

New Way to Develop Mixture of Lactic Leavens and Cardoon Flower Powder (*Cynaraca rdunculus*) in Producing Yoghurt: Approach to Immobilization

Benahmed Djilali Adiba^{1*}, Ouelhadj Akli¹, Derridj Arezki¹, Bedrani Fatiha¹ and Belkhir Fatiha¹ and Raman Yakout²

¹Faculty of Biological and Agronomical Sciences, University of Mouloud Mammeri of Tizi-Ouzou 15000, Algeria

²National School of Agronomy (ENSA), El Harrach 16000, Algeria

Abstract

The principal objective of this study was to develop a combination between lactic leavens (*Lactobacillus thermophilus*) and the cardoon flower powder (*Cynaracardunculus*) and their application on yoghurt. The milk clotting was optimized by using the two coagulant agents (with fresh and immobilized states).

The results obtained revealed that a quantity of the cardoon flower powder has a very interesting clotting rate (2.55 min) in comparison with the use of the optimized mixture M2 (75% of cardoon flower powder and lactic leavens 25%) and the optimized quantity of the leavens (0.1 g) with (3.6 and 22.58 min) as respective rates. The immobilization of the various coagulant agents improves the milk-clotting rate. Indeed, a quantity of 6 g of the beads prepared from cardoon flower powder shows a very fast speed (1.06 min) in comparison to the same quantity of the beads prepared starting from the mixture M2 (3.71) and the immobilized leavens (73 min).

The beads prepared starting from the cardoon flower powder and the mixture M2 can completely substitute the immobilized lactic leavens according to the matrix of similarity (similarity of 70%). Moreover, on the one hand, the beads containing the cardoon flower powder improve the speed of yoghurt clotting (75 min) in comparison to immobilized leaven yoghurt (270 min). On the other hand, the rheological properties were also improved (smooth structure and absence of syneresis phenomenon).

Keywords: Lactic leavens; Milk; Clotting; Cardoon; Immobilization; Substitution

Introduction

Dairy industry sector uses a number of processing aids, including lactic acid bacteria to develop fermented foods. Lactic acid bacteria are added to milk to start the fermentation process; they are used for the production of a wide range of dairy products such as cheese, yoghurt, butter and cream [1,2]. Given the high cost of these ferments, import presents a major impediment to the development of local production of dairy products. Therefore, Algeria's dependence on foreign suppliers drew our attention to search other producing local sources of milk clotting.

Although their numbers may be considered low, some work has already addressed this issue, like those conducted by the Transformation Team and Development of Agri-Food (TEPA) which covered pepsin extracted from proventricule chicken [3,4], given the importance of such enzymes, we decided to undertake an interesting result of research by exploring a different track this time; the coagulants of plant origin, which are enzymes extracted from the cardoon flowers (*Cynaracardunculus*).

The interest generated by the choice of this plant is due to its great distribution in Algeria, more precisely in the highlands, where soil and climate conditions are adequate for its development, especially during the rainy years. Rural population empirically uses its fresh or dried forms in manufacturing fresh cheese made from raw sheep milk called: djben [5].

Generally, enzymes, which have low stability and high costs, need to immobilize in porous solids and protective matrices. This technique allows dairy industries to increase their yields. Recognizing this, and in order to remedy this situation to better develop this plant, it is

useful to consider its transformation through the acquisition of new technologies, including the immobilization of enzymes and their use in the preparation of yoghurt.

This study's main objective is to substitute fully or partially lactic ferments by promoting cardoon to bring to market a new clotting agent. Optimization of milk clotting conditions has been studied.

Materials and Methods

Plant material

Dried cardoon flowers (*Cynaracardunculus*) were bought at the local markets in Tlemcen (Algeria) in May 2014. Just after being harvested, the flowers were dried at ambient air and preserved in bags at ambient temperature.

Biological material

The freeze-dried species of lactic leavens (*Lactobacillus thermophilus*) was delivered by yoghurt manufacturing in Algiers.

