



Effect of Plant Coagulant Extract on Proteolytic Activity during Ripening of A Local Soft Cheese “J’ben Elgafs”

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ABSTRACT

Background: Biological ripening of cheese is a key stage in cheese processing, a slow and costly process.

Methods: Various research projects on our local cheeses have shown that thistle extract (*Cynara cadunculus*) can accelerate ripening without causing all the defects associated with plant enzymes. To this end, we used the thistle enzyme (cyprosin), on cheeseable milk with its native lactic flora, at doses ranging from 20% to 50%, with the aim of optimizing ripening without affecting the technological and sensory characteristics of Elgafs-type soft cheese. Optimization of the ripening process is assessed by the enzyme's proteolytic activity, which is reflected in the increased level of soluble nitrogen (SN) released into the coagulum mass during ripening. Comparison of the SN/total nitrogen NT ratio enabled us to assess the difference in proteolysis and the evolution of ripening in our trials.

Result: The application 30% of cyprosin gave the expected results on the one hand on the control of proteolysis during ripening, on the other hand on the reduction of the ripening time from 12 to 09 days and finally by obtaining a cheese very appreciated by the tasting panel.

Key words: Cyprosin, J'ben elgafs, Optimization, Proteolysis, Ripening.

INTRODUCTION

The manufacture of cheese always begins with the coagulation of milk using an enzymatic coagulant preparation (Germonville, 2003). Historically, the most used was rennet, essentially extracted from the abomasum of the calf. Rennet is composed of 80% chymosin and 20% pepsin. The first enzyme, chymosin, destabilizes the casein, an insoluble protein in milk, which causes the milk to coagulate and a coagulum to be obtained by draining. The second enzyme, pepsin, continues the degradation of proteins during the ripening of cheeses, which contributes to the development of their specific textures and tastes (Collin, 2015).

Ripening corresponds to various biochemical transformations (proteolysis, lipolysis, glycolysis...) that result from the activity of enzymes native to the milk, or added for its coagulation, or from those derived from microorganisms added to it for its transformation into cheese. It is these desired modifications that are responsible for maturing the coagulum and obtaining a derivative product with desired organoleptic characteristics (Ayerbe and Gillis, 2018; Begum *et al.*, 2019; Mahaut *et al.*, 2017; Malaka *et al.*, 2024; Shinde *et al.*, 2023; Zhao *et al.*, 2019).

The growth of the cheese industry worldwide has led to the appearance of various substitutes, which has required the development of analytical methods for a better characterization of their enzymatic composition as well as the establishment of appropriate legislation (Fox *et al.*, 2005).

The search for singularity and authenticity of products encourages today to preserve ancestral know-how and to question the function of the various components of these coagulants.

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In Algeria, cyprosin is the only vegetable coagulant enzyme approved by cheese makers. This enzyme is extracted from the pistil of the thistle flower. A lot of work has been done on the effect of the thistle flower enzyme extract on the proteolysis of cow's milk cheese. Nevertheless, little information is available on the effect of the substitution of animal rennet by the coagulant extract from thistle flower on the sensory and proteolysis characteristics of these cheeses (Amaal *et al.*, 2021; Galan *et al.*, 2008).

Thus, the present work proposes to study the effect of the substitution of an animal enzyme by a plant enzyme, cyprosin, on the optimization of the ripening time and on the organoleptic properties of an experimental soft cheese.

To achieve this objective, we used an enzyme from the thistle flower “*Cynara cadunculus*” extracted and characterized in the laboratory. In order to control this optimization of the ripening time, we approached the proteolytic activity by the evaluation of the increase of the rate of soluble nitrogen (SN) released in the mass of the coagulum during the ripening of the cheese with an appreciation of the ratio of soluble nitrogen to total nitrogen SN/NT.

MATERIALS AND METHODS

Location

This study was conducted at the Research Laboratory of Sciences and Techniques of Animal Production “LSTPA” of Hassi-Mameche, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University of Mostaganem, Algeria. The experimental period was established from April 2021 to May 2024.

Samples of the plant coagulant

The thistle enzyme “Cyprosin” was extracted in the laboratory from the plant species “*Cynara cadunculus*”, according to the protocol defined by Galan *et al.* (2008), harvested from the Dahra region “The Dahra Mountains” Sidi-Ali, Mostaganem, Algeria.

Experimental milk

The experimental cheese milk was collected in the Relizane dairy basin, from a dairy farm with a herd of Holstein dairy cattle. The milk was collected aseptically in a refrigerated isothermal tank at a temperature of 04°C.

