Ann. Fac. Medic. Vet. di Parma (Vol. XXVI, 2006) pag. 145 - pag. 164

PROTEOLYSIS AND LIPOLYSIS OF PARMIGIANO-REGGIANO CHEESE AT DIFFERENT RIPENING PERIODS: 12, 24, 55 AND 96 MONTHS

Massimo Malacarne, Paolo Formaggioni, Piero Franceschi, Andrea Summer, Primo Mariani

Sezione di Scienze Zootecniche e Qualità delle Produzioni Animali,
Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti,
Università degli Studi di Parma, Via del Taglio 8, 43100 Parma, ITALY.
Phone +39 0521 032615; massimo.malacarne@unipr.it

Summary

A characterisation of the ripening phase of Parmigiano-Reggiano cheese has been reported in this note. The trend of physico-chemical characteristics and of indeces descriptive of lipolytic and proteolytic processes of cheese have been illustrated. Data were obtained from 17 wheels of Parmigiano-Reggiano cheese of different age, ranging from the cheeses of 24-48 hours to cheeses of 8 years of ripening (96 months). Proteolysis and lipolysis trends showed distinctive peculiarities throughout ripening. Proteolysis resulted significant in the first 24 months of ripening, particularly in the first 6 months. Beyond two years the proportion of pH 4.6 soluble nitrogen remains unvaried. Lipolysis processes were characterised by a more regular trend: the proportion of free fatty acids (summation of FFA * 100 / cheese fat) increase till the 55th month of ripening. Afterwards, the values of FFA/cheese fat *ratio* was almost constant . Furthermore, a higher degree of lipolysis towards *medium* chain (C10÷C14) and, particularly, short chain (C4÷C8) fatty acids was observed.

Introduction

The ripening of cheese is a complex *phenomenon*, a concatenation of physico-chemical, biochemical and biologic events strictly connected among themselves. During ripening, the curd, characterised by light taste and flavour, undergoes deeper changes until it assumes the typical rheological and sensory characteristics of the final product. The whole process of ripening consists essentially in an enzymatic "digestion" of the curd. The substrate is represented by the casein, but the process involves also the soluble components (sugar, lactic acid, citric acid, *etc.*) and, according to cheese variety, lipids. The physico-chemical and biochemical modifications evolve according to the characteristics of the substrate and to the nature of the numerous agents responsible for the transformation, and in relation to a great variety of primary and secondary products that originate throughout ripening, to their interaction and synergism. Essentially, these deeper changes consist in the loss of moisture, the fermentation of lactose, the partial metabolisation of the lactic acid and the citric acid, the more or less intense solubilisation of the casein and intermediate products, the hydrolysis of fat and the formation of the cheese rind. The main agents responsible for transformation (glicolysis, proteolysis, lipolysis) are represented by the milk enzymes, the starter, rennet preparation or substitutive, polluting microflora, secondary starter cultures used in specific dairy productions. The determinism of biotransformations is under the influence of several physico-chemical conditions which are able to influence the growth of microorganisms (bacteria, yeasts, mould), their capability to synthesize enzymes and, finally, the enzymatic activity: pH, water activity, redox potential, composition of the soluble phase, temperature, *etc*. The physico-chemical structure of the curd, of the cheese mass, exert a primary role with reference to the state and degree of dispersion of bounded water, properties of the casein *reticulum*, structure of the paracasein, physical state of fat, size of fat globules and degree of integrity of milk fat globules membrane, *etc*.

The ripening of Parmigiano-Reggiano cheese take e *minimum* period of 12 months, but, in most cases, it last until 24 months. This period represents the basic characterising element of the cheese, imparting its peculiar qualities. In fact, during this phase the cheese develops its typical flavour and aroma properties. This is related to the enzymatic activities present in the cheese which are related to the peculiarities of the cheesemaking process, such as the use of raw milk, the addition of natural whey starter culture and the use of calf rennet. The intensity and the specificity of the enzymatic action seems to be strictly conditioned by the cheesemaking technology. The higher cooking temperature and the dimension of the cheese are able to determine the formation of a temperature gradient, which decrease from the inner to outer side of the cheese mass, and which remains for about 24 hours after the extraction of the cheese mass from the vat. This temperature gradient affects the development of microbial populations and, consequently, of their connected enzymatic activities, with important repercussions on the evolution of glicolytic and proteolytic processes in the outer and in the inner zones of the cheese during ripening. On the various

"external" factors that are able to affect the processes of cheese ripening, previous research has underlined the significant role exert by the season of production.

