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# Characterization of the Purified Coagulant Extracts Derived from Artichoke Flowers (*Cynara scolymus*) and from the Fig Tree Latex (*Ficus carica*) in Light of Their Use in the Manufacture of Traditional Cheeses in Algeria

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**Abstract:** Artisanal cheeses made with milk from small ruminants form a part of the eating habits of the people in the agricultural regions of northern and southern Algeria. The products are prepared from a maceration of cultivated plants such as artichoke, pumpkin seeds, fig tree sap and sometimes from the extracts of dried rennet. A strong plant flavor and limited conservation are characteristics of these types of cheese. The properties of purified extracts were studied in this research. The process involved purification by exclusion chromatography on Sephacryl S-200 and a Sephadex G-50 gel treatment to discolor the excess pigmentation as well as to highlight the effects of the various parameters (pH, temperature, enzyme and $CaCl_2$ concentration) on the coagulant activity of the enzymes. The purified plant extracts known as *Aspartic proteases* are active in an acid medium, tolerate high milk temperatures (80°C) and have an increasing proteolytic activity on full-cream bovine casein as compared to commercial rennin. Like rennin, the extracts are relatively stable when stored at a low temperature.

**Key words:** Protease, *Cynara scolymus*, *Ficus carica*, cynarase, ficine, milk coagulation

## INTRODUCTION

The valorization of by-products of various origins (chicken pro-ventricule, goat stomachs, fish offal, bacterial proteases, plants and vegetable by-products etc.) in the preparation of milk coagulant enzymes, constitutes another alternative, which could stimulate an industrial interest in local cheese production, mores since Algeria is ranked as the greatest consumer of milk and milk products in Maghreb with 110 1/year/person according to the statistics supplied by the Ministry of Commerce for the year 2005 (Anonymous, 2005). Moreover, milk production from small ruminants is increasing and comes mainly from family dairy farms and from mountain agriculture. It remains of little value (494 and 216 mL, respectively for sheep and goat milk) (Hacin, 2007).

Since, the last decade, the interest shown towards substitute enzymes of plant origin has allowed the valorization of research, through attempts at large-scale production, of various types of local cheeses bearing the label of the region in several countries of the Mediterranean basin and South America (Lopes *et al*., 1998). Moreover, recent studies conducted on plant substrates have been published, indicating the new interest that plant based proteases have evoked (Low *et al*., 2006; Egito *et al*., 2007; Chazarra *et al*., 2007; Tejada *et al*., 2008; Pereira *et al*., 2008; Fernandez *et al*., 2008). Previously, the *Cynara* L. sp. was the subject of numerous cheese products manufactured with goat milk (Barbosa *et al*., 1976) and many plant species were identified, notably *Ananas comosus* (Cattaneo *et al*., 1994), *Cucumis melo* (Uchikoba and Kaneda, 1996) *Calotropis procera* (Sanni *et al*., 1999), *Opuntia phylloclades*, *Cereus triangularis*, *Euphorbia caducifolia*, *Ficus bengalensis*, *F. elastica*, *E. hista, Ficus carica* (Umar Dahot *et al*., 1990; Oner and Akar, 1993), *Lactuca sativa* (Lo Piero *et al*., 2002), *Cynara scolymus* (Sidrach *et al*., 2005), *Cynara cardunculus* (Sousa and Malcata, 2002), *Helianthus annuus* (Park *et al*., 2000), *Albizia lebbeck* (Egito *et al*., 2007). More advanced attempts at purification and a deeper understanding of the biochemical mechanisms of these enzymes became the center of interest of researchers. In Algeria, traditional cheese production has always involved the use of coagulant plant extracts in their crude