The milk was thermized at 63°C, enriched with calcium chloride (0.2 g/L CaCl_2) and stored under agitation at 15°C for controlled prematuration with its autochthonous lactic flora for 08 to 10 hours.

Commercial animal rennet

The animal rennet is a commercial brand CHY-MAX powder from CH Hansen, Denmark. The rennet stock solution was prepared at a concentration of 2%. The experimental dilution was established, according to the technical recommendations at 2.5%, defined by the International Dairy Federation “IDF” (2020), to obtain a flocculation time at 30°C, technological standard, between 8 and 15 minutes.

Experimental tests of soft cheese

The experimental tests of soft cheese were conducted at the research laboratory LSTPA as follows:

- Use of 20 liters of milk per dose of experimental preparation.
- 05 trials of cheese preparations were carried out in 05 replicates: A control trial with rennet and 04 trials with doses of cyprosin (ranging from 20 to 50%).
- The ripening was carried out in a laboratory incubator designed for controlled ripening of cheeses at a

temperature between 15 and 18°C and a relative humidity HR of 90 to 95%.

The preparation protocol of the soft cheese was defined by the research team of the LSTPA laboratory and established as follows:

- Preparation of thermized milk at 63°C/5 min and pre-maturation of milk for 08 to 10 hours at 15°C with its native lactic flora, as initiated by Dahou *et al.* (2021) and with the addition of 0.20 g/l CaCl_2 for a milk pH of 6.3 to 6.35.
- Additional maturation at 36°C for 1 hour to 1 hour 30 minutes to reach a pH of 6.10.
- Milk-renneting with the coagulant according to the tests defined above.
- Control of the setting time from 8 to 15 min.
- Total coagulation from 32 to 60 min.
- Slicing of the coagulated milk to obtain curd grains of 2 to 2.5 cm on a side.
- Syneresis-exudation of the whey.
- Stirring for 15 min to release 30% of whey with an acidity of 16°Dornic.
- Molding of 8 liters/kg of cheese for an initial PNM protein nitrogenous matter > to 3%.
- Draining in mold with 03 turnings, for this, the temperature of the draining room must be maintained at 28°C for 3 hours after molding to lower it 20°C after the 3rd turning.
- Demolding of the cheese at pH 5.0 to 5.2 for a total dry extract, TDE, of 40 to 42%.
- Salting in brine at 13°C for 35 minutes to obtain an average of 1.8 to 2 g per 100 g of cheese.
- Maturing in the ripening room for 9 to 12 days at 15 to 18°C and 95% relative humidity, RH.
- Packaging at D+9 or D+12.

Technical analysis

Characterization of coagulation

Determination of coagulant activity

Coagulant activity is determined by measuring flocculation time and setting time according to IDF (2020) recommendations.

Determination of flocculation time

The flocculation time is the time interval between the moment of renneting and the appearance of the first casein flakes visible to the naked eye.

Determination of the setting time

The setting time is the time when the first droplets of whey appear (beginning of the exudation of whey). The setting time is generally about twice the time of flocculation: Thus for a flocculation time between 8 and 15 minutes, the setting time is between 16 and 30 minutes.

Proteolytic activity in ripening

Measurement of the proteolytic activity of the enzyme

By measuring the rate of soluble nitrogen (SN) released into the coagulum mass, according to (Bathmanathan *et al.*, 2019).

By comparison of the SN/NT ratio to detect the efficiency of the enzyme during ripening and to evaluate the optimization of the ripening time in comparison with the standard recommended by the IDF, (2020), defined for cheeses at the end of ripening.

Observation

The determination of soluble nitrogen is estimated after precipitation with trichloroacetic acid (TCA) at 12% of the ripening cheese proteins. After filtration, nitrogen is determined by the Kjeldahl method (Sousa *et al.*, 2002; Zhao *et al.*, 2019).

The experimental milk used is of cheesable quality with a protein content above 3% and a total dry extract 12.10%.

Statistical analysis

The study of the similarity of the means of the results giving the optimization of the ripening of J'ben Elgafs type soft cheese was made possible by the use of a statistical test of the SYSTAT MYSTAT 13 SOFTWARE. The statistical significance level is estimated at $P < 0.05$.

RESULTS AND DISCUSSION

Proteolytic activity

This activity was assessed both by technological coagulation times and by the proteolytic kinetics of the coagulant.

It should be noted that during our experimentation we opted for a dilution of the enzyme extract to 2.5% following the performance tests previously conducted on the coagulant to obtain a flocculation time, consistent with a good coagulation and between 8 and 15 minutes as described by Bornaz *et al.* (2010).

