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Use of *Pergularia tomentosa* Plant Enzymatic Coagulant System in Fresh Cheese-Making

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Abstract

Introduction: *Pergularia tomentosa* is a saharan spontaneous plant belonging to *Asclepiadacea* family. It has several medicinal properties and uses in the human food as an auxiliary in cheese-making. The purpose of this study is the extraction of the coagulant enzymatic system (C.E.S) from the plant dried leaves and its use in the preparation of fresh cheese. **Materials & Methods:** The determination of the coagulant activity was carried out on a skimmed milk powder by Berridge's method. Cow's milk used for coagulant strength determination and cheese-making. Proteolytic activity. The chemical determinations of raw milk were carried out according to AFNOR analysis methods. A descriptive sensory evaluation was performed to determine the organoleptic properties of the produced cheese. The proteolytic activity was determined by measuring the aspartyl-protease units. **Results:** The main results of the physico-chemical characterization show that its coagulant activity (R.U mL^{-1}) was 97.19 ± 4.67, coagulant strength (SU) was 230.05 ± 0.37, proteolytic activity (μg·mL^{-1}) was 0.33 ± 0.04 and coagulant activity/proteolytic activity ratio was 294.51. For the cheese made, it has presented the following results as % (g/100g): titrable acidity 0.80 ± 0.005, total dry extract 30.48 ± 1.490, fat 2.32 ± 0.02, fresh yield 11.5 ± 0.09, dry yield 30.06 ± 0.05. Moreover, sensory analysis results showed that fresh cheese prepared from *Pergularia tomentosa* dried leaves diluted enzymatic extract filtrate was characterized by whitish color, smooth texture, with acceptable and satisfying lactic taste. **Conclusion:** The plant enzymatic system studied could be added to the list of plant rennet substitutes and, thus, contributes to the manufacture of enzymatic coagulation cheese.

Keywords

Pergularia tomentosa, Coagulant Enzymatic System (C.E.S), Fresh Cheese, Sensory Analysis, Yield

1. Introduction

Saharan spontaneous plants are characterized by their particular mode of adaptation to the environment. *Pergularia tomentosa*, is a perennial shrub belonging to the *Asclepiadaceae* family [1]. This family plant represents an immense reservoir of potential compounds attributed to the primary and secondary metabolites. They have the advantage of participating through their biological activities thanks to their appropriate chemical structures in several industries of food, cosmetology, and pharmacology [2].

Pergularia tomentosa is a scandent or climber arising from a woody rot-stock, in dry savanna and wooded savanna. It is widely distributed across the Sahara desert, Mauritania and Mali to North Nigeria. It extends to the Congo basin and eastward across the Horn of Africa through Sinai (Egypt), southern Israel, Jordan and the Arabian Peninsula to the deserts of southern and eastern Iran, Afghanistan, Pakistan and India. Usually, it grows in sandy and clay soil areas of deserts [3] [4]. It has a lot of medicinal efficacy. Each part of the plant has specific medicinal properties. For example, roots are used for the treatment of pulmonary disorders, the whole plant against diarrhea, and dysentery. For the plant sap, it treats cutaneous and subcutaneous parasitosis [5].

In eastern Sudan peulh, herdsmen rub the latex onto the udder of cows to increase milk production. In semi-desert areas a little milk, in which stems have been soaked, is added to milk to curdle it for cheese-making. It is also reported to be given to goats as forage. The stems are reported to be occasionally used as famine food [6]. In Algeria, this plant commonly called in southern, "Ghalga" or "H'lib Edabba grows on the rubble, the edges of the paths and the degraded steppe courses. It is used as a hemostatic substance, fights against *Leishmaniasis* and relieves dental pain [7]. In cheese technology, coagulation is the key step in making cheese. Indeed, it consists of the transformation of milk into gel following physicochemical modifications which occur on the casein micelles. It is often the result of the action of enzymes of animal, plant or microbial origin. Rennet is the main coagulating enzyme used in cheese-making, but the increase in the consumption and production of cheese causes a shortage of rennet and very large fluctuations in its price [8].

The objective of this study is the extraction of the coagulant enzymatic system (C.E.S) from the *Pergularia tomentosa* plant leaves and its use in the preparation of fresh cheese. Physicochemical and sensory characteristics are determined on the obtained products. Thus, plant enzymatic system studied could be added to plant origin rennet substitutes list to diversify the milk coagulating agents list.

