STUDIES ON ACCELERATION OF PENTELEU PASTA FILATA CHEESE RIPENING BY EXOGENES ENZYMES

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Abstract. *Cheese is the most valuable dairy product, enjoyed more or less by all the people in the world. Pasta Filata type cheeses make up the world's second largest manufacturated cheese segment after Cheddar type cheeses. Ripening is a slow and consequently an expensive process that is not fully predictable or controllable. The ripening process of most hard cheeses 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 reason of the interest in the methods for acceleration of cheeseripening, yet maintaining comparable organoleptic and physico-chemical properties of the final product.The selective methods of accelerating ripening cheese by addition of enzymes are in the center of these tests. The objectives of this study were to investigate the effects of adding exogenes enzymes on the ripening period of the pasta filata cheese – type Penteleu - by evaluating proteolitic process of samples obtained by adding different enzymes, in comparison with a sample that was not added exogenes enzymes.*

Keywords: pasta filata cheese, accelerated ripening, enzymes, proteolysis.

INTRODUCTION

Cheese ripening, one of the most complex phenomenons 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.

Acceleration of the ripening step of cheese production is an area of scientic 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 (El Soda&Pandian, 1991; Wilkinson&Fox, 1993; Fox et al., 1996).

Different methods are known for intensification of proteolysis during cheese ripening like: elevated ripening temperatures, addition of enzymes, addition of cheese slurry, attenuated starters, adjunct cultures, 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. The advantages, limitations, technical feasibility and commercial potential of these methods are to be discussed and compared.

For reduction of ripening time, flavour enrichment, a better scent (more persistent and characteristic) an addition of enzymes was used – proteinases and lipases – and the effects of these on the ripening period of the pasta filata cheese by evaluating proteolysis, lipolysis and development of microstructure was investigated. Due to its complexity, proteolysis cannot be described by only one index.

MATERIAL AND METHOD

Penteleu Pasta Filata Cheese was produced from cow milk with 3.7% fat, pasteurised at 72 °C for 15 s and cooled to 34 °C, using a small-scale cheesemaking equipment, in the ICA Research Pilot Plant and, using a semicontinuous line, in a dairy plant Lactate Bradet at Bradet Arges. Calcium chloride solution, coagulating enzyme Fromase and DVS starter culture DI-PROX LH1 and FD – DVS LH-B02 (mixture of *Lactobacillus helveticus* and *Lactobacillus lactis* lyophilises) 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. 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 85-90^{0}C, with salt 8-10%, has lead 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) was added at levels recomanded 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.

The ripening process took place in two stage: first at 18^{0}C for 15 days, and second after packing the cheese in thermoshrikable foil, at 12^{0}C for another 2 weeks.

The samples were encoded as follows:

- M – the witness sample
- A – the cheese sample with Accelase, proteolitic enzymes addition
- P – the cheese sample with Promod addition
- L – the cheese sample with a mixture of Promod and Lipomod, proteolitic and lipolitic enzymes addition.

The control sample as well as those of different treatments were analysed for chemical and organoleptic properties at different ripening times, when fresh (before and after stretchhed) and after 8, 15 and 30 days of maturation/storage and were encoded with index 1,2,8,15 respectiv 30 for each samples M, A, P, L.

The fabrication step		Encode
Before stretching		M1, A1, P1, L1
After stretching		M2, A2, P2, L2
Maturation, days	8	M3, A3, P3, L3
	15	M4, A4, P4, L4
	30	M5, A5, P5, L5

Quantitative determination of main constituents – fat, moisture content, salt concentration, dry matter, titrable acidity, pH were performed separately. When the results were compared, the same evolution of these parameters has been observed, except for titrable acidity and the moisture content on the ripening and storage period. The cheese has been analysed for protein breakdown at the same intervals as above by determining the nitrogenuous fractions using Kjeldhal analysis and different maturation indices like: total and soluble nitrogen, non protein nitrogen contents, ration soluble N/total N, non protein nitrogen as a percentage of total nitrogen were determined.

