

ORIGINAL RESEARCH PAPER

RESEARCH REGARDING ACCELERATED RIPENING OF PASTA FILATA CHEESES

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Received on 2 July 2011

Revised on 26 July 2012

The effect of adding exogenous enzymes to pasta filata cheeses was studied as a method of proteolysis' acceleration during ripening.

After stretching the curd, before being turned in forms, this was divided into four portions. Three commercial proteolytic and lipolytic enzymes (Accelase® AM250 M, Promod™215P and Lipomod™166P) were added to the first three portions of stretched cheese, and the last one was left without treatment and served as a control. Pasta filata cheese samples were taken periodically, when fresh and after 8, 15, 30 days of ripening, for analysis. The changes in titratable acidity, pH value, dry matter, content of salt and moisture, proteic content as well as the dynamics of proteolysis were studied.

Examination of the data revealed that the addition of enzymes had no significant effect on dry matter, fat, total nitrogen, salt contents of obtained cheeses. The exceptions were the titratable acidity and the content of dry matter, respectively humidity.

An intensification of proteolysis in samples with enzymes added, unlike the witness sample, has been observed, the nitrogen components in cheese and the proteolytic index presenting higher values than the witness sample. Thus, different evolutions have been observed, confirming the acceleration of the cheese ripening.

Keywords: Pasta Filata Cheese, exogenous enzymes, proteolysis, accelerated ripening

Introduction

Cheese ripening, one of the most complex phenomenon in food biochemistry, is basically about the breakdown of proteins, lipids and carbohydrates which releases flavour compounds and modifies cheese texture. The biochemical and biophysical processes involved have only partly been elucidated. The process of ripening is a long lasting process, thus expensive, since the financial expenses for the raw

material purchase, processing and treatments during ripening are refunded after a relatively long time. This fact is the reason of the interest in the methods for acceleration of cheese ripening, yet maintaining comparable organoleptic and physico-chemical properties of the final product (Kujawski *et al.*, 2003).

The first reasons for developing systems of accelerating cheese ripening have been focused on shortening the time of maturation and thereby reduce storage costs.

Acceleration of the ripening step of cheese production is an area of scientist and commercial interest. Accelerating ripening allows producers to reduce ripening space requirements and the financial cost of maintaining a large stock of cheese for long periods. This topic has been reviewed periodically (Fox *et al.*, 1996; El Soda *et al.*, 2000; Wilkinson & Kilcawley, 2005).

Different methods are known for intensification of proteolysis during cheese ripening like: elevated ripening temperatures, addition of enzymes, addition of cheese slurry, attenuated starters, use of shocked cells, use of cell-free extracts of some cheese-related microorganisms, genetically engineered starters and recombinant enzymes and microencapsulation of ripening enzymes, addition of slurry ripening system or free amino acids, the use of different packaging materials, high pressure application to cheese curds are traditional and modern methods used to accelerate cheese ripening (Fernandez-Espla and Fox, 1998; Saldo *et al.*, 2002; Borda, 2007; El-Hofi, 2010). The advantages, limitations, technical feasibility and commercial potential of these methods are to be discussed and compared (Azarnia. *et al.*, 2006).

The objectives of this study were to investigate the effects of adding exogenous enzymes during the ripening period of the pasta filata cheese by evaluating proteolytic process of the samples in which these enzymes have been added, in comparison with a sample that was not added exogenous enzymes.

Materials and methods

Materials

Cheese milk

Fresh whole cows' milk used in the present study was collected from an acquisition centers located in Bradet, Arges county.

Cheese starter culture

The starter culture FD–DVS Lh–B02 which contains pure species of *Lactobacillus helveticus*, purchased from Chr. Hansen, has been used. The addition of starter culture was carried out after cooling pasteurized milk in an inoculation doze of 50 U/500 liters of milk.