We remind that *Calotropis procera* plant of the same *Asclepiadaceae Pergula-*

ria tomentosa family was the study subject in the extraction of its calotropaïn enzyme and its use in the peulh type fresh cheese preparation [9].

2. Materials & Methods

2.1. Samples

1) Berridge's substrate: The determination of the coagulant activity was carried out on a skimmed milk powder of a medium heating quality (Molochansk Dairy; Ukraine) with moisture 3% and fat 0.15%. After its reconstitution at 12% (m/v) in $CaCl_2$ (0.01M) solution, the obtained milk, constituted the Berridge substrate. It was stored at 4˚C overnight to ensure the physicochemical balance, the stability and rehydration of micelles. To prevent microbial growth, 0.04% (w/v) sodium azide was added.

2) Cheese-making milk: Cow's milk used for cheese-making was provided from a farm located in Constantine city (Algeria). After milking, it was immediately transported to the laboratory in a cooler (4˚C).

2.2. Coagulating Enzymatic System (C.E.S.)

Pergularia tomentosa leaves were collected from *Ain Diab* region of Biskra city (south-eastern Algeria **Figure 1**). They were dried in the open air at room temperature and then powdered with a mortar. The powder obtained underwent aqueous maceration in sodium acetate buffer solution (pH = 5.5) for 2 hours at 4˚C, followed by filtration and, then, centrifugation at 4000 g for 15 minutes at 4˚C. The supernatant represents the leaves crude extract [10].

2.3. Physicochemical Composition

The chemical determinations of raw milk were carried out according to AFNOR analysis methods: dry extract, lactic acidity, pH, milk and cheese fats were determined according to (AFNOR) 1980 [11]. Total proteins were quantified by the Bradford method [12] against a calibration curve D.O (595 nm) = f [bovine serum albumin].

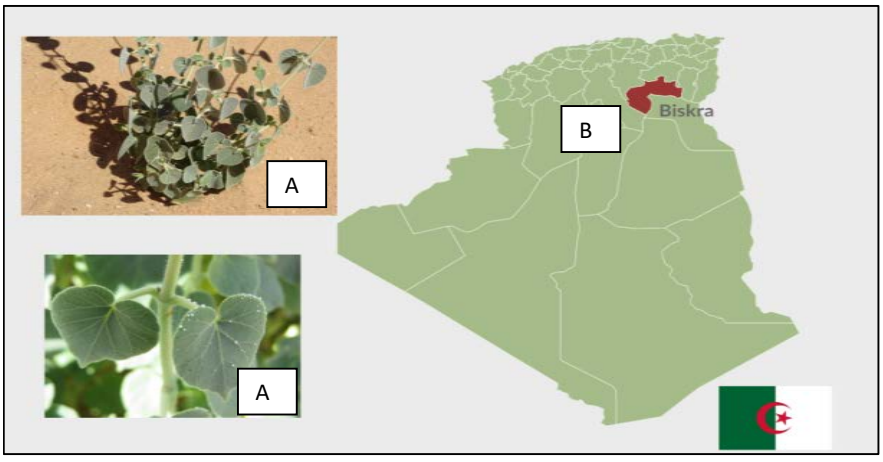

Figure 1. *Pergularia tomontosa* plant (A) and source (B), algerian Saharan Biskra city.

2.4. Sensory Analysis

A descriptive sensory evaluation was performed to determine the organoleptic properties of the produced cheese. A panel of thirty (30) subjects including fifteen (15) males rated the odor, texture, and flavor descriptors on a point scale from 1 to 15 [13].

2.5. Physicochemical Characterization of the Enzymatic Coagulant System (E.C.S.)

Ten (10) mL of Berridge's substrate (pH 6.6) contained in a test tube were maintained in a water bath at 30˚C. The timer was started when adding 1 mL of coagulant enzymatic system (C.E.S) and the tube was, then, rotated slightly. The timer was stopped as soon as the first flakes appeared on the wall of the tube and the flocculation time was noted [14]. The flocculation time was used in coagulant activity calculation (RU mL^{-1}) by the following formula:

$$\text{Coagulant activity}\left(\text{RU mL}^{-1}\right)=\frac{100\times V}{10\times T\times v}$$

where: V: volume of milk; v: volume of the enzymatic extract multiplied by the dilution factor, T: flocculation time.