In this sense, the clarified extract of cyprosin gives an average flocculation time of 65 seconds at 30°C. Dilution to 2.5% in sterile distilled water gave an average flocculation time of 420 to 510 seconds (7 to 8.5 minutes). These times give conforming setting times between 840 and 1020 seconds (14 and 17 minutes). This confirms the results obtained by Fox *et al.* (2005), Galan *et al.* (2008) and Ilboudo *et al.* (2012).

The cheese curd gels obtained show good consistency and firmness with controlled exudation of whey *i.e.* low release of soluble nitrogen SN. This gel quality is highly demanded in soft cheese industry for which the mixed lactic-enzymatic coagulation gel must be firm and tenacious (Almaal *et al.*, 2021).

Coagulant proteolysis kinetics

The kinetics of coagulant proteolysis yields a parallel release of soluble nitrogen SN, which is soluble in trichloroacetic acid TCA solution at a concentration of 12% (Fox *et al.*, 2005 and Zhao *et al.*, 2019).

This time-dependent evolutionary release of soluble nitrogen content is given in Table (1).

The SN content on cheese curds at the end of ripening varies according to the dose of the plant coagulant used, ranging from 12.50 to 14.75%, for proportions ranging from 20 to 50% cyprosin, added to the experimental milks.

The ratio of SN/NT in refining for cheeses from a coagulation based on cyprosin (20 to 30%), is in the range of 17.50 to 19.25%, which confirms the control of coagulant dilutions essential for a controlled proteolysis and for obtaining optimized technological times giving the physicochemical and rheological qualities specific to soft cheese.

Table 1: Average profile of proteolytic activity of the experimental trials at the end of the refining process.

Cyprosin dose %	20	30	40	50	Control	IDF standard
Day of ripening	D+10	D+9	D+9	D+9	D+12	D+12
FT in minutes	8,5	8	7,5	7	9	8 à 15
ST in minutes	17	16	15	14	18	16 à 30
NP%	70.10	69.80	68.75	68.10	69.45	69.50 à 70.12
SN%	12.50	13.10	14.05	14.75	13.40	12.16 à 13.50
SN/NP	17.83*	18.77*	20.44	21.66	19.24	17.50 à 19.25

D: Day, NP: Protein nitrogen, SN: Soluble nitrogen, FT: Flocculation time, ST : Setting time (*) Significant difference ($P < 0.05$).

Table 2: Tasting test results.

Jury evaluation	Jury score out of (A)	Jury score out of (B)
Visual examination		
Appearance of surface and paste	7.25±0.25	6.85±0.35
Olfactory examination		
Aromatic intensity	9.10±0.58	7.15±0.26
Taste examination		
Taste provided	9.35±0.25	7.85±0.56
Final score out of 10	8.75±0.5*	6.5±0.75*

A: Trial of cheese with 30% cyprosin, B: Control cheese.

(*) significant difference ($P < 0.05$).

The levels of SN released in the cheese paste at the end of ripening are almost identical for both enzymes. The average obtained for the cyprosin enzyme is 13.60% against 13.40% for the commercial rennet.

The proteolytic activity detected with the course of refining, by the SN rate assay is almost identical because of the control of dilutions giving a hydrolysis of proteins proportional to the doses of coagulants used. This approach is comparative to that obtained by Ilboudo *et al.* (2012).

The presence of an appreciable proportion of soluble nitrogen in the experimental cheeses at the end of the ripening period is variable according to the dose of cyprosin used. The proteolytic activity evaluated by the soluble nitrogen is perceived at a dose of 30% cyprosin giving an optimized ripening time of 09 days. This SN fraction increases with the dose of cyprosin added to the milk at renneting with compliance with the technological standard at 30%. These results are in agreement with those performed by Bathmanathan *et al.* (2019) and Galan *et al.* (2008).

Fig 1 and 2, concretize the variations that, from one stage of ripening to another, are noted on the protein nitrogen NP and on the soluble nitrogen SN, at the level of the different cheese pastes, with the different doses of the vegetable coagulant used, the commercial rennet-based control and the IDF standard. Given the proteolysis induced on the one hand by the ripening environment conducive to the development of microbial flora and the initiation of enzymatic activities. These desired changes are responsible for the maturation of the coagulum and for obtaining a by-product, the soft cheese, with desired organoleptic characteristics.

According to Ayerbe and Gillis (2018), the desired proteolysis is a progressive digestion comparable to that which occurs in the digestive tract of animals, but which remains limited. Overall, this proteolysis had two

consequences: The cheese paste becomes softer and smoother, with a production of metabolites that confer a particular aroma and flavor to the cheeses produced.

