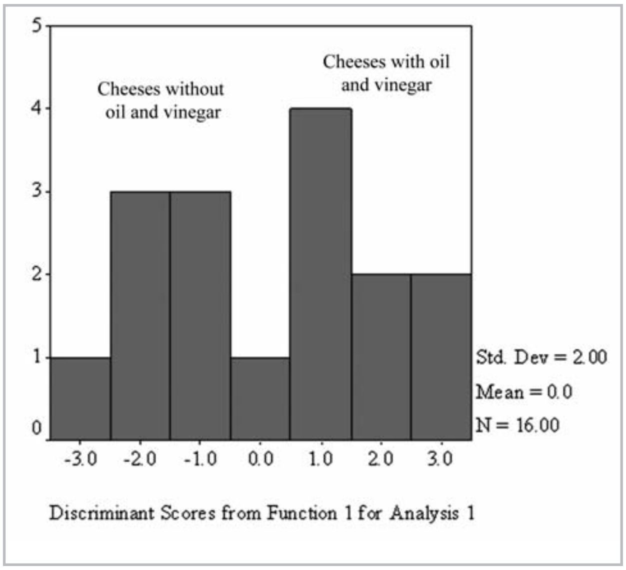


Figure 10 - The distribution of data regarding the free fatty acid content, related to cheese samples as function of rennets and treatments, expressed as discriminant scores along the first two eigenvectors (1 = pig rennet with oil and vinegar; 2 = pig rennet without oil and vinegar; 3 = calf rennet with oil and vinegar; 4 = calf rennet without oil and vinegar). 100.0% of original grouped cases correctly classified

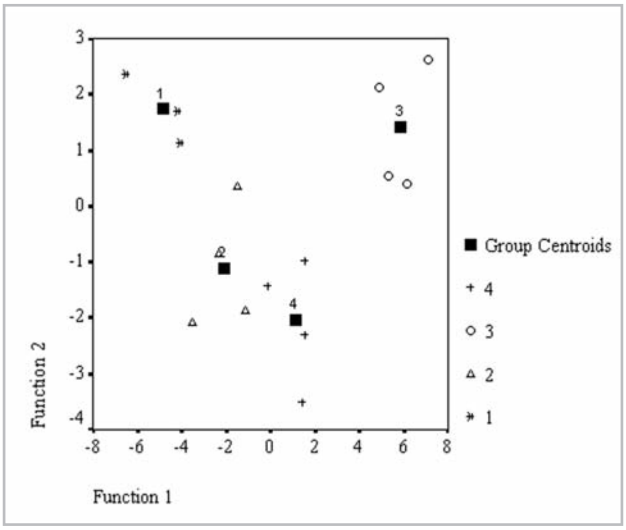
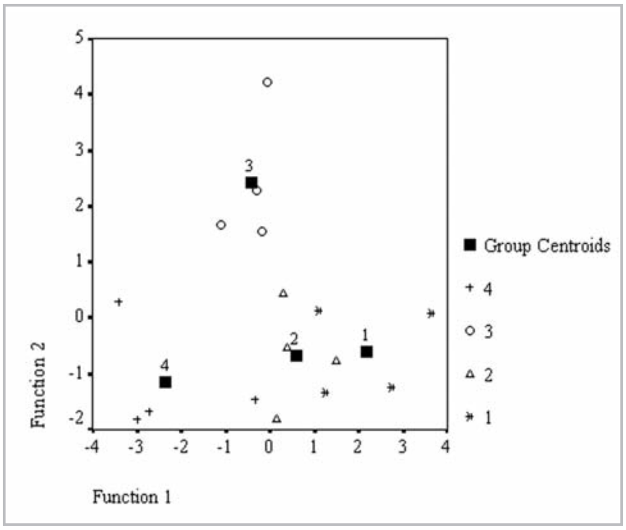


Figure 11 - Discrimination of cheeses obtained with different rennets and treatements, as a function of Panel Test, expressed as discriminant scores along the first two eigenvectors (1 = pig rennet with oil and vinegar; 2 = pig rennet without oil and vinegar; 3 = calf rennet with oil and vinegar; 4 = calf rennet without oil and vinegar).