The pasteurized milk (1.5% Lipid Matter, pH=6.7) was bought at

*Corresponding author: Benahmed Djilali Adiba, Faculty of Biological and Agronomical Sciences, University of Mouloud Mammeri of Tizi-Ouzou 15000, Algeria, Tel: 00213783012617; E-mail: adiba.benahmed@yahoo.fr

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the local markets in Tizi-Ouzou (Algeria) delivered by D.B.K milk of Tizi-Ouzou. The powders of κ -carraghenan and milk (0% Lipid Matter) used in this study were delivered by the same yoghurt manufacturing.

Methods

This part of work was carried out at the Laboratory of Microbiology (University of Mouloud Mammeri of Tizi-Ouzou); Algeria during the period of May-July 2014.

The method defined by Fernandez et al. [6] and Aquilanti et al. [7] was used to prepare the plant enzymatic extract. A quantity of 3 g of dried cardoon flower was macerated in 100 ml distilled water at 45°C for six hours and then, filtered through Watman No: 4 filter paper. The obtained enzymatic extract is conserved at 4°C before undergoing clotting tests.

κ -carraghenan solution (3%) was used as an optimal concentration in term of suitable beads elaboration. It was sterilized at 70°C for three hours and conserved at 4°C before being immobilization process.

CaCl₂ solution (4%) was used as solidification agent for elaborating different optimized coagulant beads (cardoon flower powder, lactic leavens, and mixture).

The process of immobilization of different optimized coagulant agents was achieved by inclusion κ -carraghenan solution using searing in the sterilized area. Beads solidification was achieved in CaCl₂ solution at 4°C for 3 hours. Finally, beads were washed twice with distilled water and preserved at 4°C. Clotting tests were considered according to experimental conditions summarized in Table 1.

In all studies the milk was heated to 45°C, the average time of clotting and standard deviation were calculated from three replicates.

Morphology of different coagulants agent: Environmental Scanning Electron Microscope SEM (PHILIPS ESEM XL30; Heindoven, Netherlands) was used to study the texture properties, the microstructure of differently coagulants (cardoon flower powder, lactic leavens, sour milk issued from milk clotting using cardoon beads, immobilized lactic leavens and the immobilized mixture M2). Samples were made without metallization according to the protocol recommended for 20 μ m particle size.

Statistical study: In order to show the effect of the nature of coagulants agent (free or immobilized) on the time of milk clotting, we used the test of similarity, using Statistica software (version 6). This was to compare two-to-two similarity of the different time of clotting using different coagulants.

Results and Discussion

The results obtained revealed that a quantity of 0.3 g of the cardoon flower powder has very interesting clotting speed (2.55 min) (Figure 1a) in comparison with the optimized mixture of powders M2 (75% of cardoon flower powder and lactic leavens 25%) (Figure 1b) and the optimized quantity of the leavens (0.1 g) with the respective speeds (3.6 min and 22.58 min) (Figure 1a).