Nowadays, the main research concerning the ripening of Parmigiano-Reggiano cheese were focused on the commercial ripening product, *e.g.* 24 months. Conversely, scarce information on cheese ripened over a period longer than 24 months are reported.

In this note, the ripening of Parmigiano-Reggiano cheese will be illustrated by the examination of some physico-chemical parameters and of some indeces descriptive of the protelytic and the lipolytic processes. The data showed were obtained by analysis of 17 cheese wheels of different age, ranging from extraction from the vat (0 months) to 96 months (8 years of ripening).

Materials and methods

The research was carried out on 17 Parmigiano-Reggiano cheese wheels produced in 12 cheese factories distributed in the provinces of Parma, Reggio-Emilia, Modena and Mantova.

The cheese wheels were selected according to their age, to cover to whole ripening cycle of Parmigiano-Reggiano cheese. In particular, 4 cheese wheels (one sample for season of production) were selected for each of the following age of ripening: 1, 12 and 24 months . Furthermore, 2 cheeses of 24-48 hours (defined as cheese mass at extraction from the vat), 2 cheeses of 55 months and 1 cheese of 96 months were sampled. For each cheese, the agreement to structural standard requirements was verified. Cheese was sampled according to standard FIL-IDF methods [1].

On cheese samples the following analyses were performed: pH with potentiometer; moisture by drying the sample at 102°C [2]; fat according to Gerber method modified by Siegfeld [3]; sodium chloride by titration with $AgNO_3$ [4]; for total nitrogen determination the Kjeldahl method was used; nitrogen soluble at pH 4.6 (SN), 12% trichloroacetic acid-soluble nitrogen (TCASN) and 5% phosphotungstic acid-soluble nitrogen (PTASN) were separated using the procedure proposed by Gripon *et al.* [5] and determined by the Kjeldahl method; ammonia nitrogen (NNH_3) according to Savini [3]. From nitrogen fractions, the values relative to the components of high molecular weight (Peptones N = NS - TCASN) and peptides of low molecular weight (Peptides N = TCASN - PTASN - NNH_3) were obtained. Free fatty acids were determined according to De Jong and Bandings [6] by means of capillary gas chromatography.

The data collected were subject to univariate ANOVA (SPSS 14.1, Chicago, IL), considering as a fixed factor the effect of the age of cheese (6 levels: 0, 1, 12, 24, 55 and 96 months of ripening).

Results and discussion

Physico-chemical characteristics of Parmigiano-Reggiano cheeses of different age - 0 (corresponding to the extraction of the cheese mass from the vat), 1, 12, 24, 55 and 96 months of ripening - are reported in table 1.

Table 1:
Basic composition of Parmigiano-Reggiano cheese at different age of ripening. Mean value±SD.
Tabella 1:
Composizione di base del formaggio Parmigiano-Reggiano a differente età di stagionatura. Media±DS.

Months of ripening *Mesi di stagionatura*	0(1)	1	12	24	55	96
No. of cheeses *N. di formaggi*	2	4	4	4	2	1
Moisture (g/100g) *Umidità*	39.30±0.44	37.00±0.49	33.25±0.65	31.06±0.67	26.40±2.33	25.10
Dry matter (g/100g) *Sostanza secca*	60.70±0.44	63.00±0.49	66.77±0.65	68.94±0.67	73.60±2.55	74.90
Fat (g/100g) *Grasso*	27.45±0.21	27.30±0.50	28.87±0.69	30.49±1.01	32.47±3.00	28.43
Protein (g/100g) *Proteina*	29.50±0.69	30.89±0.61	31.47±0.77	32.65±0.96	34.66±1.14	40.92
NaCl (g/100g)	0.04±0.02	0.97±0.19	1.36±0.22	1.48±0.32	1.64±0.08	1.97
NaCl/Moisture (g/100g) *NaCl/Acqua*	0.10±0.05	2.64±0.52	4.11±0.67	4.80±1.12	6.26±0.92	7.84
pH	n.d.	5.32±0.02	5.36±0.02	5.36±0.05	5.22±0.01	5.34