Rennet

Fromase 2200TL microbial cheese rennet from *Rhizomucor miehei* was used to coagulate the milk. This has been produced by Seclin, France and was purchased from Chr. Hansen.

Exogenous enzymes

For the acceleration of ripening, were added of different types of enzymes - AccelaseAM250^M, PromodTM215P and LipomodTM166P.

Experimental procedure

Pasta filata cheese manufacture

The method of manufacturing cheese is well-known and will be only briefly introduced so that the use of the experiments and their results can be better understood.

Pasta Filata Cheese was produced from cow milk with 3.5% fat, pasteurised at 72⁰C for 15 s and cooled to 34⁰C, using a semicontinuous line, in a dairy plant at Bradet Arges. Calcium chloride solution, coagulating enzyme Fromase and starter culture FD–DVS Lh–B02 were added during the cheese production. The amounts of these were to be added after a calculation, made according to the brand and preparation method. So, these were added at a level of 15g CaCl₂/100 l milk, 10U starter culture/100 l milk, 16g Fromase/1000 l milk, and coagulation took place at 32⁰C for 60–90 min.

Following the process of coagulation, the coagulum was cut into pieces and transferred into cotton bags, in perforated moulds, for whey drainage. After drainage, the curd was pressed and left at 20–25⁰C/12–24 hours, to maturate.

The milling was done when the acidity of the curd has reached pH = 5.1–5.3. The blocks are usually cut into strips by using stainless steel knife and then milled into small pieces. Stretching and kneading the curd properly, under hot water at 85–90⁰C, with salt 8–10%, has led to the fuse of the curd particles and to the formation of a smooth texture and body. After proper kneading and stretching, the curd was moulded into wheel-shaped cheese blocks of around 500 g each.

After stretching the curd under hot water, before turning it in forms, these were divided into four portions. Two different type of protease, Accelase and Promod, and a mixture of protease/lipase (Promod, Lipomod) were added at levels recommended by the producer to the first three portions of stretched cheese, and the last one was manufactured without the addition of any protease or lipase to serve as a control sample (Table 1).

The ripening process took place under controlled temperature conditions and possibly under controlled humidity in two stages: firstly at 16±2⁰C for 15 days, and secondly at 12±2⁰C for two more weeks; during storage, the relative humidity was 85%±5.

Methods of cheese chemical analysis

The control sample as well as those of different treatments were analysed at different ripening times, when fresh (before and after being stretched) and after 8, 15 and 30 days of maturation.

Pasta filata cheeses were analyzed for pH values, titratable acidity, protein and fat contents, salt, dry matter/humidity, water activity (a_w). The studies were carried out

trice and processed in the laboratories of ICA Research & Development SRL Bucuresti.

Table 1. Samples codification

Product name	Encoded samples	Type of enzymes added	Dosage gr. enzymes/ 100 kg cheese
the witness sample	M	-	-
the cheese sample with Accelase, proteolytic enzymes addition	A	AccelaseAM250-M	75
the cheese sample with Promod, proteolytic enzymes addition	P	Promod TM 215P	30
the cheese sample with a mixture of Promod and Lipomod, proteolytic and lipolitic enzymes	L	Promod TM 215P/ Lipomod TM 166P	30/6

A part of the samples were chemically analyzed using available Standard methods and others using different methods like described below.

Titrateable acidity ($^{\circ}$ T) was done with Thörner method as described by STAS 6353/85 A and the pH was measured by ExStikTM, waterproof pH Meter (from EXTECH Instruments) by STAS 8201–82. Dry matter content was performed by SR ISO 5534:2004(E) IDF 4:2004(E), fat content by SR ISO 3433:2009 Van Gulik method, sodium chloride by STAS 6354–84 and protein by SR EN 8968/2–2001. The water activity was determined with AWMD-10 from NAGY-Instruments.