The coagulant strength expressed in Soxhlet units (SU) represented the number of volumes of the mixture milk coagulated by one volume of rennet in 40 min at 35˚C. Coagulating time was estimated from the start of gel formation. The coagulant strength is expressed by the following formula [14]:

$$\text{Coagulant strength}\left(\text{SU}\right)=\frac{2400\times V}{T\times v}$$

V: volume of milk (pH: 6.6, T˚: 35˚C), T: time in seconds, v: volume of the enzyme extract multiplied by the dilution factor.

The proteolytic activity was determined by measuring the aspartyl-protease units according to the method described by Green Margaret & Stackpoole (1975), [15]. Principle was based on the measurement of the increase in the hydrolysis products obtained by enzymatic action on the casein dissolved in trichloroacetic acid (TCA) at a final concentration of 12%. The protein level was estimated by the Bradford method [12] against a standard curve: O.D. (595 nm) = f [bovine serum albumin].

2.6. Fresh Cheese-Making Diagram

The cheese-making diagram follows steps according to Sessou *et al.*, (2013) [16]. Five liters of raw cow's milk were filtered through gauze to remove large fragments and then heated to 60˚C for five minutes. The diluted enzymatic extract (1/100) of *Pergularia tomentosa* dried leaves (680 mL) was added with an increase in temperature to 75˚C. Then, the mixture was removed from the heat and was allowed to stand to favorise clotting. After firmness, of about 35 minutes, the curd was drained, then stored at 4˚C (**Figure 2**).

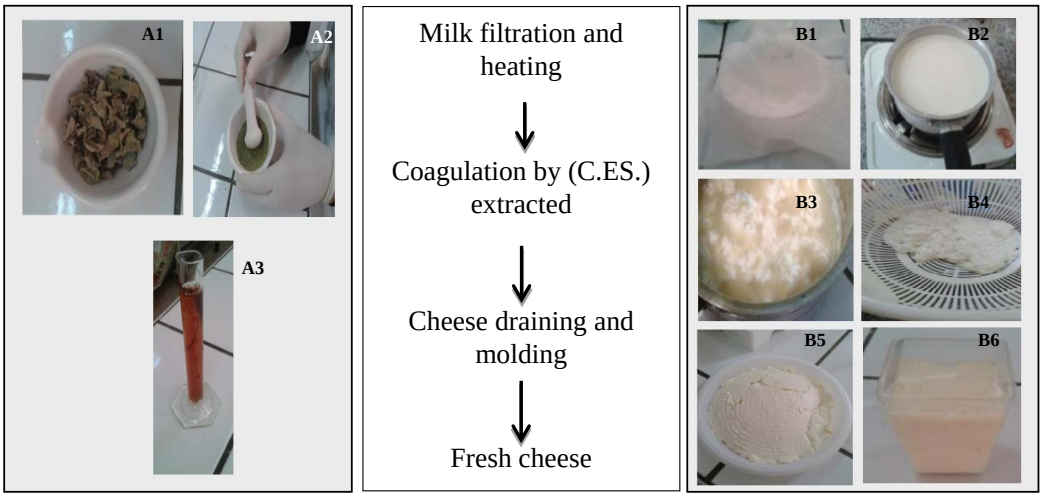

Figure 2. Manufacture of fresh cheese by the *Pergularia tomentosa* coagulant [16] (A) Coagulant Enzymatic System (C.E.S.) extraction, (B) Fresh cheese-making steps. Caption: A-1: *Pergularia tomentosa* leaves plant dried at room temperature; A-2: Reduction of leaves to powder; A-3: Coagulant Enzymatic System (C.E.S.) extract; B-1: Raw milk filtration; B-2: Raw milk heating at 60˚C for 5 min; B-3: Coagulant enzyme extract addition and curd formation; B-4: Curd draining of about 35 min. and whey recovery; B-5: Fresh cheese molding; B-6: Fresh cheese conservation.

The cheese yield as reported by Vandewegh (1997) [17] and Michalski *et al.*, (2003) [18] was calculated on a wet and dry basis according to the following formulas:

$$\text{Fresh yield} = \frac{\text{fresh curd quantity}}{\text{milk quantity}} \times 100$$

$$\text{Dry yield} = \frac{\text{fresh curd quantity} \times \text{curd total dry extract}}{\text{milk quantity} \times \text{total milk dry extract}} \times 100$$

3. Results & Discussions

3.1. Physico-Chemical Characterization of Cow's Milk

Physicochemical characteristics of the raw milk used in the manufacture of fresh cheese are mentioned in **Table 1**.