(1) Cheese mass extract from the vat (24-48 hours); *Massa caseosa estratta dalla caldaia (24-48 ore)*
n.d. not determined; *n.d. non determinato*

Moisture content of cheese decreased significantly from the extraction of the cheese mass from the vat (39.30 g/100g) to 96 months ripened cheese (25.10 g/100g). An accentuated decrease of cheese moisture was observed during the first 12 months of ripening; in this period the loss of 6.05 g/100g of moisture was registered ($P<0.05$), corresponding to 43% of the whole cheese moisture variation from 0 to 96 months of age. In the following 12 months of ripening the loss of moisture resulted less accentuated and corresponded to 2.19 g/100g (15% of 0-96 months variation; $P<0.05$). From the cheeses of 24 months to those of 55 months, the moisture value recorded a further drop ($P<0.05$), corresponding to 4.66 g/100g (33% of the whole variation). No significant differences ($P>0.05$) of cheese moisture were observed between 55 months ripened cheeses and 96 months ripened cheese.

In general, the content of cheese protein and fat has underlined an increasing trend as the age of cheese increased. This trend has to be related to the corresponding variations of cheese moisture. The protein content is varied between 29.50 g/100g as

observed in the cheese mass after the extraction from the vat to 40.92 g/100g reported for the 96 months ripened cheese ($P<0.05$). Concerning the cheese fat, the increase of its content with cheese age was observed until 55 months of ripening ($P<0.05$). On the other hand, the 96 months ripened cheese (data obtained from a single cheese sample) showed a fat value (28.43 g/100g) clearly lower than that observed in 55 months ripened cheeses ($P<0.05$) but no different ($P>0.05$) with respect to 0-1-12 and 24 months ripened cheeses (27.24-27.30-28.87 and 30.49 g/100g).

The content of sodium chloride (NaCl) is reported both on 100 g of cheese and on 100 g of cheese moisture. This latter value represents an indication of the saltiness of the aqueous phase in which cheese enzymes are present. These enzymes are involved in the chemical and biochemical reactions that are the basis of cheese ripening. The content of NaCl on 100 g of cheese moisture is markedly increased ($P<0.05$) from the extraction of the cheese mass from the vat (0.10 g/100g) to one month ripened cheese (2.64 g/100g), in correspondence with the end of the brining phase. Even if less accentuated, a clear increase of salt to cheese moisture was observed as cheese age increased: in 12, 24, 55 and 96 months ripened cheeses, it reached values, respectively, of 4.11-4.80-6.26 and 7.84 g/100g of cheese moisture. This trend is related to the loss of moisture during the ripening of cheese.

The pH values of cheeses varied significantly according to cheese age ($P<0.05$). An increase of pH values was observed from 1 to 12 months ripened cheeses (5.32 *vs* 5.36). No differences ($P>0.05$) were registered between 12 and 24 months ripened cheeses. Then, after 55 months of ripening, the pH value of cheese (5.22) was clearly lower ($P<0.05$) than in 24 months ripened cheese. Ninety-six months ripened cheese showed a pH value (5.34) markedly higher ($P<0.05$) than 55 months ripened cheese. The increase of pH values in the firsts months of ripening was reported in other studies carried out on hard and semi-hard cheeses, such as Cheddar, Gouda and Gruyére, ranging from 5 to 6 months of age [7, 8]. According to McSweeney and Fox [9], pH tended to increase during ripening because of the formation of alcaline nitrogen compounds (*e.g.* ammonia) and the catabolism of lactic acid. Zapparoli and Dugoni [10], in a study carried out on 60 Parmigiano-Reggiano cheese wheels of different age, had reported a decrease of cheese pH after 9 months of ripening They concluded that this was probably due to the accumulation of free fatty acids during ripening. In our study, the decrease of cheese pH was observed after 24 months of ripening. Actually, as reported in the follows paragraphs, proteolysis, in general, and the production of ammonia, in particular, seems to stop after the 24th month of ripening, while the production of free fatty acids continue progressively until the 55th month of ripening.