Secondary proteolysis was measured by nitrogen fraction in cheese. Total nitrogen content (TN) and soluble nitrogen (SN) were determined according to Kjeldahl's method, by STAS 6355–89 and non-protein nitrogen (NNP) by SR EN ISO 8968–4/2002. Soluble nitrogen in fosfowolframic acid (N_{fws}) was determined also through by Kjeldahl analysis, from filtrate after precipitation of N-components from solution with fosfowolframic acid.

Results and discussion

Results presented in Figures 1–8 indicate that the cheese chemical composition was affected by the exogenous enzymes and/or the ripening period. This may be due to the production of some acidic compounds as a result of the enzyme action, and also due to the probable stimulatory action of some compounds produced by protein hydrolysis. This means that the protein degradation and formation of soluble nitrogenous contents of pasta filata cheese were further enhanced by the addition of different types of proteolytic enzymes (Zaharia and Rotaru, 2011).

The acidity and pH dynamics

Cheese moisture, mineral content, texture and flavour are all influenced directly by the activity of free hydrogen ions (i.e. pH). It must be emphasized that the most important factor available to the cheese maker to control spoilage and pathogenic organisms is pH control. The pH history during and after cheese manufacture is the

most important trouble shooting information. The titratable acidity and pH evolution during the maturation of pasta filata cheese are shown in Figures 1-2.

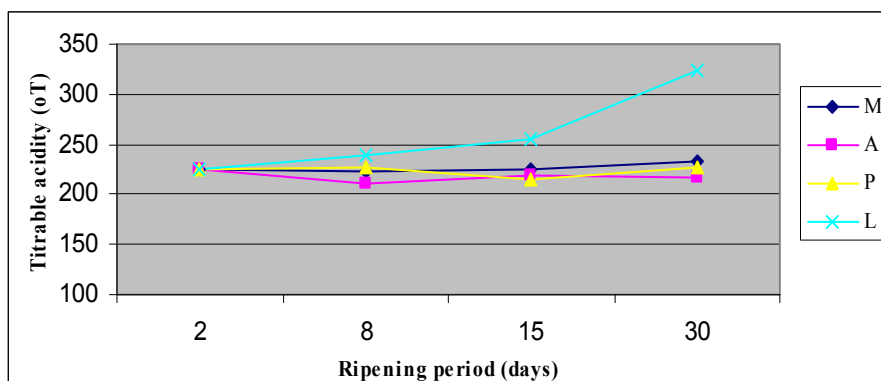


Figure 1. Titratable acidity (TA) of witness and enzymes treated cheese during ripening period

The rate of acid formation, which is a critical factor in the manufacture of cheese, was not affected by the addition of proteolytic enzymes. So, if in the samples M, A, P the titrated acidity stays practically constant, after stretching, until day 30, in the case of sample L acidity increases continuously between 8–15 days and very quickly starting on the 15th day of maturation until the 30th.

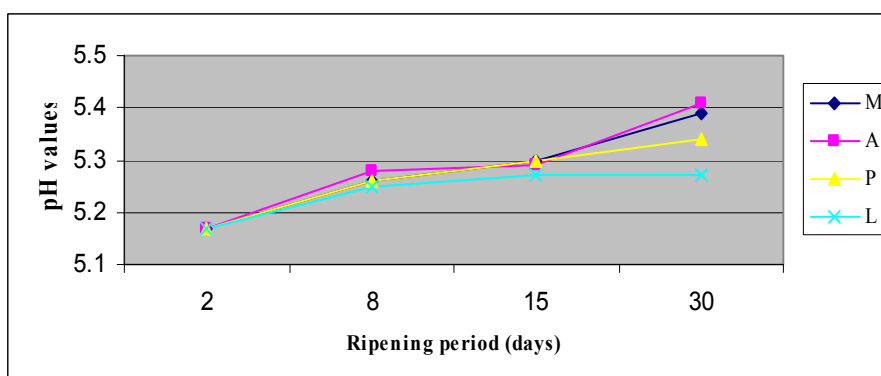


Figure 2. pH values of witness and enzymes treated cheese during ripening period

The changes in pH value prove that the enzymatic degradation occurs during ripening. The cheese pH starts from 5.17 and increases in the first 8 days of ripening up to about 5.25 for all samples. After that moment the evolution was slightly increasing and after 30 days of ripening, the respective pH values were close to 5.4 for samples M and A, 5.34 for P and 5.27 for sample L (Figure 2). This proves that the enzymatic processes were intensified in the samples with enzymes.