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state, obtained from fig tree sap, artichoke and cardoon flowers or pumpkin seeds for the preparation of fresh cheeses. It was for the Djeben, made from goat and sheep milk in northern Algeria and Kemaria, made with cow and goat milk and sometimes camel milk in the south, that Quezel and Santa (1962) established the list of flora with coagulant qualities in the northern and desert regions. The products are intended for the market and for local consumption and remain a form of valorization of milk derived from different livestock. However, the products are characterized by a mediocre micobiological and sensorial quality, which remains dependent on a better understanding of the characteristics of protease because several factors such as temperature, pH, enzyme concentration and the concentration of $Ca^{++}$ ions control the milk coagulation mechanism (Okigbo *et al*., 1985; Bringe and Kinsella, 1986; Payne *et al*., 1993; Picon *et al*., 1995; Gunasekaran and Ay, 1996; Daviau *et al*., 2000). If the use of ficine in traditional cheese production presents a certain reticence on account of the few studies carried out, the use of cyranase from artichoke is actually recommended (Verissimo *et al*., 1998; Silva and Malcata, 2000; Sidrach *et al*., 2005; Chazarra *et al*., 2007). This current piece of research is submitted with the aim of examining the aptitudes of milk coagulation, stability during conservation and the physic-chemical properties of purified coagulases extracted from artichoke flowers (*Cynara scolymus*) and from the fig tree latex (*F. carica* L.) as compared to rennet, with a view to promoting traditional cheese-making through improvement in the quality of artisanal cheeses for future production.

## MATERIALS AND METHODS

**Biological equipment and extraction of coagulant enzymes:** Artichoke flowers from fresh, well-developed capitula are derived from the winter (February) produce and come from the market. The variety used in our study is the cultivar called violet. This variety is very much exploited in Algeria between the months of January and June and generates large quantities of residue in consumption. The flowers are put to dry for about three weeks at a room temperature not exceeding 25°C and shielded from light, scattered on filter paper according to the process used by Tsouli (1974). Extraction is performed on 10 g of dried flowers using a sodium acetate buffer solution at 0.1 M, pH 5 adulterated with boric acid at 0.2% depending on the optimized process in the laboratory. After macerating for 24 h under gentle agitation, then freezing and thawing, the solution obtained is centrifuged at 1000 rpm for 45 min at 4°C. The recovered supernatant goes through 2 successive filtrations, on filter paper, then a vacuum filtration on a 0.4 microns membrane. The crude, enzymatic extract is adjusted to pH 5, a pH with enzymatic stability (Laurent, 1974; Tsouli, 1974). In order to eliminate excessive browning of the extracts, discoloration was achieved by passing the enzymatic solution through a semi-preparatory column (pharmacia, 40×2.5 cm) of the Sephadex G-50 gel.

The crude sap of *Ficus carica* is derived from the Onk l'hmam variety, which is widespread in Northern Algeria and the coastal regions. The sap is collected from all parts of the plant. Ten mililiter of latex are centrifuged at 3200×g for 15 min so as to eliminate the gum (Riffaat *et al*., 1970; Low *et al*., 2006). The supernatant, which constitutes the crude enzymatic extract is adjusted to pH 5 with the help of a concentrated solution of hydrochloric acid and stored at -18°C until it is used. Depigmentation of the extracts was performed as previously described.

The rennet referred to is a standard liquid enzyme of bovine origin (80% chymosin and 20% pepsin, Chr Hansen, Denmark), of 1/10,000 strength, used by the Boudouau (Algeria) cheese dairy in the production of Edam cheese. The protein concentration is adjusted to 1.23 mg $mL^{-1}$ according to Dehove (1990).

**Purification of coagulant extracts:** The crude enzymatic extracts were purified using a high resolution gel Sephacryl S-00 on a column such as Spectra/chrom™LC, 80×1.5 cm, which was equilibrated beforehand using a 0.01 M, pH 5 acetate buffer solution with an elution flow of 0.2 mL $min^{-1}$. The active fractions are reassembled and conserved at -18°C. The recovered active fractions constituting the purified enzyme are set aside for the study of characterization without analyzing the chromatographic profiles.

**Measure of the coagulant activity:** Coagulant activity is determined according to the method of the Berridge (1952) and as modified by Collin *et al*. (1977). This method enables one to express the activity of the enzymatic extract in coagulant strength. It represents the volume of milk coagulated per unit of volume of the enzymatic extract in 40 min, at 35°C and at pH 6.4 according to the formula:

$$2400\ V/tv$$

where:

V = Volume of milk in mL
t = Clotting time in seconds
v = Volume of the enzymatic extract in mL

Berridge's substrate is composed of milk powder at 120 g $L^{-1}$ (low heat milk) at 0% of fat) watered down with 0.01 $CaCl_2$ moles.