The pH of raw milk used in cheese-making is 6.62. This value agrees with those given by Malacarne *et al.*, (2013) [19] of 6.72; Nian *et al.*, (2012) [20] of 6.76 and Vignola (2002) [21] of pH between 6.6 and 6.8. On the other hand, the titrable acidity is 17.51˚D, it is higher than that reported by Labioui (2009) [22] of 16.75˚D, and than of Rouissi (2018) [23] 15˚D. These results remain in conformity from 16 to 18˚D as declared by (AFNOR 1993) [24]. The average total dry extract content is 11.66%. It is lower than that given by FAO (1995) [25] of 12.8% and similar to that of Labioui (2009) [22] which is 11.75%. For fat, a value of 32 g/L is noted, slightly lower than cited by (AFNOR 1993) [24] for milk from 34 to 36 g/L and the value reported by Rouissi (2018) [23] of 42.6 g/L. However, it is close to 31.45 g/L, value announced by Labioui (2009) [22]. These differences

Table 1. Physicochemical characteristics of cow's milk.

Characteristics	Mean ± Standard deviation
pH	6.62 ± 0.01
Titratable acidity (˚D)	17.51 ± 0.50
Total dry extract (%)	11.66 ± 1.04
Fat (g/L)	32 ± 0.98

in pH, acidity, total dry extract and fat content are influenced by many factors such as season, stage of lactation, number of milkings, type of forage and availability of water [26] [27] [28]. Thus, the fresh milk used in the production of cheese meets the quality criteria related to fresh, non-acidic milk, with a dry extract and a fat content suitable for a satisfactory cheese yield.

3.2. Physico-Chemical Characteristics of *Pergularia tomentosa* Leaves Extract

Any coagulant enzyme is primarily characterized by its coagulant strength and proteolytic activities. The characteristics of the coagulant enzyme crude extract (C. E.S.) from *Pergularia tomontosa* plant leaves are shown in **Table 2**.

In coagulant activity terms, the obtained results are superior to that of calotropaïn coagulant enzyme of *Calotropis procera Asclepiadaceae* family, estimated at 33.22 R.U. mL^{-1} given by Djeddi & Misky (2014) [29]. On the other hand, this activity is lower than the results obtained for ficin coagulant enzyme extracted from *Ficus carica L.* reported by several authors [30] [31] [32] averaging 200.76 R.U. mL^{-1}. For the coagulant strength, it is higher than that found by Necib & Tabet (2018) [33] estimated at 66 SU for the raw extract of *Pergularia tomentosa* fresh leaves. On the other hand, this strength seems to be lower than that found by Djeddi & Misky (2014) [29] estimated at 3780 of *Calotropis procera* leaves filtrate and lower than that of the latex extract of this same plant estimated at 4953 SU by Bakou & Wodo (2014) [34], and 5188 SU by Djeddi & Misky (2014) [29].

The protein content is greater than that of the enzymatic extract of *Calotropris procera* estimated at 3.74 mg/mL as reported by Aworh & Nakai (1986) [35] and also up than 26 mg/mL cardosine protein coagulant extract of *Cynara cardunculus* flowers [36]. For the proteolytic activity of 0.33 µg/mL, it is relatively lower than that given by Necib & Tabet (2018) [33] of 61 µg/mL for *Pergularia tomentosa* fresh leaves raw extract.

A comparison of the results obtained for the *Pergularia tementosa* dried leaves extract with other plant enzymes extracts indicates that its activity and its coagulant strength are technologically acceptable for cheese-making. This could be confirmed by the high ratio (coagulant activity/proteolytic activity) of 294 widely sought after by cheese makers for an interesting and advantageous milk coagulation. Differences in enzyme characteristics are mainly due to botanic physiology,

Table 2. Proteolytic and coagulant characteristics of *Pergularia tomentosa* enzymatic extract.