i.e. $C_{4:0}$ to $C_{10:0}$ (31). Long chain FFA plays a minor role in cheese flavor, whereas short chain has a considerably lower perception threshold and gives a characteristic flavour note: for example butanoic acid contributes "rancid" and "cheesy" flavour, hexanoic acid has a "pungent" flavor note, and so on. The more extensive proteolysis also explains the bitter taste. In fact, as it is known, a positive correlation was found between b-casein degradation and the bitter taste (32) and the main peptides responsible for the bitter taste in cheese appear to be those corresponding to the C-terminal portion of b-casein (33- 34). Other authors have attributed bitter flavor in cheese to the concentration of arginine (35).

These characteristics of Farindola ewe Cheese, which make it sweeter and less spicy and bitter than cheeses made with calf rennet, explain its prestige and popularity with consumers. This performance confirms widely what is reported by Pasta et al. (36). These researches have been demonstrated that food linked to the biodiversity is better evaluated than industrial products thank to their qualitative components that are immediately recognizable by the consumers, independently from the presence of external cues.

References

1. Merker HM. Pepsin versus rennet in cheese making. Journal of Dairy Science 1919; II (6): 482-6.
2. Schirone M, Tofalo R, Mazzone G, Corsetti A, Suzzi G. Biogenic amine content and microbiological profile of Pecorino di Farindola cheese. Food Microbiology 2011; 28 (1): 128-36.
3. Dalgleish DG. The enzymatic coagulation of milk. In Cheese: Chemistry, Physics and Microbiology, Vol. I, General Aspects. Edited by Fox, 1999: Aspen Publication, Maryland, Second Edition.
4. Fox PF. Milk-clotting and proteolytic activities of rennet, and of bovine pepsin and porcine pepsin. Journal of Dairy Science 1969; 36: 427-33.
5. Foltmann B, Jensen AL, Lonblad P. A developmental analysis of the production of chymosin and pepsin in pigs. Comp Biochem Physiol 1981; 68B (1): 9-13.
6. Krause VW, Partzsch M, Hassan ZME. Zur hydrolyse von aS-casein durch aspartatproteasen aus milchgerinnungsenzympräparaten. Milchwissenschaft 1996; 51 (4): 205-9.
7. Green ML. Assessment of swine, bovine and chicken pepsin as rennet substitutes of Cheddar cheese-making. Journal of Dairy Science 1972; 39: 261-73.
8. Attallah AG. Characters of Chymosin gene Isolated from Different Animal Sources at molecular level. J Applied Sci Res 2007; 3 (9): 904-7.
9. Larráyoz P, Martímez MT, Barrón LJR, Torre P, Barcina, Y. The evolution of free fatty acids during the ripening of Idiazábal cheese: influence of rennet type. Eur Food Res Technol 1999; 210: 9-12.
10. Vicente MS, Ibáñez FC, Barcina Y, Barron LJR. Changes in the free amino acid content during ripening of Idiazabal cheese: influence of starter and rennet type. Food Chemistry 2001; 72: 309-317.
11. Bustamante MA, Virto M, Aramburu M, et al. Lamb rennet paste in ovine cheese (Idiazabal) manufacture. Proteolysis and relationship between analytical and sensory parameters. International Dairy Journal 2003; 13: 547-57.
12. Virto M, Chávarri F, Bustamante MA, et al. Lamb rennet paste in ovine cheese manufacture. Lipolysis and flavour. *I*nternational Dairy Journal 2003; 13: 391-9.
13. Prieto B, Franco I, Fresno JM, Gonzáles Prieto J, Bernardo A, Carballo J. Effect of ripening time and type of rennet (farmhouse rennet from kid or commercial calf) on proteolysis during the ripening of León cow milk cheese. Food Chemistry 2004; 85: 389-98.
14. Yvon M, Rijnen L. Cheese flavour formation by amino acid catabolism. International Dairy Journal 2001; 11: 185-201.
15. Barlow I, Lloyd GT, Ramshaw EH, Miller AJ, McCabe GP, McCabe L. Correlations and changes in flavour and chemical parameters of Cheddar cheeses during maturation. The Australian Journal of Dairy Technology 1989; May: 7-18.
16. Di Giacomo F, Del Signore A, Giaccio M. Farindola Sheep Cheese. J Commodity Sci Technol and Quality 2009; 48 (II): 177-97.
17. Clarke AP, Jandik P, Rocklin RD, Liu Y, Avdalovic N. An Integrated Amperometry Waveform for the direct, sensitive detection of aminoacid and amino sugars following anion exchange chromatography. Analytical Chemistry 1999; 71: 2774-81.
18. Hui JY, Taylor SL. High pressure liquid chromatographic determination of putrefactive amines in foods. JAOAC 1983; 66 (4): 853-7.
19. Mietz JL, Karmas E. Polyamine and histamine content of rockfish, salmon, lobster and shrimp as an indicator of decomposition. JAOAC 1978; 61: 139-45.