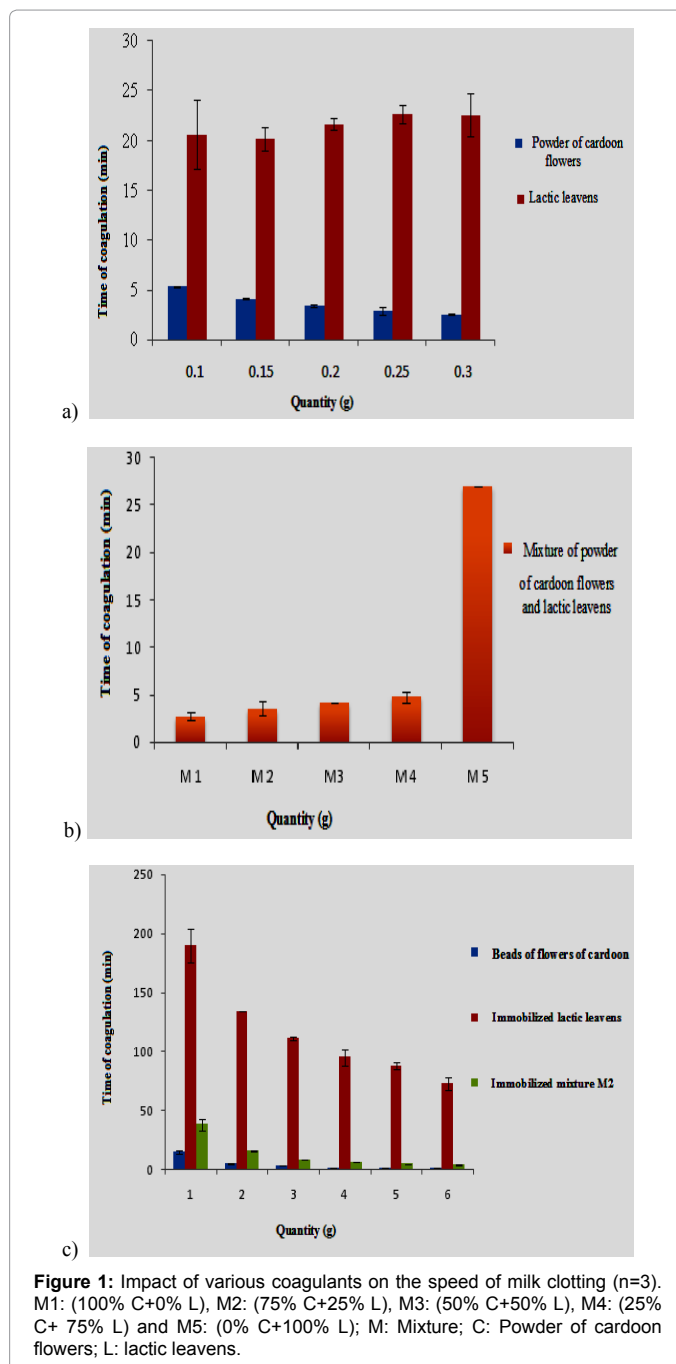
These results are similar to those reported by Feranndez -Salguera et al. [8] working on the same cardoon enzymes. Other researchers, such as Chazarra et al. [9] worked on *Cynarascolymus*; and Yousif et al. [10] on *Solanum dudium*, demonstrated the effect of using enzymes extracted from plants.

Comparison between the various coagulant agents in terms of pH, acid clotting using lactic acid bacteria revealed a slight increase in pH

Nature of coagulants agent	Quantity (g)	Clotting Conditions
Powder of cardoon flowers	(0.1-0.3)	10 ml. of milk, T=45°C
Lactic leavens	(0.1-0.3)	
Mixture (cardoon flower powder+Lactic leavens)	(0.3-0) to (0-0.1)	
Beads of cardoon flower powder	(1-6)	
Beads of lactic leavens	(1-6)	
Beads of optimized mixture M2	(1-6)	
Number of Recycling beads	(1-10)	
Water extract of cardoon flowers	(1-10 ml)	

M2=(25% lactic leavens+75% of cardoon flower powder).

Table 1: Various clotting tests.



(4.68 to 4.81). For cons, the cardoon flower powder leads to a slight decrease in pH (6.53 to 6.28). Our results are in agreement with the results of David and Forte [11], who reported that getting a good curd obtained from the enzyme clotting was observed at pH ≥ 5.2 and a curd from acid clotting under the action of lactic acid bacteria was observed at a pH of ≤ 4.6 .

Also, the mixture of various coagulant agents promotes a decrease in pH. Indeed, with such a combination, there are two types of proteolytic hydrolyzed: 1) partial hydrolysis (at the surface of the casein) with the enzymes from the cardoon flower powder or 2) complete hydrolysis (whole casein) by enzymes ferments. It was shown that enzymes derived from plants had a broad spectrum of activity in medium acid [9] and in a basic medium [12].