Characteristics	Mean ± Standard deviation
Coagulant activity (R.U mL^{-1})	97.19 ± 4.67
Coagulant strength (SU)	230.05 ± 0.37
Protein ($mg{\cdot}mL^{-1}$)	53.86 ± 0.54
Proteolytic activity ($\mu g{\cdot}mL^{-1}$)	0.33 ± 0.04
Coagulant activity/proteolytic activity	294.51

climatic, soil and even leaf conditions (dry or fresh). In fact, each enzymatic extract has thermal and chemical stability and an adaptation with its catalytic medium linked to the appropriate reaction factors. Thus, these characteristics may be potential parameters for the *Pergularia tomentosa* enzyme use in the coagulation of milk and, therefore, in the manufacture of cheese.

3.3. Physico-Chemical Characteristics of Manufactured Fresh Cheese

The main physicochemical characteristics of fresh cheese are mentioned in **Table 3**.

The study results) show a pH of 6.38, relatively close to the pH 6.28 given by Benyahia-Krid *et al.*, (2016) [9] for the "peulh" cheese type prepared from the coagulation of milk by the filtrate of *Calotropis procera* leaves. However, Necib & Tabet (2018) [33] give an average pH of 5.89 for fresh cheese prepared from *Pergularia tomentosa* latex.

This variation could be explained by the initial pH of the milk used and the coagulation method followed. Regarding the titratable acidity of 1.8 g/L, it is higher than that found by Necib & Tabet (2018) [33] of 1.6 g/L, for fresh cheese prepared from the filtrate of *Pergularia tomentosa* fresh leaves.

The fresh cheese obtained after milk coagulation with *Pergularia tomontosa* dried leaves enzymatic extract has a total dry extract of 30.48%. In comparison with those of authors, we note that this rate is lower than cheese total dry extract prepared by Nouani *et al.*, (2009) [32] based on the enzymatic extract of *Pergularia tomentosa* fresh leaves estimated at 40.73% while it is close to those cited by Bakou & Wodo (2014) [34] of 34.80% and Djeddi & Misky (2014) [29] of 33.14% prepared from *Calotropis procera* enzymatic extract.

This difference in the total solids content could mainly be due to curd types obtained which are dependent on the initial milk composition, the type of coagulation as well as the type of drainage [14]. The fat content of 2.32 percent grams of cheese agrees with the value given by Codex Standard (283-1978) [37] which should be less than 20% for fresh cheese.

According to Vierling (1999) [38], fresh cheeses have a solid content from 10 to 15%. Ramet, (1997) [39] gives an amount ranging from 11.5% to 35%, while

Table 3. Physico-chemical characterization of fresh cheese.

Characteristics	Mean ± Standard deviation
pH	6.38 ± 0.015
Titratable acidity % (g/100g)	0.80 ± 0.005
Total dry extract (%)(g/100g)	30.48 ± 1.490
Fat % (g/100g)	2.32 ± 0.02
Fresh yield % (g/100g)	11.5 ± 0.09
Dry yield % (g/100g)	30.06 ± 0.05

Veisseyre (1979) [40] and St-Gelais & Collet, (2010) [41], report that fresh cheese from cow's milk is always moist with a solid content of 20% - 40%. However, Fox *et al.*, (2004) [42], report water content greater than 40%.

The average produced cheese (fat/dry) extract ratio of 7.61% confirms its belonging to the lean fresh cheese category according to the classification of Codex Standard (283-1978) [37], which stipulates a lower fat/dry extract ratio at 10%. The same source indicates that the unripened cheese of which the fresh cheese is ready for consumption shortly after its manufacture.

For the fresh cheese yield produced, it is 11.50% and 30.06% on a wet basis and on a dry basis, respectively. Necib & Tabet (2018) [33] present fresh cheese yields of 15.35% for cheese obtained by *Pergularia tomentosa* latex milk coagulation and 15.27% for that obtained by *Pergularia tomentosa* leaves filtrate milk coagulation. On the other hand, Benyahia-Krid (2016) [9], have presented a cheese yield on a fresh basis of 17.69% and on a dry basis of 55.45% for the Peulh type fresh cheese obtained by calotropaïn milk coagulation of *Calotropis procera*. For this same last plant and for the same Peulh type fresh cheese, Djeddi & Misky (2014) [29] noted a cheese yield of 7.69%, and Chella & Mamou (2018) [43] of 24.9%.

The yield depends closely on the type, quality of cheese produced, the milk composition used and the followed unit operations, mainly the draining method.

3.4. Sensorial Characteristics

Pergularia tomentosa fresh cheese profiles mean sensory odor taste and texture) are presented in **Figure 3**. *Pergularia tomentosa* fresh cheese presented principally, a firm texture, (9.96 ± 1.7), followed by a crumbly (7.20 ± 0.88), and a smooth (5.66 ± 0.99) aspects.