**Measurement of the specific activity:** The specific activity is calculated according to the formula: Coagulant activity/mg of protein.

**Measurement of the proteins:** The measurement of proteins in the coagulant extracts is carried out according to the method of Bradford (1976) in a range of sensitivity of 0-10 μg. The concentration is measured from a standard curve of bovine albumin (SAB, Sigma chemical).

**Characterization of purified coagulant extracts and rennin:** The optimal coagulant activity on the milk of purified enzymatic extracts and rennin is determined according to the standard measuring conditions of the coagulant activity (35°C, pH 6.4) by observing the time of the coagulation of milk by varying the values of the parameter studied. It is expressed in relative activity (%).

**Optimal pH:** The optimal pH of milk coagulation is determined by observing the shortest coagulation time with a pH between 5 and 8.

**Optimal temperature:** The enzymatic activity of the extracts is determined within the milk coagulation temperature between 25 and 90°C.

**Optimal concentration of $CaCl_2$:** The optimal concentration of $CaCl_2$ is determined by observing the milk coagulation time watered down with $CACl_2$, at concentrations between 0.001 and 0.5 moles.

**Optimal enzyme concentration:** The volumes of enzymatic preparations added to the milk vary according to the suitable dilution factor of between 0 and 500 of the initial enzymatic extract.

**Measurement of proteolytic activity:** Measurement of the proteolytic activity of coagulant extracts allows for the evaluation of the rate of degradation of casein (Sigma, Biochemical and reagents) during the primary reaction. It consists of measuring, the increase of Non-Proteinic Nitrogen (NPN) in Trichloracetic Acid (TCA) at 12% of the final mixture (Houins *et al*., 1973).

**Stability of the enzymes**

**Thermal stability and pH:** Enzymatic stability was determined by way of the residual activity according to the standard conditions of measurement of the coagulant activity of the purified extracts and rennet after preincubation at temperatures ranging between 25 and 45°C for 48 h. The effect of the pH was measured by adjusting the enzymatic extracts according to the range of pH, citrate (pH 2 and 3), acetate (pH 4 and 5) and phosphate (pH 6 and 7) after 24 h of incubation at 4°C.

**Conservation of enzymes:** The coagulant extracts are stored at different temperatures, +4°C and at room temperature (+18/20°C). The residual activity is measured according to the conservation time depending on the standard conditions of measurement of the coagulant activity.

## RESULTS AND DISCUSSION

**Results of extractions and physico-chemical characters:** The average quantity of artichoke flowers, which can be recuperated varies according to the size of the capitula. It is approximately, 6 g $kg^{-1}$ of capitula that is 0.6%. The advantage of using artichoke to obtain coagulant enzymes is the easy obtainment of flowers from the capitula. Its major inconvenience rests in its scant availability, which is limited during the winter season in Algeria and in its weak coagulant activity. Nevertheless, the development of dairy products based on this cultivar is dependent on the agricultural nature of the region. For the fig tree sap, the recuperation of the enzymatic system is very important at the crude state as well as at the purified state with a yield of 80%. On the other hand, its production remains delicate due to its highly viscous texture but its use necessitates purification to improve the sensory properties of the Djeben. The physicochemical characteristics of this crude material are represented in Table 1.

The total protein concentration of the crude enzymatic coagulant preparations is relatively low compared to the mass of the crude material used. That of the fig tree is about 4 times higher than that of artichoke (22 mg $mL^{-1}$ against 5.6 mg $mL^{-1}$) with a proteinic yield of 11 and 2.02%, respectively (Fig. 1).

Moreover, the coagulant activity obtained is closely linked to the proteinic levels with the exception of rennet where the opposite effect is noticed due to the fact that it is marketed as a protein enzyme (Table 1).

In fact, in its crude state, the fig tree sap extract represents the greatest activity in a ratio of 1/50 compared to the coagulant extract of artichoke (Fig. 2). This parameter combined with the apparent characteristics of the crude sap (Table 1) affects the sensory properties of fresh cheeses, which produce a very strong odor and bitterness, as reported also in the research of Sgarbieri *et al.* (1964), Genin and Johri (1968), Fahmi (1973), Poznanski *et al.* (1975), Garg *et al.* (1994) and Walstra *et al.* (1999).