No significant differences were found to occur between the pH values of cheeses manufactured with enzymes and mator sample. However, after 30 days, witness sample and probe with Accelase showed a slightly higher pH value (5.40) than cheeses with Promod and Promod and Lipomod.

However, it should be noted that the pH of the cheese is determined not only by the amount of lactic acid produced in situ by the microflora but also by the buffering capacity of the curd, which arises primarily from the quantities of casein and partially soluble anorganic phosphate and citrate (Macedo and Malcata, 1997).

The evolution of the dry matter and fat content

The dry matter of cheese is continuously increasing by 11%, compared to the beginning of maturation, for all samples and reached 59.5%.

After stretching the cheese, a higher moisture content was also observed, which seems to be related to a change in the structure of the paracaseinate network. During ripening, the fat content on a dry matter basis has practically remained constant (50.4%) for all experimental samples.

The activity of water

The water activity of a food refers to this unbound water and is not the same thing as its moisture content. The determination of a_w values of kashkaval may be used for predicting the shelf life, safety, texture, flavour and smell of the product.

During cheese maturation, the water activity (a_w) values decreased, as shown in Table 1, because, through ripening, result various soluble breakdown products of acids, sugars, proteins and lipids.

Table 1. The values of the water activity during ripening period

a_w , %	2 days	8 days	15 days	30 days	60 days
M	0.985	0.965	0.947	0.918	0.909
A	0.985	0.947	0.921	0.907	0.889
P	0.985	0.957	0.932	0.927	0.921
L	0.985	0.959	0.941	0.929	0.926

The water activity is not constant in ripening time. Thus, after stretching, the value of a_w was 0.985 and it decreased continuously until reaching a balance between the surface of the product and the surrounding atmosphere. As it is also mentioned in the speciality literature (<http://www.foodsafetysite.com>) in this study it can be observed that at the same humidity values the water activity is not identical. Also, it is observed that during the maturation period, a decrease in the humidity content by 12–13%, while the water activity presents decreases between 5.6% and a maximum of 8%, proving the fact that the two parameters and their evolution are not identical.

According to Cogan (2000) a depression in a_w during cheese ripening occurs due to water loss by evaporation, salt, and the hydrolysis of proteins to peptides and amino acids and triglyceride to glycerol and fatty acids; the hydrolysis of each

peptide or ester bond requires one water molecule. Since significant proteolysis occurs in cheese, the unbound moisture level will decrease during maturation (Beresford *et al.*, 2001).

The ratio of salt to moisture

Other critical process control parameter is the ratio of salt to moisture (S/M).

As it can be observed from Figure 3, no significant differences were found in the salt-to-moisture ratio for cheeses produced with different enzymes compared to the witness cheese.