Furthermore, the effect of the immobilization had the major impact on the speed of milk clotting. A quantity of 6 g of the beads prepared from the cardoon flower powder shows a very fast speed (1.06 min) in comparison with the same quantity of the beads prepared starting from the mixture M2 (3.71) and the immobilized leavens (73 min).

It is worth noting that recycling provides better enzyme activity in the case of using ball base of powder of cardoon flowers (Figure 1c). A decrease in the activity was observed from the 5th recycling. The recycling of immobilized enzymes causes an increase in the latter passing from one cycle to the other (Figure 1d).

According to the similarity test, we can replace the enzymes of *Cynaracardunculus* immobilized enzymes, immobilized lactic acid bacteria (71.4% similarity) and the balls of the mixture M2 by immobilized enzymes (69.29% similarity).

The beads prepared starting from the cardoon flower powder and the mixture M2 can substitute completely call the immobilized lactic leavens according to the matrix of similarity (similarity of 70%).

(Figure 2) shows the appearance of the curds obtained by varying the amount of the beads prepared using the powder flowers thistle. It appears that the coagulation rate is directly related to the availability and the diffusivity of the enzymes within the beads to the surface. These results are reinforced with those obtained by Arima et al. [13], which proved that residual activity of the immobilized rennet was low during reuse. Similarly, Bartolini et al. [14] showed that the enzyme activity in the immobilized state remains stable.

Recycling can generate strong fermentation activity due to the multiplication of bacteria in the balls. Thus, Stenroos et al. [15] demonstrated the possibility of reusing in several cycles beads prepared from low cell concentrations.

SEM Results

Figure 3 shows the microscopic structures of the different coagulants used in this study. SEM results show that the cardoon flower powder has a homogeneous layered sheet structure (Figure 3a) whilst lactic acid bacteria contain a heterogeneous tube structure (Figure 3b).

Curds from milk clotting using cardoon flower powder or the mixture M2 were characterized by non-porous structures as various-size dense flakes (Figure 3c and 3e).

The curd from milk enzymes had a micro-lump structure (destructured flakes associated with pores) (Figure 3d). The breakdown could be due to lack of training of the network, which provides the gel permeability but the elasticity and plasticity are virtually nil [16].

Yoghurts results

Table 2 presents the main characteristics of some yoghurt. It is worth highlighting the positive effect of that cardoon flower powder on the quality and time of yoghurt clotting.

The comparison between these yoghurts also reveals that the yoghurt (2) and (3) (Figure 4) form opaque firm gels with good cohesion (lack of syneresis phenomenon). This phenomenon has been observed in the yoghurt (1).

In order to better compare the yogurt quality, it was interesting to prepare three gels and make a thorough characterizing study of the rheological parameters (viscosity, elasticity, ...). Whilst, from pharmacological point of view, it also was interesting to characterize the two yoghurts to recommend them for people who suffer from stomach acid problem.

By comparing structures of prepared yoghurts (Figure 5), it was

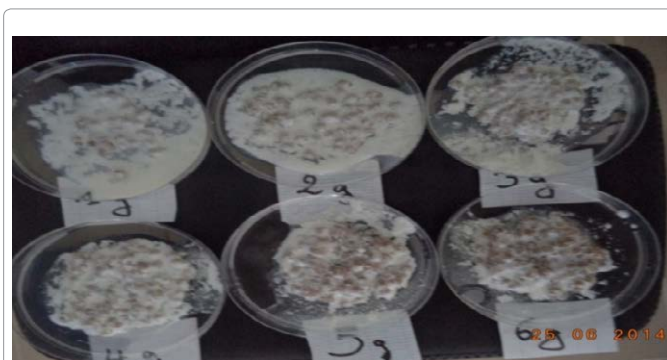


Figure 2: Curds from the milk clotting by the beads of cardoon flower powder.