The excess oxidation of the crude extracts leads to browning and this constitutes a major inconvenience for

Table1: The physicochemical characteristics of the raw materials

| Raw material | Obtaining period | Recover mode | Availability | Crude amount | Net amount | Viscosity | Smell | Colour | Strength | Dried raw |
|---|---|---|---|---|---|---|---|---|---|---|
| Artichoke | Seasonal | Easy | Important | 6 kg | 3 kg of flowers | No viscous | vegetal | Dark brown | 1/400 | 3 |
| Fig | Annual | Slow | Low | 12 mL | 10 mL | Viscous | Pronounced fruity | Clear brown | 1/40,000 | 10 |
| Rennet | Annual | Easy | Very low | - | - | No viscous | Average | Clear to brown | 1/10,000-1/ 100,000 t | 2 |

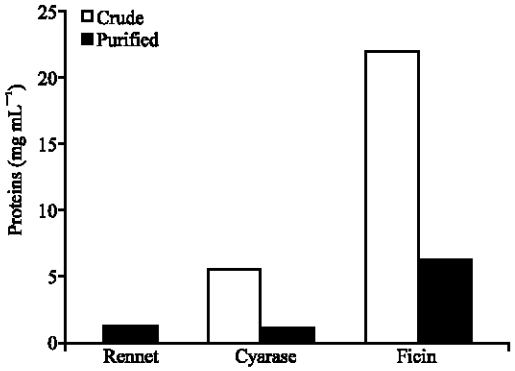


Fig. 1: Proteins concentration of the crude and purified extracts from artichoke, fig and rennet

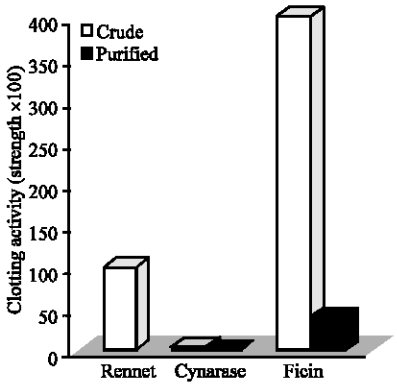


Fig. 2: Clotting activity (expressed in strength ×100) of the crude and purified extracts

use in cheese technology. Clarification by passage through sephadex G-50 led to the elimination of a significant portion of the pigments and probably of the peptides of small molecular weight associated with the enzyme and the recuperation of a mass of purified enzyme, significant for the continuation of the study. The various adsorbents (bentonites, PVP and ion exchangers) and the ammonium sulfate precipitation used by Sidrach *et al.* (2005) to clarify the crude extract of cultivated artichoke did not produce satisfactory results except for an average discoloration by ultrafiltration through a membrane with a cut-off of 10 kDa and an activated carbon adsorption (Lorente *et al.*, 2004). This discoloration prevents the coagulant extract from transmitting an undesirable color and odor to the milk used in production and also to the cheese.