Proteolysis

Proteolysis is a basilar *phenomenon*, which is able to characterise the whole ripening process of cheese [11-14]. The biochemical reactions of proteolysis, which, starting from the curd, deeply modify the casein *reticulum*, is determined by an enzymatic system composed of proteases of different origin: milk (plasmin), rennet (chymosin), milk natural microflora (proteases from mesophilic lactic bacteria) and whey starter bacteria (proteases from termophilic lactic bacteria). The cheese proteolysis is based essentially on enzymatic processes and its evolution depend on the action and interaction of those factors which are able to influence the specific enzymatic activities that take place during the whole ripening, such as the moisture content of cheese, pH values, salt content, free fatty acids, temperature, *etc*. The degradation of protein does not proceed necessarily from high to low molecular weight substances. The first step concerns the break down of single caseins by endopeptidase with the formation of small units (peptides of elevated molecular weight) which are, however, insoluble at pH 4.6, as the undigested casein [15]. These peptides are the substrate of esopeptidase, carboxipeptidases and aminopeptidases leading to the formation of medium-low molecular weight peptides and free aminoacids, all entities soluble at pH 4.6 [16].

a) *Ripening index* - The ripening index is represented by the percentage of pH 4.6 soluble nitrogen on the total nitrogen of cheese. This index is descriptive of the proportion of casein which is progressively digested by proteolytic enzymes. The values of the ripening index of Parmigiano-Reggiano cheeses of different age - 0, 1, 12, 24, 55 and 96 months of ripening - are reported in table 2.

Table 2:
Ripening index and soluble nitrogen composition of Parmigiano-Reggiano cheese at different age of ripening. Mean value±SD.
Tabella 2:
Coefficiente di maturazione e composizione dell'azoto solubile del formaggio Parmigiano-Reggiano a differente età di stagionatura. Media±DS.

Months of ripening *Mesi di stagionatura*	0[1]	1	12	24	55	96
No. of cheeses *N. di formaggi*	2	4	4	4	2	1
Ripening index[2] *Coefficiente di maturazione*[2]	5.10±0.18	9.16±1.34	26.47±2.50	33.22±0.61	33.55±1.65	35.90
pH 4.6 soluble nitrogen composition (%) *Composizione dell'azoto solubile a pH 4,6 (%)*						
Peptones N/SN *N peptoni/NS*	51.05±2.08	26.48±5.33	9.33±1.17	10.65±1.37	10.23±1.71	10.12
Peptides N/SN *N peptidi/NS*	21.67±9.85	22.34±2.75	14.91±5.87	17.34±4.09	15.66±3.21	18.80
AA N/SN *N aa/NS*	24.74±7.79	47.65±5.10	69.04±5.37	63.70±3.00	66.03±3.33	62.14
NH_3 N/SN *N NH_3/SN*	2.54±0.03	3.53±0.70	6.72±1.26	8.30±1.00	8.07±1.60	8.94

SN = pH 4.6 soluble nitrogen; *NS = Azoto solubile a pH 4,6*
AA N = free amino acids nitrogen; *N aa = azoto degli amminoacidi liberi*
NH_3 N = ammonia nitrogen; *N NH_3 = azoto ammoniacale*
(1) Cheese mass extract from the vat (24-48 hours); *Massa caseosa estratta dalla caldaia (24-48 ore)*
(2) SN *100/Total nitrogen - *NS *100/Azoto totale*

At the extraction of the cheese mass from the vat, 5% of cheese casein was solubilised. The degree of casein solubilisation increased progressively with cheese age (P<0.05). The values of the ripening index were 9.16-26.47-33.22-33.55 and 35.90%, respectively, in 1-12-24-55 and 96 months ripened cheeses. The solubilisation of casein was particularly remarkable during the first period of ripening, *e.g.* from the extraction of the cheese mass from the vat to 12 month aged cheeses (+21.37 percentage unit, P<0.05). Then, from 12 to 24 months ripened cheeses, the degradation of casein resulted as reduced in intensity (+6.75 percentage unit; P<0.05). After 2 years of ripening (24 months) no significant variation (P>0.05) of the ripening index was observed. When compared to shorter ripening cheeses, ranging from some months to one year, such as Emmental, Gruyère, Sbrinz, *etc.*, Parmigiano-Reggiano cheese had showed an intense solubilisation of casein, comparable to that in Emmental cheese one, but lower than that reported for an Asiago cheese, a semi-hard cheese [17, 18].

b) *Composition of pH 4.6 soluble nitrogen* - The composition of pH 4.6 soluble nitrogen of Parmigiano-Reggiano cheeses of different age - 0, 1, 12, 24, 55 and 96 months of ripening - is reported in table 2.