RESULTS AND DISCUSSION

Results presented in Figure (1-10) indicate that the cheese chemical composition was affected by the exogenes 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 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 Penteleu cheese were further enhanced by the addition of different type of exogenous proteolitic enzymes.

The pH and acidity 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.

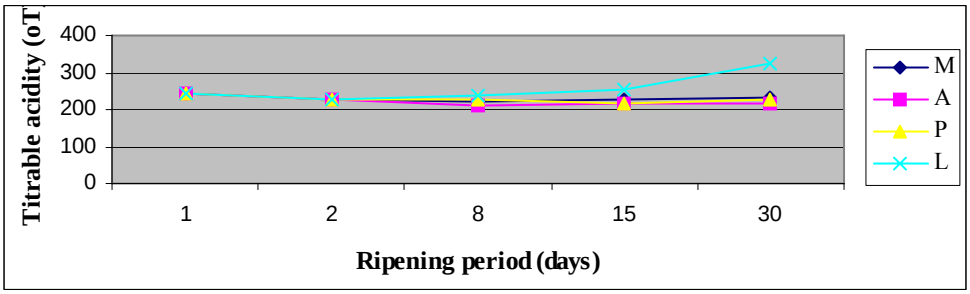

Fig. 1. Titrable acidity (TA) of witness and enzymes treated cheese during ripening period

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 continuous between 8-15 days and very quickly starting by the 15th day of maturation until the 30th.

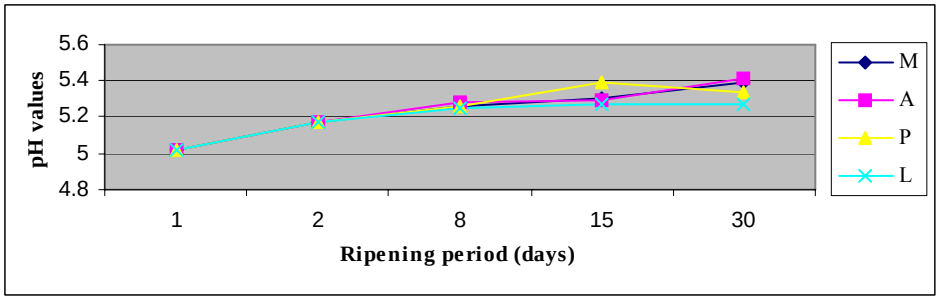

Fig. 2. pH values of witness and enzymes treated cheese during ripening period

The changes in pH value prove that the enzymatic degradation occurs during ripening. A clear relationship has been observed between the P and L samples and the acidity of M witness cheese. Despite similar acidity of cheeses after stretching (the pH value was the same 5.17), the ion hydrogen concentration was the lowest for L cheese and as it can be seen in table 1, after 8 days of maturation, the value remaining at the same level(5.25-5.27) until day 30. This valoue is corelated with the titrable acidity raise. Changes in pasta filata cheese acidity (pH) depending on type of enzymes. An instantaneous increase in pH cheese after stretch treatment has been related to the release of colloidal calcium phosphate into the aqueous phase of the cheese.

The evolution of moisture

After stretching the cheese, a higher moisture content was also observed, which seems to be related to a change in the structure of the para-caseinate network. Salt diffusion in stretched cheese may lead to a higher moisture content.

