Characterization and Application of Phytochemicals Substances of the Fig Tree: Biological and Sensory Characterization of Ficin and Cheeses "Fresh and Soft"

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ABSTRACT

The manufacture of cheese based on goat's milk is a traditional practice using the fig tree latex. The objective of this paper is to study the possibility of substituting rennet with ficin as a vegetable coagulant milk and its characterization. In addition, two trials of manufacturing a fresh and soft cheese using ficin were made. The results obtained show that the coagulant activity of ficin is optimal at pH = 5, at a temperature of 75°C and a $CaCl_2$ concentration of 0.03 M with 2097.90 UR., 911.79 UR., and 192.30 UR respectively. The coagulant force of this enzyme is 1/21250.911 with a proteolytic activity estimated at 139 µg of tyrosine equivalent per ml of ficin extract. The fresh cheese is white with a creamy appearance and great solubility in the mouth. The ficin soft cheese has an organoleptic quality better than that of control cheese and a softer with more viscous texture in comparison to that of rennet. The ficin soft cheese has a penetrometry of 2.5 mm in comparison with that of rennet of 3.6 mm. Ficin can replace rennet in cheese making and deserves to be expanded in the manufacture of different cheeses.

Key Words: Ficus carica L., Latex, Ficin, Protease, Caseines, Cheese

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INTRODUCTION

Mountain agriculture in Algeria is very diverse in the richness of its products. The cultivation of the fig tree as a hardy species and goat breeding are part of this heritage which, like other Mediterranean countries. Several research projects have been conducted (Solomon et al., 2006; Oliveira et al. 2012) in order to safeguard and improve the fig genetics heritage. This is achieved by valuing the know-how of this species so as to meet to the needs of the food industry and especially the cheese industry

Enzymes are currently widely used as processing aids. Most of these enzymes are proteases. These enzymes have been used in a wide variety of applications such as meat tenderizing and cheese making (Feijoo and Villa, 2010). These proteases have several microbial, animal or vegetable origins such as ficin obtained from fig tree latex.

The latex of the fig tree, which contains ficin, has been used since ancient times for the manufacture of cheese or as an antihelminthic (Feijoo et al., 2014). Traditionally, in the mountains of Algeria, particularly Kabylia, fig tree latex (raw ficin) is used as a coagulating agent for the preparation of a cheese known as "Agugli".

Ficin, is the name given for the proteolytic enzyme (endopeptidase) isolated from Ficus tree latex. It belongs to the family of cysteine proteases (Zare et al., 2013).

The cheese industries use rennet as a coagulant agent which is traditionally obtained from the abomasum of young, unweaned calves, consists of 80% chymosin and 20% pepsin and is sold in powder form (Huppertz et al., 2006). In recent decades, there has been a worldwide shortage of rennet due to zootechnical and economic reasons leading to increased demand because of a steady increase in world cheese production. This is why research has been actively pursued to find new potential sources of enzymes that can be substituted for animal rennet. Among these substitutes, the oldest are proteases of plant origin used in traditional preparations such as those from the thistle, the latex of fig and papain (Biswajit et al., 2013). The enzyme that has been the subject of this work is extracted from fig tree latex (Ficus carica) in Bouira Province. The main of this study is the valorization of ancestral know-how about traditional practices of Algerian mountain pastors, who made their own goat cheese on the spot during grazing, and characterization of the crude extract of latex " the ficin ", as well as the study of the possibility of its use as rennet substitutes in the cheese industry to make the fresh and soft cheeses.

MATERIAL AND METHODS

Reconstituted milk made from 12 g of skimmed milk powder in 100 ml of 0.01M $CaCl_2$ solution was used to study the characteristics of ficin according to the Berridje protocol (1955). It is then stored at 4°C for 12 h. Goat's milk used for the production of fresh cheese was kindly provided by a family from Bouira Province. The manufacture of fresh cheese from goat's milk was done at Institut National de Alimentation, la Nutrition et des Technologies Agro-Alimentaires (INATAA), Constantine Province. Cow's milk used for soft cheese processing was obtained from a cheese dairy "La Vallée" in Tazmalt, Bejaia Province. The latex was picked from figs of different varieties and from different regions at the end of April. It was collected in clean plastic containers and kept cold so that it does not lose its freshness during storage.