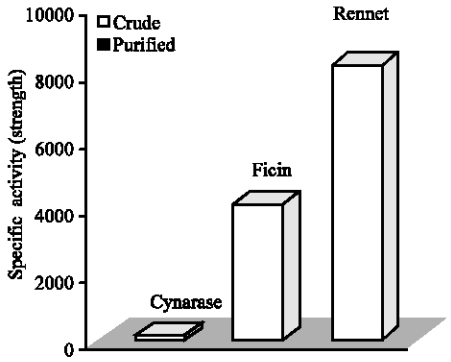


Fig. 3: Specific clotting activity of the purified extracts

**Coagulant activity of the purified extracts:** The measurement of the coagulant activities of the enzymatic extracts constitute an important aspect of cheese technology. The purified fractions endowed with enzymatic activity present proteinic rates and forces, which are lower than in the crude state because of dilution, but which are clear of inactive proteins. Gel filtration chromatography (profile not studied) revealed only 1 form of cynarase (*Cynara scolymus*) and 2 active forms of ficine (*Ficus carica*) unlike the research of Verissimo *et al.* (1995) and Sidrach *et al.* (2005), who observed 3 forms (cyranase A-C). Lorente *et al.* (2004) isolated 2 very active forms. In the fig tree sap, several peaks endowed with coagulant activity were identified by Oner and Akar (1993), through ion exchange chromatography. The protein concentrations (Fig. 1) are 6 and 1.05 mg $L^{-1}$, respectively for the fig tree and artichoke extract (0.105 and 0.60%) and that of rennet is 1.23 mg $L^{-1}$ (0.123%) consisting of 100% enzymatic proteins. These values are either close to or higher as compared to rennin but this does not correspond to a high specific activity. In fact, the specific activity actually reflects the strength of the coagulant protease and expresses the level of enzymatic protein. The specific activity of the fig tree represents about 40 times that of the artichoke, only 50% of the activity of commercial rennin, the preparation, of which is done in conditions of adequate stability (Fig. 2 and 3).

**Proteolytic activity:** It is certain that the coagulant proteases present a double activity: one very specifically

on κ-casein, the other of general proteolysis likely to appear during the maturing process. Proteolysis is one of the most important phenomena of the maturing process, because it affects not only the flavor of the cheese but also, its appearance and texture. It results in the successive release of peptides followed by amino acids. The latter can be degraded into varied compounds, thus, contributing to the appearance and the flavor (Fig. 3).

The proteolytic activity of rennin is less significant than that of the plant enzymes, which were studied, while that of the fig tree has a more marked kinetic on casein compared to artichoke (Fig. 4). According to Paquet (1977), the initial slope observed during the first 20 min of casein hydrolysis corresponds to the primary reaction (cleavage Phe105-Met106). From then on there is no more release of non-proteinic nitrogen. In our study, this initial slope is not observed due to the fact that the action of the enzymes was only measured after 1 h of hydrolysis but the overall proteolytic activity with regards to rennet has a similar behavior. The breaking off of the Phe105-Met106 bond from κ-casein by aspartyl proteases such as cynarase and ficine is similar to that of chymosin and the other proteases of fungal and bacterial origins (Sidrach *et al.*, 2005) and the proteolytic effect is identical to that of the extracts of *Cynara cardunculus*, which are largely used in the manufacture of cheeses (Campos *et al.*, 1990; Silva and Malcata, 1999; Silva *et al.*, 2002) (Fig. 4).

Previously, Heimgartner *et al.* (1990) and Cordeiro *et al.* (1992), observed during a comparative study of the coagulant and proteolytic activity that the crude extracts and forms 1 and 2 of cyranase manifest an excessive proteolytic activity compared to chymosin due to the non-specific action of the proteases towards the other milk caseins ($\alpha_s$ and β-caseins). The more intense action observed in the fig tree ficine probably produce the same effects on milk caseins (Fox and McSweeney, 1998; Walstra *et al.*, 1999). Observations indicated that the type of milk influences the proteolytic activity of plant enzymes. It is more significant in cow's milk as compared to sheep's milk (Marcos *et al.*, 1980), less significant when the dried milk extract is increased (Renner and Abd El-Salam, 1991a, b; Walstra *et al.*, 1999) and a less pronounced bitterness in sheep's cheese (Barbosa *et al.*, 1976, 1981).

**Properties of purified extracts**

**Optimal temperature and heat stability of purified extracts:** The enzymes of plant origin are characterized by optimal activity temperatures, which are quite higher than those observed in the case of rennet. They are between 80 and 82°C, respectively for artichoke and fig tree protease as opposed to 42°C for rennet (Fig. 5).