20. Bárcenas P, Pérez-Elortondo FJ, Albisu M. Selection and screening of a descriptive panel for ewe's milk sensory profiling. Journal of Sensory Studies 2000; 15 (1): 79-99.
21. Anderson TW. An Introduction to Multivariate Statistical Analysis. New York, NY: John Wiley & Sons, 1984.
22. Lebart L, Morineau A, Warwick KM. Multivariate Descriptive Statistical Analysis, New York, NY: John Wiley & Sons, 1984
23. Mardia KV, Kent JT, Bibby JM. Multivariate analysis, London, UK: Academic Press, 1993.
24. Kubícková J, Grosch W. Evaluation of Flavour Compounds of Camembert Cheese. International Dairy Journal 1998; 8: 11-16.
25. Woo AH, Lindsay RC. Concentrations of major free fatty acids and flavor development in Italian cheese varieties. Journal of Dairy Science 1984; 67: 960-8.
26. Freitas AC, Malcata FX. Lipolysis in Picante cheese: influence of milk type and ripening time on free fatty acid profile. Lait 1998; 78: 251-8.
27. House KA, Acree TE. Sensory impact of free fatty acids on the aroma of a model Cheddar cheese. Food Quality and Preference 2002; 13: 481-88.
28. Collins YF, McSweeney PLH, Wilkinson MG. Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. International Dairy Journal 2003; 13: 841-66.
29. Fox PF, Guinee TP, Cogan TM, McSweeney PLH. Fundamentals of cheese science. Gaithersburg, MD: Aspen Publishers Inc., 2000.
30. McSweeney PLH, Sousa MJ. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Le Lait 2000; 80: 293-324.
31. Nelson JH, Jensen RG, Pitas RE. Pregastric esterase and other oral lipases – a review. Journal of Dairy Science 1977; 60: 327-62.
32. Menéndez S, Centeno JA, Godínez R, Rodríguez-otero JL. Effects of *lactobacillus* strains on the ripening and organoleptic characteristics of Arzúa-Ulloa cheese. Int J Food Microbiology 2000; 59: 37-46.
33. Sullivan JJ, Jago GR. The structure of bitter peptide and their formation from casein. Aust J Daury Technol 1972; 27: 98-105.
34. Visser S, Hup G, Esterkate FA, Stadhouders J. Bitter flavour in cheese. 2. Model studies on the formation and degradation of bitter peptides by proteolytic enzymes from calf rennet, starter cells and starter cell fractions. Neth. Milk Dairy J 1983; 37: 169-82.
35. Barcina Y, Ibáñez FC, Ordóñez AI. Evolution of free amino acids during Idiazábal cheese ripening. Food Control 1995; 6 (3): 161-4.
36. Pasta C, Cortese G, Campo P, Licitra G. I fattori della biodiversità incombono realmente sulle preferenze dei consumatori? Progress in Nutrition 2009; 11 (1): 3-11.