Extraction of ficin was done by centrifugation of fig latex at 3.200 g for 15 mins at 4°C to recover the enzyme extract and, then it was stored at 18°C for subsequent uses.

The coagulant activity was expressed as the speed at which the enzyme coagulated the milk and was determined according to Berridj's method (Libouga et al., 2006). It was based on visual evaluation of the first appearance of Berridge substrate coagulation flakes and expressed in terms of unit of coagulant activity (U.A.C) or unit of rennet (UR). The latter is defined as the amount of enzyme contained in 1 mL of the enzyme solution that can coagulate 10 mL of standard Berridge substrate in 100 s at 30°C.

$$U.A.C = (10 * V) / (T * v)$$

Where: U.A.C. = unit of coagulant activity; V = volume of milk; 10 = volume of the standard substrate (10 mL); v = volume of the enzyme extract, T = flocculation time in seconds;

The coagulant force represents the volume of the coagulable milk per unit volume of an enzyme or an enzyme extract, in 40 min, at 35°C and pH equal to 6.4. The coagulating force is expressed by the following formula:

F = (2400.V) / (T.v)

Where: F: Strength of the enzyme V: Adjusted milk volume (pH: 6.4, T: 35°C); v: Volume of the enzyme solution, T: Milk coagulation time (in seconds), Standard test time = 2.400 s.

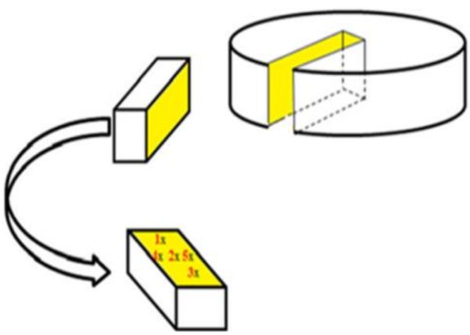
To determine the influence of temperature on coagulation, the coagulation time for the Berridge solution at a temperature range of 30 to 85°C was calculated. The activity of enzymatic extract obtained, the changes in coagulating activity at a well-defined pH range of 5 to 7 were used. $CaCl_2$ concentration in the Berridge solution ranging from 0.005 M to 0.05 M was analyzed in order to determine the optimal $CaCl_2$ concentration to achieve enhanced activity of the enzyme extract at 30°C and fixed pH. The tests were repeated thrice at each parameter and the activity was determined from their averages.

The tasting panel consisted of 20 peoples from Bouira and Constantine trained to assess the samples. To carry out the intensity test, soft cheeses with ficin and rennet and fresh cheese prepared with ficin cut into small cubes, which were presented to the tasters to score on a scale of 0 to 9 the 13 organoleptic criteria (elasticity, texture mellow, rough texture, smooth texture, dispersion in the mouth, adherence, solubility in the mouth, bitter taste, sweet taste, acid taste, astringency taste, yellow color and lactic odor) presented on an evaluation grid established especially for this analysis.

The results obtained from the intensity test were processed by Excel, then graphically represented in order to visualize the organoleptic characteristics of the cheeses manufactured and to facilitate their interpretation.

The penetrometer test was performed on a slice of soft cheese, after it was equilibrated at room temperature. A penetrometer (PNR10) was used for this measurement, it is equipped with a cone which penetrates in free fall in the sample, for 5 s, the average of 5 penetration measurements in mm was calculated, thus gives an idea on the firmness of the cheese. Figure 1 shows the sample preparation method for penetrometer. The penetrometer test gives the distance traveled by the penetrometer cone in the mass of a sample under the effect of its own weight; this distance is a function of the hardness of the cheese (Fox Patrick et al., 2000; Laitier et al., 2009).

Figure 1: Preparation of the sample for the penetrometer test



The principle of measuring the viscosity is to apply a force of movement to a product in rotation. The viscosity of soft cheeses after ripening was measured at ambient temperature (25°C). In this test a VT550 viscometer with a cone / plane geometry at shear rate of 0 to 500 1/s for 300 s was used.