During the maturation of our soft cheese tests in the incubator used in a controlled environment, there is a progressive enzymatic hydrolysis of caseins into peptides (of variable sizes) and free amino acids.

An increasing dispersion of nitrogen fractions was observed in cheese curds coagulated with cyprosin, with a progressive rate of increase in soluble nitrogen with ripening time. Proteolysis is clearly active, becoming more regular as the dose of vegetable coagulant increases. The plant enzyme optimized the refining times at the different doses used. A similarity is therefore observed between the evolution of protein nitrogen in NP and soluble nitrogen in SN, as already described by Nicosia *et al.* (2022) and Slamani (2018). The ripening of cheese with 30% cyprosin is almost the same as for the control. On the other hand, the cheese with cyprosin (30%) reached the IDF standards in NP and SN at day 9.

Optimization of proteolytic activities was ensured by controlling the incubation temperature of the cheeses between 15 and 18°C and the relative humidity between 90 and 95%. In addition, the start of maturation of the cheese curd was ensured at a pH of 5.0 to 5.2, which had an influence on microbial development on the one hand and on enzymatic activity on the other, which was particularly decisive.

The organoleptic quality of cheese prepared with 30% cyprosin, tested by the tasting panel on a series of characteristics, was judged to be good. The results in Table (2) show a significant difference from the control. Scores of 8.75 ± 0.5 were awarded to the cheese prepared with 30% cyprosin and 6.5 ± 0.75 to the control respectively. In fact, visual examination yielded the following results:

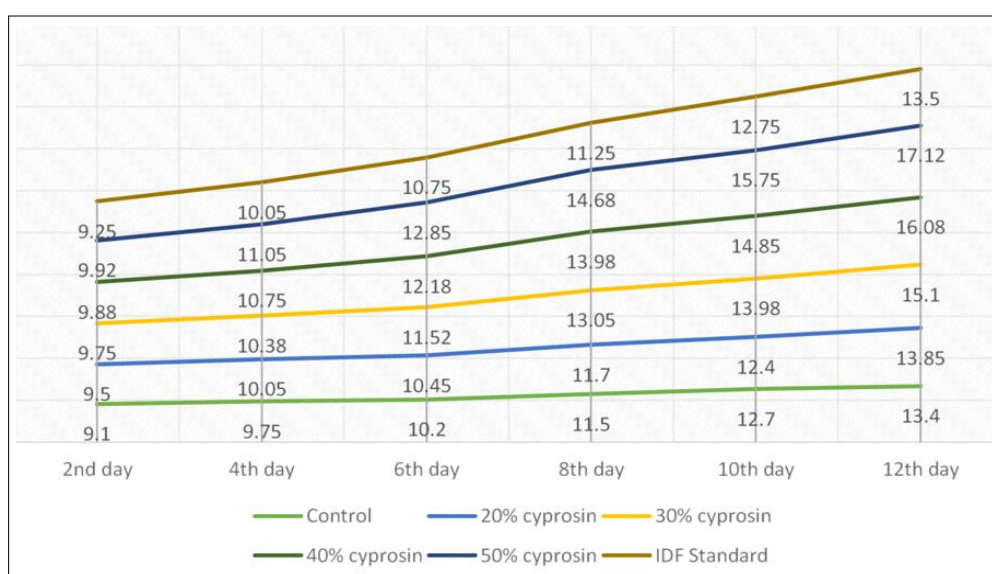


Fig 1: Evolution of SN during ripening.