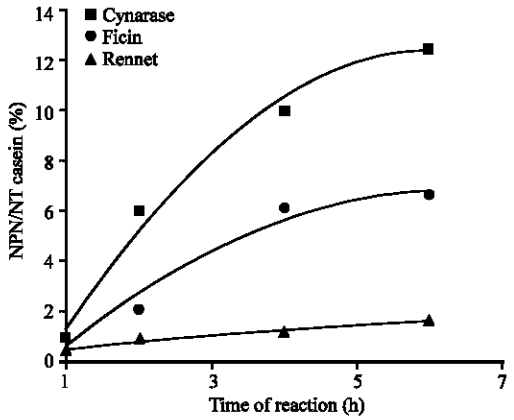


Fig. 4: Proteolytic activity of the clotting extracts

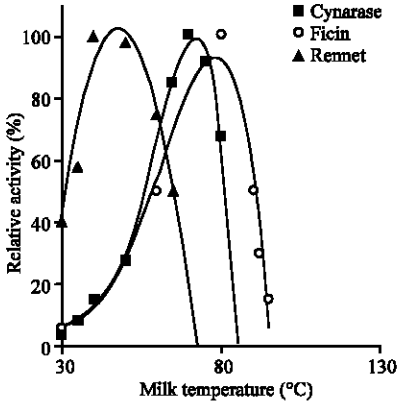


Fig. 5: Effect of temperature on milk-clotting activity of purified

The thermophilic nature of plant proteases was reported differently by Sidrach *et al.* (2005) and Chazarra *et al.* (2007) on cynarase (70°C), Raposo and Domingos (2008) on the protease from *Centaurea calcitrapa* (52°C), Lo Piero *et al.* (2002) on the lettucine from *Lactuca sativa* (50°C). The study of thermal stability indicates that all the enzymes studied are sensitive to high temperatures. They lose their activity depending on incubation time and the temperature of the reactional medium (Fig. 6a-c). At 45°C, the loss of activity is very rapid during the 8 h of incubation. It is about 80% for rennet and ficine and 30% for cynarase. At 55°C, they are totally inactive. The thermostability seems to express a varietal nature. Similar, results are reported by Lo Piero *et al.* (2002), Lorente *et al.* (2004) and Sidrach *et al.* (2005) on proteases, which were incubated for 1-4 h between 40 and 50°C. On the other hand, the *Centaurea calcitrapa* protease retains 100% of its initial activity at 70°C after 6 h of incubation (Raposo and Domingos, 2008).

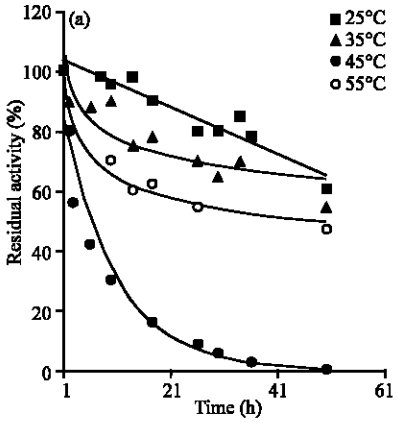


Fig. 6a: Heat stability of the purified cynarase

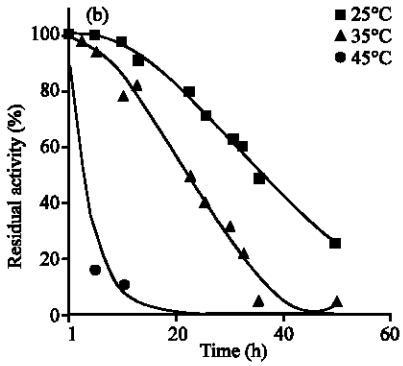


Fig. 6b: Heat stability of the purified ficin

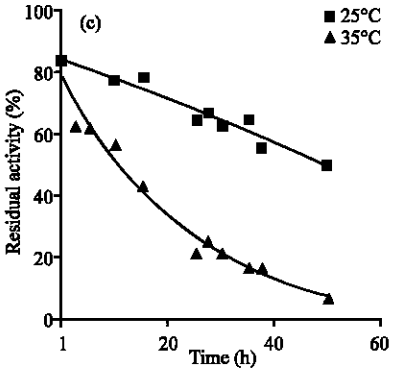


Fig. 6c: Heat stability of rennet

**Optimum pH and stability of purified extracts:** Figure 7 shows that optimum activity falls within the range of the pH acids, 5 for the fig tree enzyme and rennet and 5.5 for the artichoke protease, which seems greater compared to the pH reported by Lorente *et al.* (2004) and Sidrach *et al.* (2005) and lower compared to the pH 6 of cardosin (Chen *et al.*, 2008). At a pH close to neutrality, the loss of activity is very significant (80-90%). A similar result on