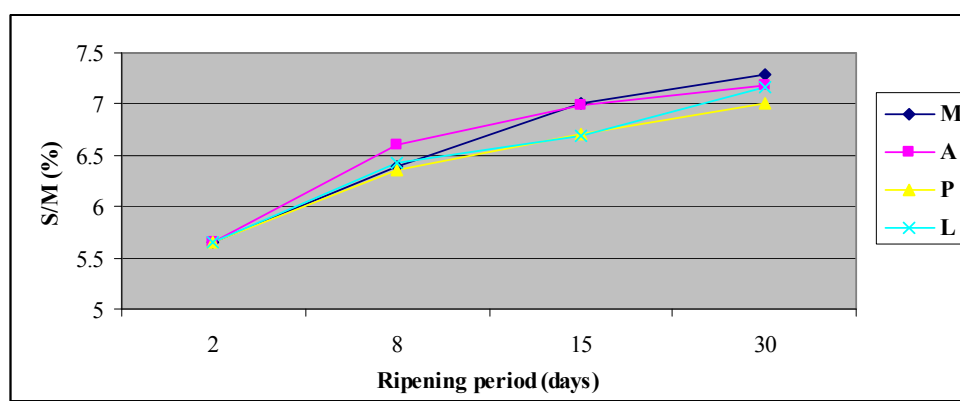


Figure 3. The salt to moisture ratio of witness and enzymes treated cheese during ripening period

The salt-to-moisture ratio increased continuously throughout ripening. A much higher increase of this ratio from 5.64% after stretching, to 6.7–7.0% after 15 days of ripening was observed for all samples. These values are slightly lower than those measured for 30-day-old cheeses (7–7.2%).

The profile of salt-to-moisture concentration versus ripening time can be explained by water evaporation from the surface of the cheese, which accounts for the decreased moisture content.

Salt uptake is affected by the quantity of added salt, size of curds, moisture content of curds, and acidity but it should be said that S/M varies widely even within a single cheese.

Salt and moisture content have a major effect on water activity, and thereby exert control over microbial growth, enzyme activity and biochemical changes during cheese ripening (McSweeney, 2007).

When the results were compared, the same evolution of cheese parameters has been observed, except for titratable acidity and the dry matter content during the ripening and storage period.

The nitrogen fraction dynamics

The degree of paracasein degradation was evaluated by measuring the changes in the content of individual nitrogen forms: total nitrogen, soluble nitrogen, non-protein nitrogen, fosfowolframic and the results being expressed in percentage of the total nitrogen as well.

During maturation, qualitative and quantitative changes of nitrogenous substances occur. The relative proportions of the main components change constantly, both from the total nitrogen and the dry weight basis.

The same forms of the curbs is observed, the total nitrogen having a slightly rising evolution due to the humidity content decrease, approximately identical during the cheese ripening at all four experimental samples (Figure 4).

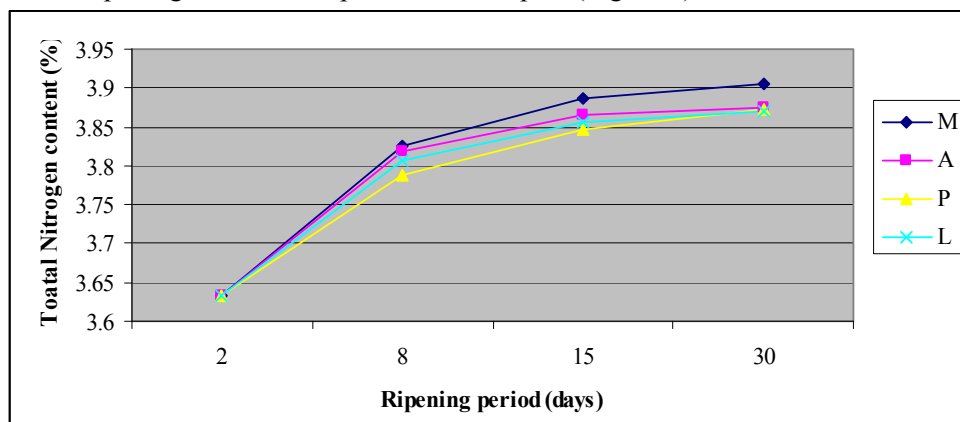


Figure 4. Total nitrogen (TN) content of witness and enzymes treated cheese during ripening period

During ripening, it was observed a continuous increase in the content of soluble nitrogen, being a measure of the ripening intensity (Figure 5).