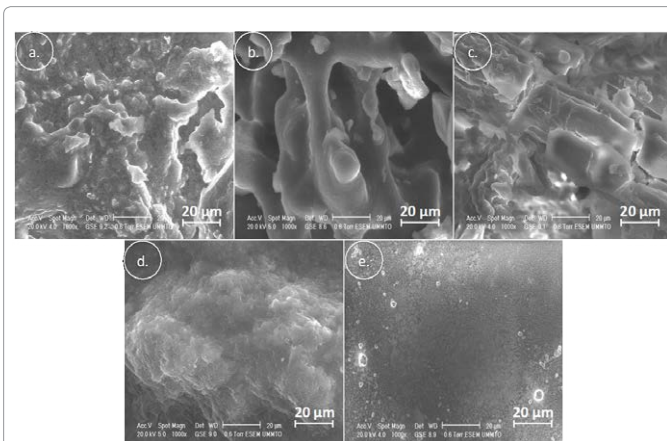


Figure 3: Microscopic structures for various sour milk and coagulants:(a): Powder of cardoon flowers; (b): lactic leaven; (c): Sour milk issued for milk clotting using cardoon flowers; (d): Sour milk issued for milk clotting using lactic leavens; (e): Sour milk issued for milk clotting using the immobilized mixture M2.

Prepared yoghurts	Time of coagulation	pH at 4°C
Yoghurt (1)	75 min	6.61
Yoghurt (2)	270 min	5.66
Yoghurt (3)	90 min	6.57
Yoghurt standards by Codex (2003)	150 to 300 min	3.6 to 4.6

Table 2: Some parameters of various prepared yoghurts.



Figure 4: Aspects of yoghurt. 1) Yoghurt fermented using sour milk issued from clotting milk by beads of cardoon flowers 2) Yoghurt fermented using sour milk issued from immobilized lactic leavens Yoghurt fermented using sour milk issued from the immobilized mixture M2.

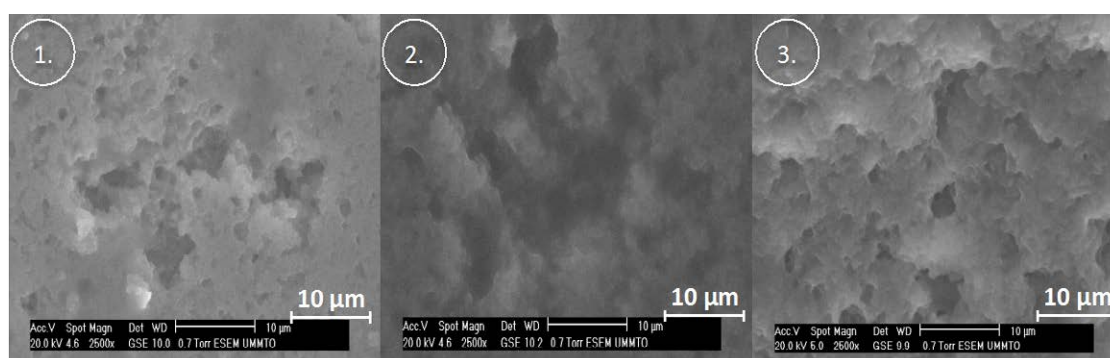


Figure 5: Microscopic structure of various yoghurts ($\varnothing=10 \mu\text{m}$).

confirmed that the yoghurt (1) is characterized by a smooth structure somewhat porous. By cons, yoghurt (2) has a rough structure in the form of multi-lump (destructured flakes). It also turns out that the combination of the two coagulants gives a firm gel form a dense, porous flake structure.

Conclusion

This work aimed at studying the feasibility of the immobilization of clotting by beads of cardoon flowers and comparing it to other conventional agents. Beads had an advantage on milk coagulation rate, and, moreover, it could be used ten times. This work allowed us to prepare such yoghurt, completely and thoroughly study its behavior and quality from physical and chemical aspects, nutritional values (lactic acid content, protein, etc.), and organoleptic and rheological attributes.

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