These aspects could be a result of the *Pergularia tomentosa* enzyme acts on milk caseins and minerals at high temperature over 58˚C, which confers a moderate compactness a firm (9.96 ± 1.71) and a crumbly (7.20 ± 0.88) structure. This organization structure gives the fresh cheese dispersion (11.30 ± 1.14) and persistence in mouth (11.53 ± 1.33) over 15 seconds.

On the other hand, and during cheese making, the heat clotting temperature might relatively mask the herbal leaves odor and taste. On the contrary, it might

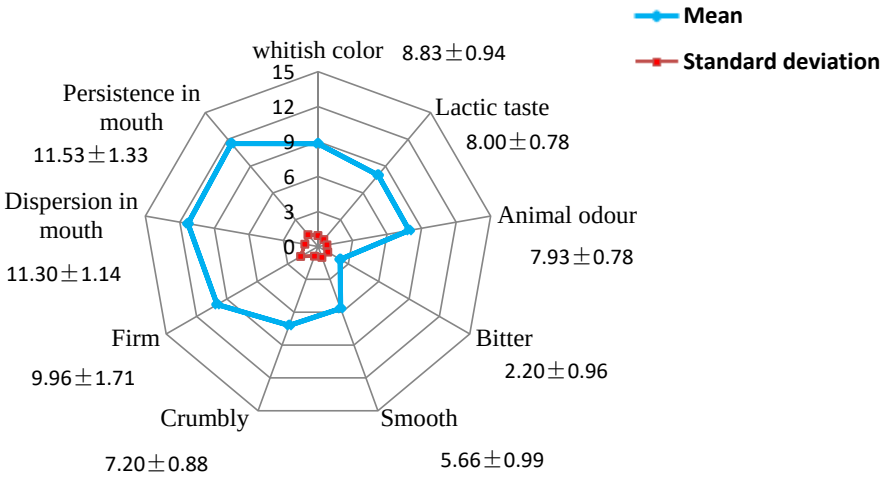

Figure 3. Sensory profile of fresh cheese from *Pergularia tomentosa* dried leaves extract.

develop lactic taste (8.00 ± 0.78) which is the main characteristic of fresh cheeses. Also, this cheese has an animal odour (7.93 ± 0.78) provided from bovine milk used for fresh cheese making.

A slight non-unpleasant bitterness (2.2/15) (is) reported. This descriptor is intense (10/15) as reported by [33] for fresh cheese prepared from raw undiluted filtrate from *Pergularia tomentosa*. Several studies on the phytochemical composition of *Pergularia tomentosa* extract show its richness in polyphenols, saponins, amides, glucosides, tannins and alkaloids. These substances impart a bitter taste to products derived from this plant when they are found at a high concentration [44] [45] [46]. However, this bitterness seems to disappear—one of the main objectives of this study—by diluting the crude extract of *Pergularia tomentosa* and, consequently, reducing the concentrations of the substances responsible for the bitterness.

Although this bitterness is unpleasant to the taste, we would like to remember that bitter substances stimulate the function of liver and allow the body to food absorbed optimally process [47].

The sensory results obtained from the fresh cheese produced are similar and agree with those given by Benyahia-Krid *et al.*, (2016) [9] for the Peulh type fresh cheese concerning its firmness, smooth appearance, dispersion and persistence in the mouth. This could be explained by the fact that the two plants *Pergularia tomentosa* and *Calotropis procera* are from the same *Asclepiadaceae* family and, therefore, have similarity in the properties and characteristics of their enzymes.

4. Conclusions

Fresh cheese made with the diluted enzymatic extract of *Pergularia tomentosa* plant has appreciable organoleptic characteristics for its whitish color, its smooth texture, as well as for its smell and its lactic taste. In addition, the disappearance of the relative bitterness in the mouth following the dilution of the leaves extract gave it a remarkable perception and an appreciable evaluation.

In fact, *Pergularia tomentosa* plant appears to be an interesting source of coagulant enzyme that could be added to the plant-derived rennet substitutes list. Thus, the physicochemical characteristics of the extract plant leaves reveal a significant coagulant potential which deserves to be exploited in the coagulation of milk and therefore in the manufacture of certain fresh or even ripened cheese.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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