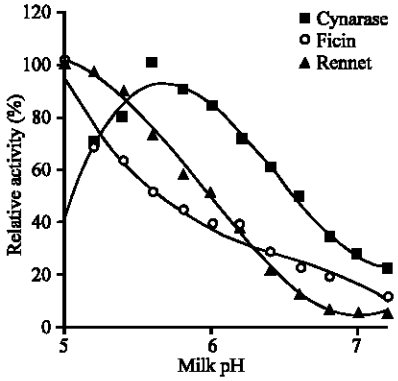


Fig. 7: Effect of milk pH on the milk-clotting activity of cynarase ficin and rennet

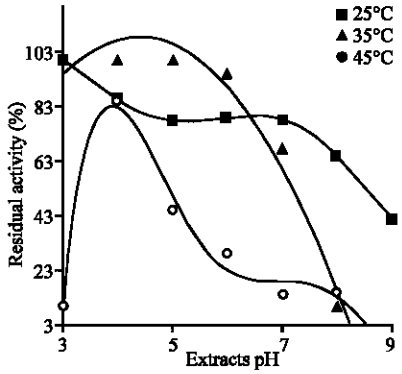


Fig. 8: pH stability of cynarase ficin and rennet

the protease of *Cynara cardunculus* was observed by Heimgartner *et al.* (1990) and Chazarra *et al.* (2007). Fresh traditional cheeses of a soft paste such as *djeben* made from the milk of small ruminants are better able to withstand the coagulation of these pH where the traditional manipulation accelerates the process of acidification of the milk. In addition, the extracts, which were studied are stable in the pH range from 3-7 and 70-100% of the initial activity is maintained (Fig. 8) after 24 h of incubation at 4°C and beyond this pH, the loss of activity is begun, fast for the fig tree extract and slow for the artichoke extract. On the other hand, the rennet activity decreases from pH 5. Similar results are reported by Sidrach *et al.* (2005) after 60 h of incubation at laboratory temperature.

**Effect of enzyme and $CaCl_2$ concentration:** The appearance of the curves in Fig. 9 shows that coagulation time increases linearly with the inverse of the enzyme concentration (expressed in enzymatic dilutions), nevertheless this is really verified only for rennet, which shows great activity at low doses (dilution <200).

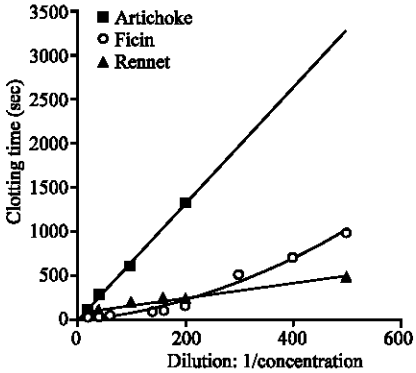


Fig. 9: Relation between coagulation time and the reverse of the extract coagulant concentration

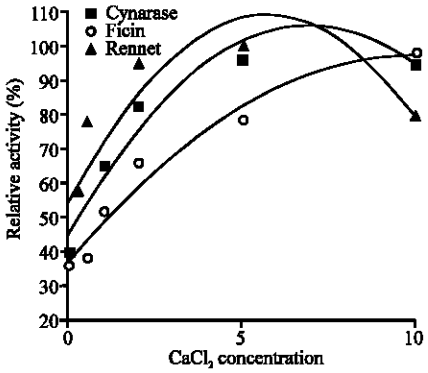


Fig. 10: Effect of milk $CaCl_2$ concentration on milk-clotting activity of cynarase ficin and rennet

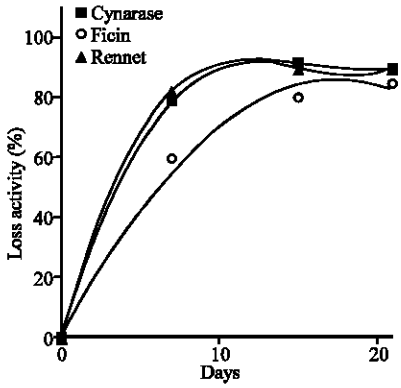


Fig. 11: Lost activity during storage at room temperature (20-22°C) of Cynarase ficin and rennet