RESULTS AND DISCUSSION

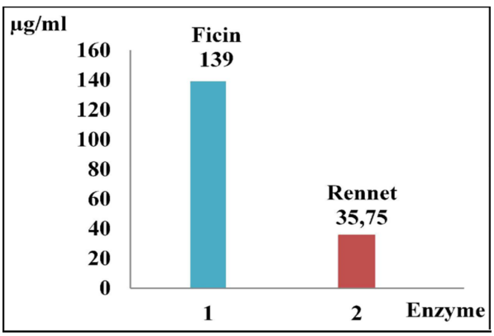
The ficin extract obtained was a light brown viscous solution of that was sticky between the fingers. These characteristics are similar with the results of Nouani et al. (2009). The protein content was 41.75 ± 0.39 mg/ml (Table 1). The enzymatic extract of fig tree latex had a coagulant activity of 103.458 ± 1.69 UR. This value is slightly lower than those reported by Williams Donald et al. (1968) who found 188, 89 UR.

Table 1: Characteristics of the crude enzymatic extract of Ficus carica

Characteristics	Ficin
Yield (%)	75%
pH	5,8±0,1
Dry Matter	20±0,01
Protein level (mg/ml)	41,75 ± 0,39
Coagulating activity (UCA) (UR)	103,458 ± 1,69
Coagulating force	21258,911
Specific activity UR/mg	2,478

The coagulating force, expressed by the volume of coagulated milk by a volume of coagulant, the extract obtained, it is 1/21250,911. This force is lower than that obtained by Nouani et al. (2009) evaluated at 1/40000. This difference can be explained by the climatic differences between the latex collection regions as well as the soil characteristics that can influence the composition of the latex. Comparing these results with those of other vegetable enzymes traditionally used in cheese making such as cardosines, we find that ficin has a higher coagulating activity, coagulant force and protein content than cardosines obtained by different authors (Nouani et al., 2009). Coagulant enzymes are able to hydrolyze casein κ, and thus causing coagulation of milk. Consequently, they are used in the cheese industry, but for the production of quality cheeses, it is necessary to consider their high additional non-specific proteolytic activity which gives them the power to hydrolyze α and β caseins (Vignola, 2002).

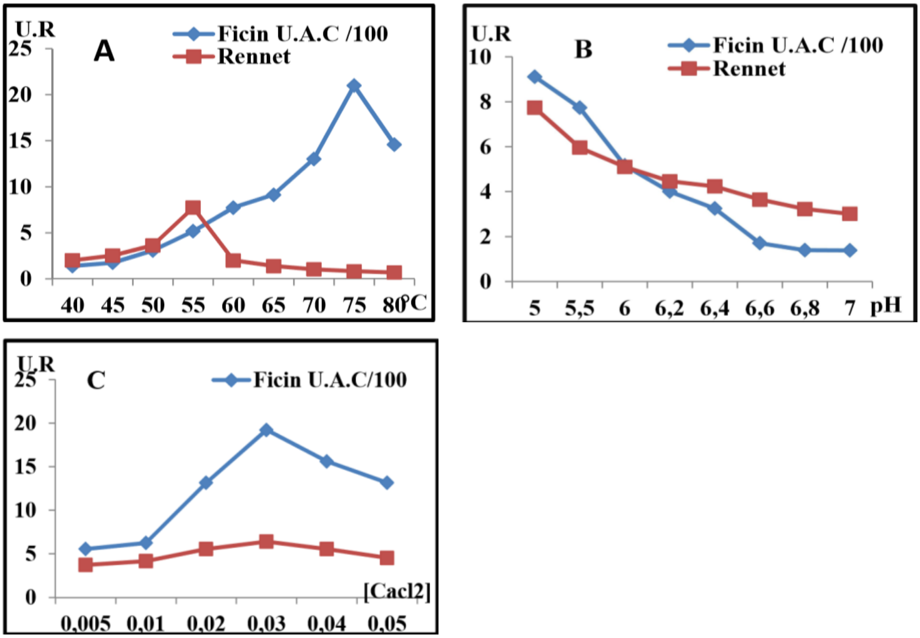
Figure 2: Proteolytic activity of ficin ■ and rennet ■



The results obtained in this work (Figure 2) show that the proteolytic activity of ficin extract is very high (4 times higher) than that of rennet; 139.094 µg/ml for ficin extract against 35.75

µg/ml for rennet. This excessive proteolytic activity of ficin has been reported by several authors (Oner and Akar, 1993; Fadyloglu, 2001; Nouani et al., 2009; Faccia et al., 2012).