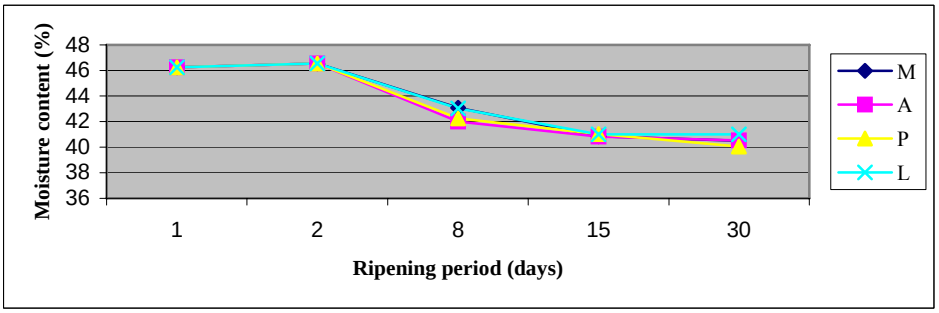

Fig. 3. Moisture content of witness and enzymes treated cheese during ripening period

The moisture content is continuously decreasing in the first 15 days by 12%, comparing to the maturation beginning, and reached 41% . In this moment the cheese was packed in thermoshrikable foil and the moisture constantly maintained until day 30.

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 and the results being expressed in percentage of the total nitrogen as well.

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

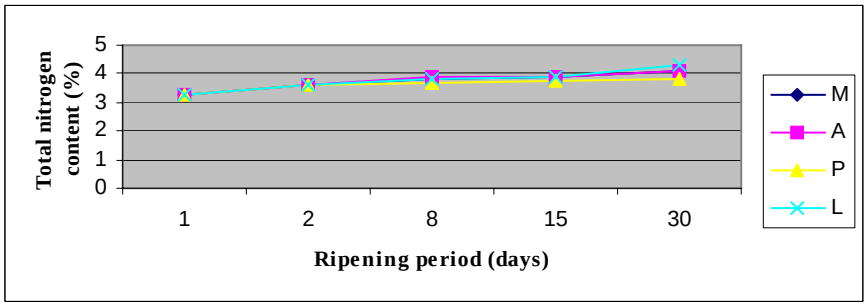

Fig. 4. Total nitrogen (TN) content of witness and enzymes treated cheese during ripening period

It is observed that the increase in total N is much lower during ripening of Penteleu Cheese in sample P, than in cheese with added enzimes and the witness sample. The greatest differences were observed between the contents of soluble nitrogen after 30 days of ripening. The average content of these compounds was by 36.4% higher for A than M, 61% for P, being respectively 70.57% for L.

The percentage content of soluble nitrogen compounds expressed in per cent of total nitrogen N_t increased from 6.915% after stretched to 8.916% after 8 days ripening for M sample and from 6.915% to 9.074 for A, whereas it increased from the same value to 18.465% for P and to 18.538% for L. The last values for P and L are specifical maturated 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 were 10.539% N_t for M and 16.552% N_t for A cheese aproximately as much as the registrated values at day 15 of ripening.

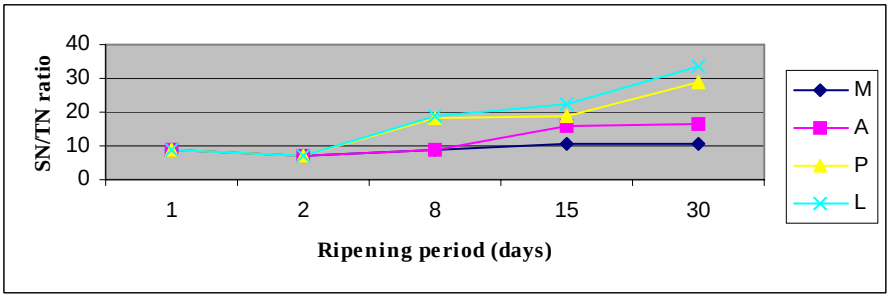

Fig. 5. Soluble nitrogen as a percentage of total nitrogen (SN/TN) content of witness and enzymes treated cheese during ripening period

Just after 15 days of ripening sample A was more superior as it had an acceptable taste and flavour (acceptable acidity).

Just like the level of soluble nitrogen (SN) after day 30, the non protein nitrogen (NPN) content or (NPN/NT) in the control cheese were lower than those of treated cheeses, as shown in Figure 6.