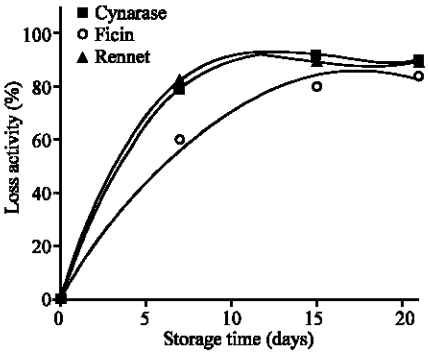


Fig. 12: Loss activity during storage at 4°C of cynarase ficin and rennet

This corresponds to the doses used in cheese making (Garnot and Martin, 1979). These observations are confirmed by Yousif *et al.* (1996),Chitipinityol and Crabbe (1998), Bencini (2002) and Chazarra *et al.* (2007), who explain that this phenomenon corresponds to the increase in the speed of proteolyse of κ-casein similar to the action of chymosin. The results are consistent with the model of the kinetic of milk coagulation proposed by Hooydonk and Walstra (1987) and taken up again by Verissimo *et al.* (1995) on chymosin and by Yousif *et al.* (1996) on plant coagulant extracts. In the standard conditions of milk coagulation, the low specific activities of the enzyme necessitate the use of high concentrations to attain the formation of gel. Taking into account these observations, it is clear that the *Cynara scolymus* extract requires significant doses for cheese-making and at low doses the coagulation time would be too slow, unlike the *Ficus carica* extract.

During the time of the $CaCl_2$ concentration of milk in used in cheese-making (0-20 mM) the coagulant activity increases progressively (parabolic speed) according to the $CaCl_2$ concentration (Fig. 10). It is fast for commercial rennet (85-90%) and slow for plant extracts (from 50-80%), for the fig tree sap coagulase and from 12-50% for the coagulase from artichoke flowers. The coagulation of milk by the enzymes studied is slow at concentrations <10 mM of $CaCl_2$. The artichoke enzyme is the most sensitive. Our results are consistent with those obtained by many authors who have highlighted the effect of $Ca^{++}$ ions in the process of enzymatic coagulation of milk (Bencini, 2002; Najera *et al.*, 2003; Lagaude *et al.*, 2004; Chazarra *et al.*, 2007; Lo Piero *et al.* (2002), who reports that the addition of $CaCl_2$ does not affect the catylatic activity of lettucine on whole casein.

**Effect of conservation conditions on the coagulant activity of purified extracts:** In laboratory preparation conditions, the conservation of purified extracts shows that at room temperature varying from 20-22°C, all the proteases gradually lose their activity depending on time (Fig. 11). After 15 days of storage, the protease from the fig tree conserves about 20% of its activity and 10% for that of artichoke and rennet. At low temperatures (4°C), the loss is less pronounced, 45% (fig tree), 25% (artichoke) and 18% (rennet) (Fig. 12).

The origin of the extract probably remains a criterion of conservation. Lopez *et al.* (1998) show that the loss of activity of the extracts is varies widely among the plant species studied.

## CONCLUSION

The enzymatic extracts, which were the object of our study have for a long time been used in the crude form in the manufacture of traditional cheeses. They are used in the form of a maceration of completely mature flowers of cultivated artichoke or a direct application of the crude sap of the fig tree in the coagulation of bovine milk or that of small ruminants, more particularly goat and sheep milk. This study allowed us to evaluate the main characteristics of enzymes purified by filtration gel compared to rennet, their coagulant activity, their protein composition and the characterization of their activity according to different parameters. A better understanding of the properties of extracted proteases was established for a better and future application in local cheese-making. The major inconvenience of excessive pigmentation of the extracts was removed by the use of a purification gel and the study of the stability of enzymes in various conditions (pH, temperature, enzyme concentration and $CaCl_2$ added to the milk) showed similarities with rennet. The purification of crude extracts, the elimination of pigment colorations from enzymatic preparations and a better understanding of coagulant properties are factors, which contribute to the valorization of milk, especially those produced by small ruminants of private dairy farms, mountain agriculture and from the regions of South Algeria

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