Figure 3: Effect of milk temperature (A), pH (B) and $CaCl_2$ concentration (C) on the coagulant activity of ficin ectract (x100) (-♦-) and rennet (-■-)



The optimal conditions of ficin activity determined in this work (Figure 3) show that the coagulant activity of the ficin strongly depends on the pH, temperature and $CaCl_2$ salt concentration. The optimum coagulant activity for ficin extract was obtained at 75°C milk temperature, at pH 5 and at a concentration of 0.03 M $CaCl_2$ with values of 2097.90 UR, 911.79 UR and 192.30 UR, respectively. These results are similar to those given by Nouani et al. (2009) evaluated at 80°C, Fadyloglu, (2001) estimated at 60°C and Payne, (2009) who observed optimum coagulant activity of ficin at 65°C. These results confirm the high thermal stability of ficin already reported by several authors (Bekhit et al., 2013). According to these results, it was found that pH plays a very important role in milk coagulation, solubilization of micellar calcium phosphate, decrease in net charge of casein molecules and dissociation of micelle caseins with optimum pH for κ-casein hydrolysis between 5.1 and 5.3.

The fresh and soft cheeses prepared with ficin had the best elasticity, soft texture (Figure 4 "A") and good solubility during mastication as reported by the tasters. However, they reported very pronounced acid and bitter taste, perhaps due to the shelf life of fresh cheese and camembert ripening. The penetrometer results were 3.6 mm for soft cheese from rennet and 2.5 mm for that obtained with ficin extract. The latter represents the depth of penetration of the cheeses at the final stage of ripening, which means softening of the dough. These results were the average of three repetitions of five penetration measurements shown in Fig.1. In fact, the rennet cheese had a firmer texture than the cheeses obtained from ficin extract whose texture was creamier and unctuous. These results are consistent with those obtained by Oner and Akar, (1993) and Faccia et al. (2012), who observed the softening of cheese made with ficin extracts. This loss of hardness was due, in large part, to the proteolytic activity of the enzymatic extracts studied.

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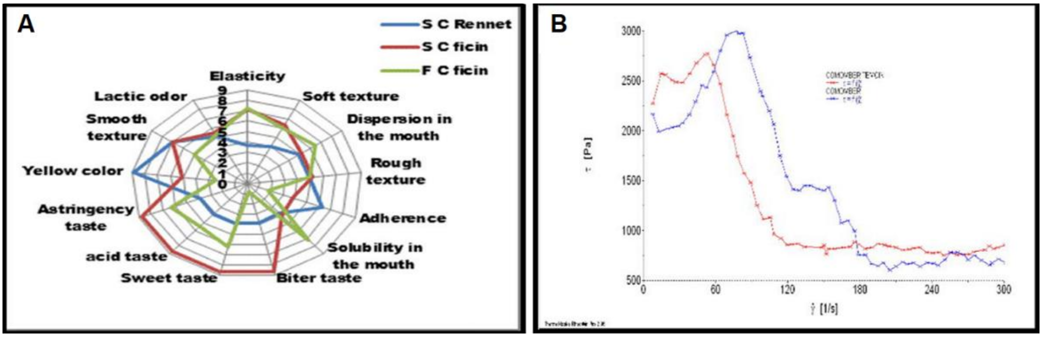
Figure 4: Organoleptic characteristics of cheeses made: **[A]** Sensory profiles of soft cheese (SC) at day 15 of ripening obtained with rennet (—), with fecin (—) and fresh cheese obtained with fecin (FC) (—), **[B]** The viscosity of cheeses soft cheese with rennet (-x-) and with ficin (-x-)



In Figure 4 "B" which represents the stress applied to the soft cheese samples as a function of shear rate during the compressions-relaxation test. The curves of the two soft cheeses have two main parts, the compression phase; during which the force increases rapidly to a shear rate of 60 t /s for the soft control cheese with rennet and 80 t/s for the ficin soft cheese. The relaxation phase, during which the stress decreases rapidly then it continues to decrease gradually until its value tends to 0. The decrease in strength could be explained by the relaxation of the cheese sample while dissipating the energy that was provided during the first phase. The decrease in the relaxation rate could be explained by cheese texture formation which was higher in ficin cheese resistance to its higher viscosity compared to the rennet cheese.

Conclusion

These results demonstrate that ficin extract can replace commercial rennet in cheese making while guaranteeing organoleptic qualities that are appreciable for the consumer.

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