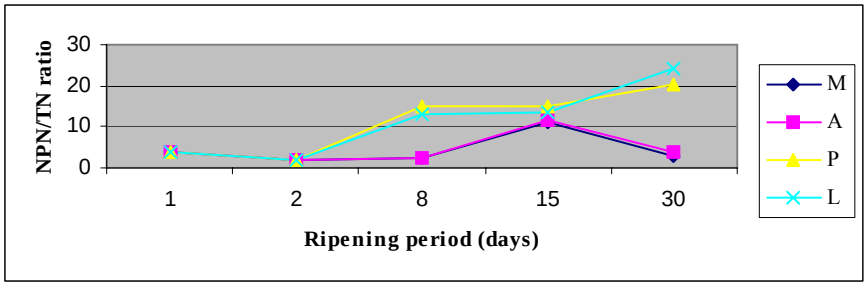

Fig. 6. Non protein nitrogen as a percentage of total nitrogen (NPN/TN) content of witness and enzymes treated cheese during ripening period

If at 15 days of maturation the levels of non protein nitrogen is aprovimative the same for all samples, 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 .

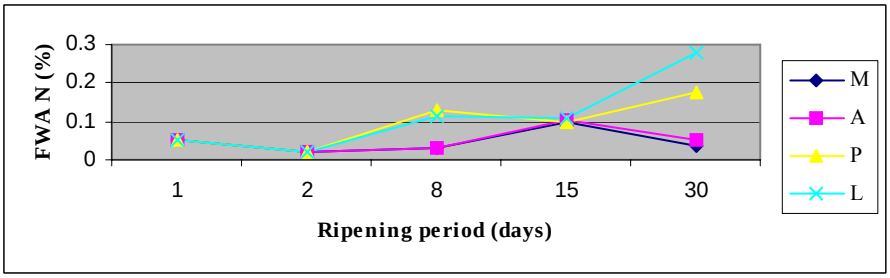

Fig. 7. Soluble nitrogen in Fosfowolframic acid content of witness and enzymes treated cheese during ripening period

The soluble nitrogen in fosfowolframic acids normally contains mostly free amino acids and very small peptides (<600Da). The rate these compounds were increasing was quicker and until a similar level for all samples at 15 days of ripening, decreasing during the last two week of ripening for control cheese and cheese containing Accelase, most probably, because of degradation of amino acids through decarboxylation, deaminaton and desulfuration. At 30 days of maturation, large differences in amounts of fosfowolframic solubil N occurred in cheese containing Promod or Accelase.

The experimental cheeses with Promod and mixture of Promod and Lipomod contained higher levels of amino nitrogen and N soluble in fosfowolframic acid than in the control cheese.

The obtained results pointed out that the samples of Promod and Lipomod-treated-cheese exhibited higher values of soluble nitrogen/total nitrogen ratio (SN/TN), N_{NH3} /NT, titratable acidity (TA), and the flavour was developed faster as compared with the samples of control.

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. 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 Penteleu cheese, without the appearance of off flavour.

CONCLUSIONS

By examinating the effect of addition different enzymes on the maturation time of Pasta Filatta Cheese it has been reported that used proteolitic/lipolitic enzymes had positive effects on the maturation time. As a result, Pasta Filata Cheese Penteleu has maturated in shorter time comparing to the traditional one.

Therefore, the aim of this study was to investigate the use of proteolitic/lypolitic enzymes and the evaluate the organoleptic properties of Pasta Filata Cheese. On the other hand I investigate the modality „cantitative" and moment of adding enzymes to eliminate inactivations before actions and without the appearance of off flavour/taste in cheese.

Research has been focused on finding new proteolitic and/or lipolytic enzymes suitable for maturation of cheese as a method to shorten the time of maturation; this reduces costs for both producers and consumers, in addition to preserving the environment.

However, more work is needed to develop the right dosage and moment to adding enzymes which contribute to eliminate the bitterness of cheese and to obtain a good texture.

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