The proportion of peptones - among soluble nitrogen fractions, the primary substrate of the proteolytic activity - was decreased throughout the first 12 months of ripening, from a value of 51.05% in the cheese mass, to a value of 9.33% in 12 months ripened cheese (P<0.05). No further variations (P>0.05) of the peptone pro-

portion were observed in 24, 55 and 96 months ripened cheeses.

On the contrary, the proportion of phosphotungstic acid (PTA) soluble nitrogen - which represent the free amino acids contents of cheese - showed a sudden increase in the first month of ripening ($P<0.05$), from a value of 24.74% in the cheese mass extracted from the vat, to a value of 47.65% in one month ripened cheese. A further increase ($P<0.05$) of PTA nitrogen proportion was recorded between 1 and 12 months ripened cheese (+21.39 percentage unit). No differences ($P>0.05$) of PTA nitrogen proportion were registered among 12, 24, 55 and 96 months ripened cheeses. Actually, free amino acids are the final products of the proteolytic processes and, consequently, they accumulate during the ripening of cheese.

No significant variations of the peptide proportion was reported amongst cheese of different age (average value 18.34%; $P>0.05$). Actually, peptides represents an intermediate product of the proteolytic process: they are produced from the break down of peptones and are hydrolysed to produce smaller peptides and free amino acids. Amino acids and peptides affect sensory, nutritional and biological function properties of cheese. They represent over the 4/5 of the whole soluble nitrogen of commercial ripened cheese (24 months).

The proportion of ammonia nitrogen was increased progressively during the first 12 months of ripening, from 2.54 to 6.72%, respectively, in 0 (cheese mass extraction from the vat) and 12 months ripened cheeses. No variations ($P>0.05$) were observed among 12-24-55 and 96 months ripened cheeses (6.72-8.30-8.07 and 8.94%).

In conclusion, the ripening "cycle" of Parmigiano-Reggiano cheese is characterised by a relevant solubilisation of the casein, with deep modifications of the rheological properties of the cheese. This *phenomenon* concurs to define the peculiar organoleptic attributes of commercial ripened cheese (24 months). The proteolysis of casein - mainly due to natural milk enzymes [16] - was particularly accentuated during the first 6 months of ripening, whereas it decreased in intensity during the intermediate and, above all, final phases of ripening. Proteolytic degradation of intermediate substrate and production of free aminoacids, with the contribution of bacterial proteases [19], results earlier and just as intense. From the third month of ripening, in fact, most of cheese soluble nitrogen is represented by amino acids and smaller peptides. However, after a deep modification in the first phase of ripening, from the 6th month of ripening an equilibrium among peptones, peptides, amino acids in the soluble nitrogen of cheese was observed. Ammonia nitrogen is the final product of nitrogen catabolism and its content is increased progressively until the 24th month of ripening. After this period, no further variation of ammonia nitrogen were reported.

Lipolysis

The hydrolysis of lipids does not affect the rheological and textural properties of cheese. However, it plays a relevant role in the development of cheese flavour. This phenomenon is much more marked in blue and in soft cheeses, while it is considered of moderate entity in hard cheeses [15]. The biochemical agents responsibile for lipolysis are essentially represented by lipases. Besides the lipoprotein lipase of milk (LPL, which is less active at pH below 6.5), lipases produced by several micro-

organisms, such as those from mould and micrococci [15], seem to play a key role in cheese lipolysis throughout the ripening process. LPL is denatured by the effect of milk pasteurisation (above 60°C). On the contrary, most of microorganism lipases are still active in milk pasteurised at 76°C [15]. The specificity of the lipolysis is strictly dependent on the cheesemaking technology [20]. Moreover, it may vary in intensity in relation to several factors, such as the enzyme concentration, temperature, water activity, salt content, *etc*. However, as opposed to proteolysis, lipolysis should not be inhibited significantly by the salt content of cheese. In general, lipolysis is influenced by storage conditions and by all those operations that interfere with cheese ripening [21]. During Parmigiano-Reggiano cheese ripening lipolytic enzymes, particularly those of bacterial origin [22], produce variable amounts of free fatty acids, which are subjected to oxidative degradation and form low molecular weight carbonilic compounds characterised by certain aromatic properties [23], particularly if derived from insaturated fatty acids. Besides this, cheese fat, by means of direct and derived products of lipolysis contribute to the rheological properties and concur in the formation of organoleptic and flavour characteristics of cheese as well, either in medium and in long ripened cheeses; minor or not well defined should be their role in the formation of cheese taste. However, free fatty acids seems to exert a meaningful role in the definition taste in some cheeses like Gouda, Gruyére de Comté and Emmental.