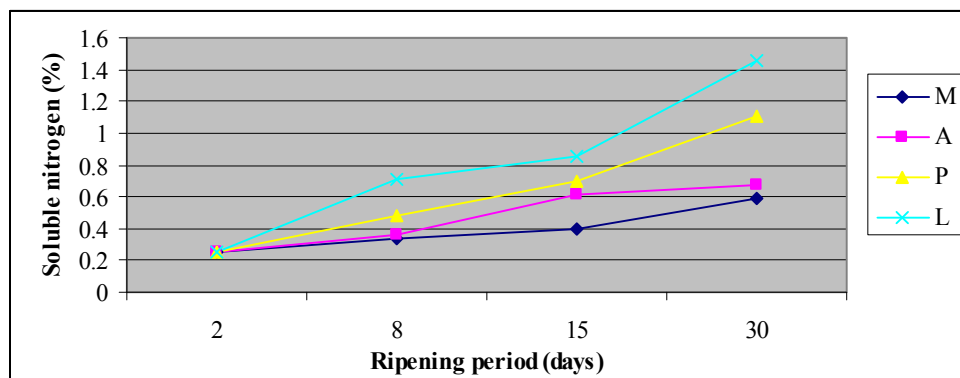


Figure 5. Soluble nitrogen (SN) content of witness and enzymes treated cheese during ripening period

Marking differences during the proteolysis are observed after 30 days of ripening between the two types of cheese, samples P and L which have been examined during this experiment. An emphasis of the proteolysis in sample L has been observed, as a consequence of the synergistic effect of the lipases and proteinases added to cheese. In this sample (L), the soluble nitrogen content, at this moment is of 1.4614% compared to 0.593% (sample M), 0.676% (sample A) and 1.102% (P). In conclusion, it can be said that soluble nitrogen having a constant increase during the observation period, has lower values in the sample M's case compared to the other 3 samples.

The soluble nitrogen in fosfowolframic acids (N_{fws}) normally contains mostly free amino acids and very small peptides (<600Da).

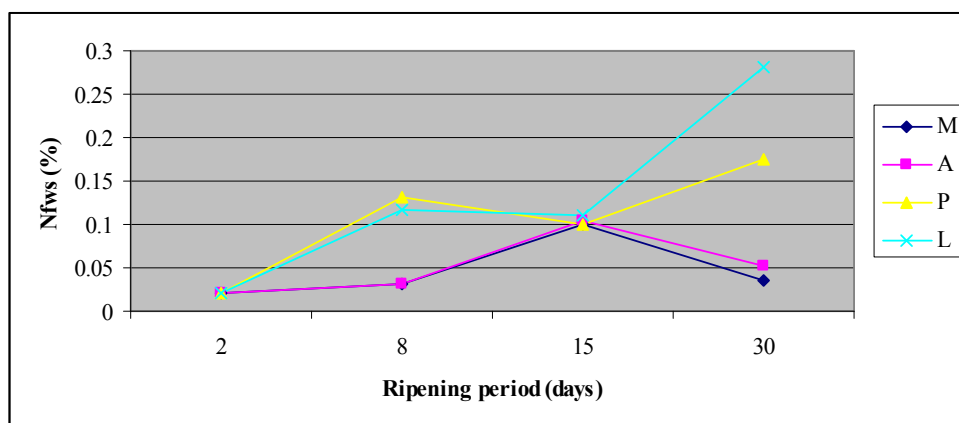


Figure 6. Soluble nitrogen in Fosfowolframic acid (N_{fws}) content of witness and enzymes treated cheese during ripening period

The rate at which these compounds were increasing was quicker until a similar level for all samples was achieved, at 15 days of ripening. After 2 more weeks, the content of soluble nitrogen in fosfowolframic acids for the control cheese and the cheese containing Accelase has decreased, most probably, because of degradation of amino acids through decarboxylation, deamination and desulfuration (Figure 6), unlike the other 2 samples, which had an ascendant evolution. At 30 days of maturation, large differences in amounts of fosfowolframic solubil nitrogen occurred in cheese containing Promod or Accelase.