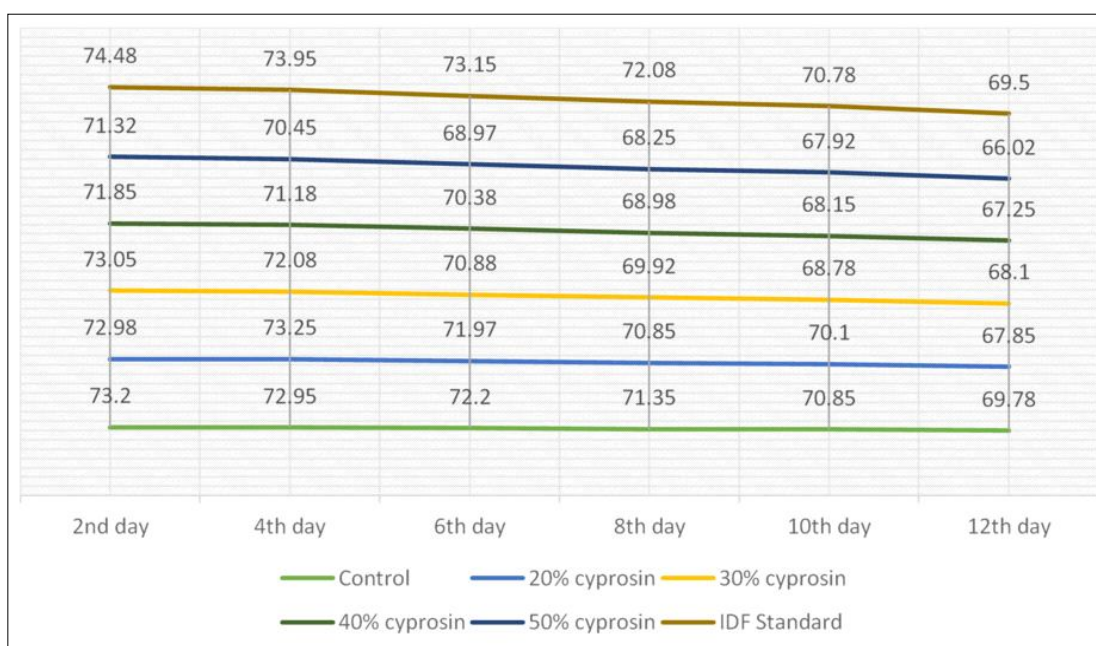


Fig 2: Evolution of NP during ripening.

For the visual aspect of the surface and paste of the cheese

A score of 7.25 ± 0.25 was given to the test with 30% cyprosin compared to the control evaluated at 6.85 ± 0.35 .

For the olfactory examination (aromatic intensity)

A score of 9.10 ± 0.58 was attributed to the cheese test with 30% cyprosin compared to the control 7.15 ± 0.26 .

Taste examination (flavor contribution)

A score of 9.35 ± 0.25 was assigned, for the flavor contributed by the cheese trial made with 30% cyprosin compared to the control 7.85 ± 0.56 .

From this test and the results obtained by Galan *et al.* (2008), it appears that cheese made from milk renneted with cyprosin has superior qualities to that made with commercial rennet. This indicates that the vegetable enzymatic coagulant has a proteolytic activity during coagulation and ripening, giving the qualities required for this type of cheese and optimizing the technological time in production and maturation.

According to the criteria defined for soft cheeses and with Controlled Designation of Origin, “CDO”, our experimentally produced J’ben Elgafs cheese meets the physico-chemical and organoleptic characteristics of this cheese.

The understanding of the evolution of the proteolytic activity of the endogenous enzymes and those brought by the cheesemaker during the ripening process influencing the organoleptic qualities of the soft cheese is thus of capital importance to improve its intrinsic value. Technological innovations to control proteolysis in milk processing and ripening, far from tradition, have been

imposed to facilitate the control of manufacturing times, stabilize cheese productions and their technology by a standardization of organoleptic and sensory characteristics of cheeses with controlled designation of origin (Raynaud and Donneaud, 2016; Ayerbe and Gillis, 2018; Mahaut *et al.*, 2017).

Statistical analysis of the results, carried out using SYSTAT SOFTWARE MYSTAT 13 statistical software, gave significance values below 5% for refining optimization resulting from the use of cyprosin at values between 20 and 30%. On this point, the SN/NT ratio (indicator of good ripening) is in the range of 17.50 to 19.25%, confirming the mastery of coagulant dilutions essential for controlled proteolysis and for obtaining optimized technological times giving the physico-chemical and rheological qualities specific to soft cheeses. The correlation approach enabled us to conclude that the 30% dose of cyprosin used in the coagulation of cheeseable milks seems to have a significant effect both on ripening time and on the appearance and organoleptic quality of J’ben Elgafs soft cheese.

CONCLUSION

Our results show no disadvantage of substituting a plant coagulant “cyprosin” for rennet during the coagulation phase of milk intended for the manufacture of a soft cheese of the J’ben Elgafs type. The application dose of 30% cyprosin gave the expected results on the one hand on the control of proteolysis during ripening, on the other hand on the reduction of the ripening time from 12 to 09 days and finally by obtaining a cheese very appreciated by the tasting panel. The study has experimentally shown the interest of vegetable coagulants in improving the proteolytic

activity of soft J’ben Elgafs-type cheese and thus optimizing the ripening time, which is very much sought after by industrialists in the field. The relatively recent evolution of the methods of transformation of the milk for the improvement of the technological times of manufacture passes by a modification of its natural enzymatic activity and consequently, those of the cheese curd put in affinage. This study is a contribution in the sense that it provides manufacturers with the scientific approach they need to master cheese processing technology.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare no conflict of interest.

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