a) *Lipolysis index* - The progress of lipolysis during Parmigiano-Reggiano cheese ripening was analysed considering the *ratio* between the content of free fatty acids and that of cheese fat (lipolysis index). The values of the lipolysis index of Parmigiano-Reggiano cheese of different age - 0, 1, 12, 24, 55 and 96 months of ripening - are reported in table 3.

Table 3:
Lipolysis index and free fatty acids composition of Parmigiano-Reggiano cheese at different age of ripening. Mean value±SD.
Tabella 3:
Indice di lipolisi e composizione degli acidi grassi liberi del formaggio Parmigiano-Reggiano a differente età di stagionatura. Media±DS.

Months of ripening *Mesi di stagionatura*	1	12	24	55	96
No. of cheeses *N. di formaggi*	4	4	4	2	1
Lipolysis index(1) *Indice di lipolisi(1)*	0.75±0.20	2.35±1.00	4.15±0.99	7.84±2.19	8.80
Free fatty acids composition (%) *Composizione degli acidi grassi liberi (%)*					
C4÷C8	1.58±0.98	5.12±2.00	6.01±1.12	10.84±4.13	14.83
C10÷C14	10.17±1.44	13.29±1.62	13.67±1.85	18.22±5.44	23.72
C16÷C18	88.25±0.56	81.58±3.02	80.31±2.58	70.93±9.58	61.44

(1) Free fatty acids content* 100/cheese fat; *Contenuto degli acidi grassi liberi*100/grasso del formaggio*

During the first two years of ripening, free fatty acids had represented a higher proportion of fat as cheese age increased ($P<0.05$), reaching values of 0.75-2.35-and 4.15% in 1-12 and 24 month ripened cheese, respectively. Fifty-five months ripened cheese was characterised by a lipolytic index (7.84%) higher than the value observed in 24 months ripened cheese ($P<0.05$). The value reported for 96 months ripened cheese was not different ($P>0.05$) with respect to 55 months ripened cheese. Trends and values observed here agree with the results of previous studies [24]

b) *Free fatty acids composition* - The composition of free fatty acids of Parmigiano-Reggiano cheese of different age - 0, 1, 12, 24, 55 and 96 months of ripening - is reported in table 3.

Single free fatty acids were grouped according to their number of carbon atoms: short chain fatty acids, C4÷C8, *medium* chain fatty acids C10÷C14, long chain fatty acids C16÷C18.

As cheese ripening progressed, a sensible increase in the proportion of short chain free fatty acids was observed ($P<0.05$). In fact, the values observed in 1-12-24-55 and 96 months ripened cheeses were 1.58-5.12-6.01-10.84 and 14.84%, respectively.

Even the proportion of *medium* chain free fatty acids registered an increased with cheese age ($P<0.05$). The values reported in 1-12-24-55 and 96 months ripened cheese were 10.17-13.29-13.67-18.22 and 23.72%, respectively.

Conclusion

The trends of the proteolytic and lipolytic showed distinctive peculiarities during Parmigiano-Reggiano cheese ripening.

The cheese proteolysis resulted significant in the first 24 months of ripening and, particularly, in the first 6 months. After 2 years of ripening, no more solubilisation of casein was observed, consequently, the proportion of 4.6 pH soluble nitrogen tends to be constant. From a qualitative point of view, amino acids and peptides represent about 4/5 of the soluble nitrogen of the commercial ripened cheese (24 months). Both fractions concur, in a significant measure at the determination of organoleptic, nutritional and biological properties of cheese.

On the other hand, cheese lipolysis was characterised by a more regular trend: the proportion of free fatty acid on cheese fat increased progressively until 55 months, then it tended to stabilise. A higher degree of lipolysis toward short fatty acids (C4÷C8) than *medium* (C10÷C14), and, particularly, than long chain fatty acids (C16÷C18), was observed during ripening.