The percentage content of soluble nitrogen compounds expressed in per cent of total nitrogen (SN/TN) increased throughout the whole period of ripening, as it can be observed in Figure 7.

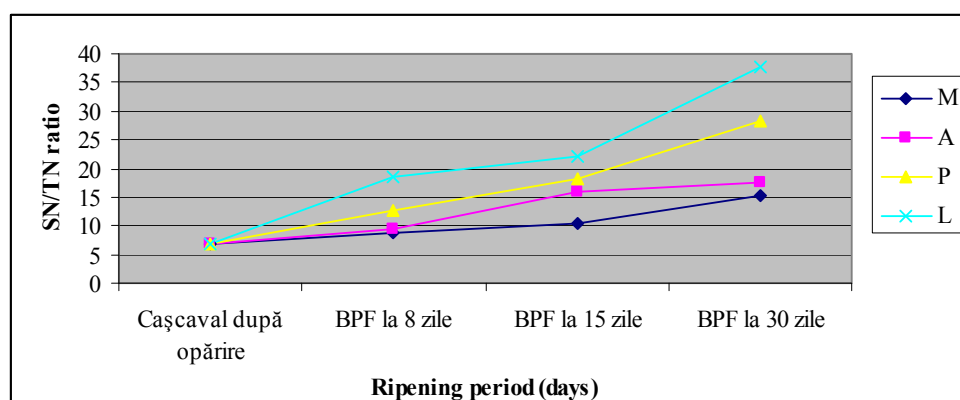


Figure 7. Soluble nitrogen as a percentage of total nitrogen (SN/TN) content of witness and enzymes treated cheese during ripening period

The ratio SN/TN increased from 6.915%, after stretching, to 8.916% after 8 days of ripening for M sample and from 6.915% to 9.312% for A, whereas it increased from the same value to 12.699% for P and to 18.538% for L sample. The last values for P and L are specific to matured cheeses, but the taste was bad.

The M and A samples showed slower rate of increase in low molecular weight compounds and their maximum content after 30 days of ripening NS/NT was 15.181% for M and 17.449% for A cheese approximately as much as the values determined at day 15 of ripening.

After just 15 days of ripening, sample A was superior to the witness sample as it had an adequate taste and flavour (acceptable acidity).

Another way for evaluating the proteolysis is to appreciate the evolution of the non-protein nitrogen as a percentage of the total nitrogen (NPN/TN) content during the ripening period, and used as an index of ripening. The closer the ratio is to 100%, the more advanced the proteolysis is (Costin, 2003).

Just like the level of soluble nitrogen (SN) after day 30, the non-protein nitrogen (NPN) content or ratio (NPN/NT) in the control cheese were lower than those of treated cheeses, as shown in Figure 8.

If at 15 days of maturation the levels of non-protein nitrogen is almost the same for samples with exogenous enzymes, at 30 days a significant difference was observed between samples P, L, which presented a much higher level than the contents from samples M and A indicating the fact that for cheese with Promod and Lipomod mixture added, the ripening is more advanced. This aspect can also be deduced from the shape of the curbs (Figure 8).

The greatest increases of the ratio non protein nitrogen as a percentage of the total nitrogen NPN/TN, which corresponded to a final value of 27.3% occurred in L cheese. Differences in the levels of NPN/TN between the cheeses were significant; the values of this ratio are of 20.1% for P sample, 13% for A sample and 7.5% in witness sample after 30 days of maturation.