Differences observed between the trends of the two enzymatic processes are probably related to the different activity that the respective group of enzymes show in relation to physico-chemical variation of the cheese paste throughout ripening. In this concern, a main role is exert by the salt content of cheese. The activity of proteolytic enzymes, in fact, is negatively affected by the increase the salt concentration of the water.

Our results clearly showed that the cheese fat undergoes important modifications even after 24 months of ripening. This is an "objective" data that allows for a differentation of long ripened (>24 months) from commercial ripened (≤24 months) cheeses from a chemical point of view.

References

1. Emmons D.B. (1999) Sampling and analysis. Practical Guide for control of cheese yield, s.i. 0001, 74-86. International Dairy Federation, Brussels, Belgium.
2. Norma internazionale FIL-IDF 4/A:1982 Fromages e fromages fondus - Détermination de l'extrait sec totale.
3. Savini E. (1946) Analisi del latte e dei latticini. Ed. Hoepli, Milano.
4. Norma internazionale FIL-IDF 88/A:1988 Fromages e fromages fondus - Détermination de la teneur en chlorures (méthode par titrage potentiometrique).
5. Gripon J.C., Desmazeaud M.J., Le Bars D., Bergère J.L. (1975) Étude du rôle des micro-organismes et des enzymes au cours de la maturation des fromages. II. Influence de la présure commerciale. Lait, 55: 502-516.
6. De Jong C, Bandings HT (1990). Determination of free fatty acids in milk and cheese: procedure for extraction, clean up and capillary gas chromatographic analysis. J. High Resolution Chromatography, 13, 94-98.
7. Zerfiridis G.K., Vafopoulou-Mastrogiannaki A., Litopoulou-Tzanetaki E. (1994) Ripening changes of Gruyère cheese. J Dairy Sci, 67: 1397-1405.
8. Lawrence R.C., Creamer L.K., Gilles J. (1987) Texture development during cheese ripening. J Dairy Sci, 70: 1748-1760.
9. McSweeney P.L.H., Fox P.F. (1993) Cheese: methods of chemical analysis. In: Cheese: chemistry, physics and microbiology, Ed. Fox P.F., second edition, Vol 2, 341-388. Chapman & Hall, London, UK.

10. Zapparoli G.A., Dugoni F. (1997) Quadro analitico di acidi grassi valotali, pH ed umidità in formaggio Grana "scelto" da 1 a 20 mesi di stagionatura. Sci Tecn Latt-Cas, 48: 297-304.
11. Desmazeaud J.M., Gripon J.C. (1977) General mechanism of protein breakdown during cheese ripening. Milchwissenschaft, 32: 731-734.
12. Pélissier J.-P. (1984) Protéolyse des caséines. Sciences des aliments, *4*: 1-35.
13. Grappin R., Rank T.C., Olson N.F. (1985) Primary proteolysis of cheese proteins during ripening: a review. J Dairy Sci, 68: 531-540.
14. Fox P.F. (1989) Proteolysis during cheese manufacture and ripening. J Dairy Sci, 72: 1379-1400.
15. Alais C. (1984) Scienza del latte. Ed. Tecniche Nuove, Milano, Italy.
16. Addeo F., Moio L., Stingo C. (1988) Caratteri tipici della proteolisi nel formaggio Parmigiano-Reggiano: composizione della frazione caseinica. Proceedings: Giornata studio composizione e caratteristiche del Parmigiano-Reggiano, pp. 21-40. Ed. Consorzio Form. Parmigiano-Reggiano, Reggio Emilia, Italy.
17. Panari G., Mongardi M., Nanni M. (1988) Determinazione con metodi chimici delle frazioni azotate del Parmigiano-Reggiano. Proceedings: Giornata studio composizione e caratteristiche Parmigiano-Reggiano, pp. 85-95. Ed. Consorzio Form. Parmigiano-Reggiano, Reggio Emilia, Italy.
18. Bütikofer U., Rüegg M., Ardö Y. (1993) Determination of nitrogen fractions in cheese: evaluation of a collaborative study. Lebensm.-Wiss. u.-Technol., 26: 271-275.
19. Bottazzi V. (1993) I batteri lattici nella maturazione del formaggio Grana. L'Industria del Latte, 29(2): 73-88.
20. Benassi R. (1964) Sui processi lipolitici del formaggio. Il Latte, 38: 335-338.
21. Collins Y.F., McSweeney P.L.H., Wilkinson M.G. (2003) Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. Int Dairy