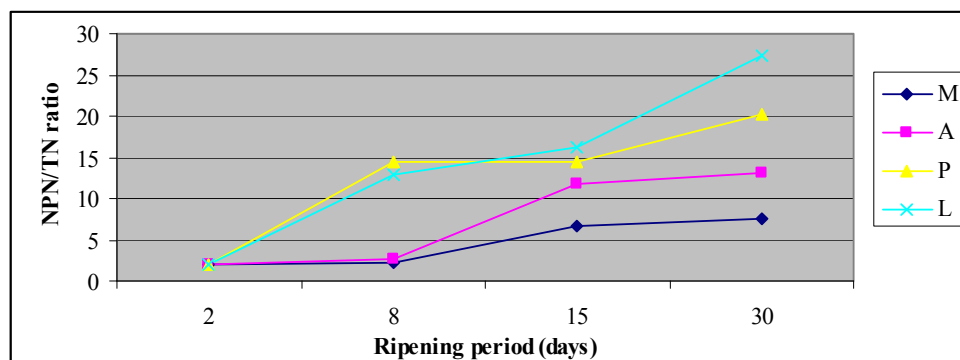


Figure 8. Non protein nitrogen as a percentage of total nitrogen (NPN/TN) content of witness and enzymes treated cheese during ripening period

The experimental cheeses with Accelase, Promod and mixture of Promod and Lipomod contained higher levels of nitrogen fractions acid than in the control cheese, at all maturation stages.

Although the cheese containing enzyme Protease or a mixture of Promod and Lipomod had an atypical flavour, the addition of Accelase reduced from 30 to 15 days the ripening period of pasta filata cheese, without the appearance of off flavour.

Conclusions

The levels of fat and protein were essentially similar in all cheeses.

The mean total solids content of pasta filata cheese increased from 51.35% to 59.5% during 30 days of ripening. As a result, moisture decreased, the concentration of other milk components increased proportionately. The mean salt to moisture content increased from 5.6% to about 7–7.2% after 30 days of ripening. By examining the effect of adding different enzymes on the maturation time of pasta filata cheeses it has been reported that the used proteolytic/lipolytic enzymes had positive effects on the maturation time. As a result, cheese has matured in shorter time comparing to the traditional one.

Out of the four types of used enzymes, of different sources, one has corresponded to the goal pursued. Thus:

- In the case of the sample that has been added Accelase, by using enzymes, the acceleration of the pasta filata cheese maturation process has been successful; some optimal sensorial characteristics of colour, taste, flavour, structure, consistency have also been obtained. A good quality Pasta Filata Cheese could be produced, with a high acceptability, when Accelase was used at level of 0.75 g/kg of cheese and the cheese was ripened for 15 days only. So, a maturation accelerated by 50% was observed, and excellent qualities in cheeses were obtained.
- In the case of samples which have been added Promod, respectively a mixture of Promod and Lipomod, a more accentuated reduction of the

maturation period has been realised but, pasta filata cheeses with flaws in taste and flavour have been obtained.

- Even though the maturation parameters suggest the obtaining of a matured product for all the experimental samples with addition of enzymes, in a time shorter compared to the witness sample, the sensorial analysis has demonstrated that not all of these cheeses can be commercialised, due to the unpleasant alterations of taste, smell and even consistency in some cases. This represents a disadvantage of the results obtained in the research.

In the case of adding a mixture of lipases and proteinases, increased proteolysis has been observed, compared to the one found using only proteinases, suggesting a possible synergetic effect. The same results were obtained by Lin, Jen, Roberts, Milliken (1987) and Kheadr E., Vuillemand, El Deeb (2003) who analysed the impact of different enzymes on Cheddar cheese ripening (Wilkinson, 2005).

In the case of exogenous enzymes added in order to accelerate ripening, as solutions, by spraying, no data exists regarding the factors influencing their distribution or the retention of the enzymes in the cheese. The efficacy of enzyme treatment depends upon the degree of diffusion, which is influenced by the size of cheese, probably temperature, and the proteolytic specificity of the enzymes. On the other hand researches should be continued in order to find the optimum quantities of enzymes and to evaluate the residual activity of the added enzymes that will ensure a reduction in the levels of bitterness, enhance specific flavour, and finally the quality of cheese.

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