

Optimization of a local *Ficus carica* plant-based coagulant for fresh goat milk transformation in southern Tunisia

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Abstract. Milk coagulation represents the fundamental phase-separation unit operation in cheesemaking, determining both the final yield and curd quality. This study optimized the enzymatic coagulation process of high-solids goat milk (16.3% total solids) from the Arab breed, indigenous to the arid climate of southern Tunisia, using a local plant-based coagulant from *Ficus carica* latex. A three-factor, three-level Box–Behnken Design (BBD) was employed to evaluate the simultaneous effects and interactions of temperature (40–60 °C), coagulant dosage (2–4 mL/50 mL milk), and latex dilution (0–75%). The experimental results were modeled using a second-order polynomial equation, and the model's adequacy was confirmed via Analysis of Variance (ANOVA). A complex three-way interaction was identified, particularly highlighting a "yield valley" at intermediate factor levels. This phenomenon is attributed to a kinetic imbalance between the enzymatic proteolysis and the protein aggregation phases, modulated by the hydration state of the enzyme. Optimization was successfully achieved, identifying the conditions required to maximize cheese yield while overcoming the high buffering capacity of the high-solids goat milk. These findings provide a technical framework for the valorization of local dairy and botanical resources in arid regions.

1 Introduction

At the core of cheese manufacturing lies the enzymatic coagulation of milk, a complex phase-separation unit operation that directly influences the texture and economic yield of the final product. This process involves a sol-gel transition where the destabilization of casein micelles allows for the separation of curd and whey [1]. Traditionally, this process has relied on animal rennet; however, ethical concerns, religious restrictions, and the high economic cost of imported enzymes have led to a growing interest in plant-derived coagulants as sustainable alternatives.

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Among the most promising vegetal sources, cardoon (*Cynara cardunculus*) and fig latex (*Ficus carica*) are the most widely studied [2, 3, 4, 5, 6]. Cardoon, rich in aspartic proteases known as cyprosin, offers enzymatic specificity similar to bovine chymosin, promoting a robust protein network [7]. In contrast, fig latex contains ficin, a potent cysteine protease characterized by its high proteolytic power [10, 8]. While ficin ensures rapid clotting, its non-specific action can lead to excessive hydrolysis of the protein network if not properly controlled, which can compromise curd structure and final yield [8, 9, 11]. In the context of Southern Tunisia, fig latex represents a strategic choice due to its natural abundance in the local oases. The fig tree is a cornerstone of the Saharan agricultural system, making its latex a readily available and cost-effective bio-resource. These characteristics are particularly critical when processing the dense and nutrient-rich milk produced by the local Arab goat breed, which is remarkably adapted to the harsh environmental conditions of the region characterized by an arid climate.

Despite its potential, the use of fig latex for processing this specific milk remains under-exploited due to the complexity of mastering its enzymatic kinetics. Therefore, this study aims to optimize the coagulation process of Arab-breed milk in the Kebili region (Southern Tunisia) using Response Surface Methodology (RSM). By modeling the simultaneous interactions between three critical processing variables—temperature, coagulant dosage, and latex dilution—we seek to define the technical optimum that ensures maximum yield and processing efficiency, thereby enhancing the artisanal dairy sector and valorizing dairy biodiversity in Tunisian arid zones.

2 Methods and materials

2.1 Raw materials: milk and vegetal biomass

2.1.1 Goat milk origin and physicochemical analysis

Raw goat milk was sourced from the Arab breed, raised under the arid climatic conditions of the Kebili region (Southern Tunisia). Samples were collected under hygienic conditions and stored at 4 °C. The technological suitability of the milk was validated through a comprehensive physicochemical characterization, including pH, titratable acidity, density, and dry matter content, following AOAC standard methods. These parameters were systematically compared with FAO reference standards to ensure the milk's representativeness for high-quality cheesemaking.

2.1.2 Vegetal biomass preparation and enzymatic integrity

Fresh latex from *Ficus carica* was collected directly from the tree stems and stored in its crude, undiluted form at 4 °C to preserve its native enzymatic potency. Dilution was performed strictly at the time of the experiment to ensure maximum catalytic activity and to prevent any pre-experimental degradation of the ficin. To assess the technological efficiency of the coagulant, the Milk-Clotting Activity (MCA) and coagulating strength were determined according to the method of Berridge (1952) [12]. These preliminary assessments, which focused on the pH and enzymatic force of the latex, were instrumental in defining the experimental boundaries (factor levels) for the subsequent process optimization using Response Surface Methodology (RSM).

2.2 Statistical design and process optimization

The optimization of the coagulation process was conducted using Response Surface Methodology (RSM) based on a three-factor, three-level Box-Behnken Design (BBD). Implemented via Design-Expert® software, this approach allowed for the evaluation of simultaneous effects and interactions between critical processing variables. To achieve a comprehensive understanding of the enzymatic behavior of *Ficus carica* latex on the high-solids milk of the Arab breed, indigenous to the arid climate of Southern Tunisia, three independent variables were investigated:

- **Temperature (A):** Explored between 40 °C and 60 °C to identify the thermal optimum for enzyme-substrate interaction.
- **Coagulant Dosage (B):** Defined as the volume of the prepared coagulant added per unit volume of milk, explored between 2 and 4 mL per 50 mL of milk (corresponding to a concentration of 40 to 80 mL/L).
- **Latex Dilution (C):** Representing the specific dilution levels (v/v) for the crude fig latex, tested at 0% (pure latex), 37.5% (central point), and 75% (high level).

The experimental design resulted in 15 randomized trials, including three replicates at the center point to estimate pure experimental error. The cheese yield (R), calculated as the weight ratio of curd to milk (%), was selected as the primary response and modeled using a second-order polynomial equation. While the model integrates all three factors (A, B, and C) simultaneously, the 3D response surfaces are generated by plotting the interaction of A and B while holding C at fixed levels. This approach allows for a precise visualization of the modulating effect of dilution on the high-solids 'Kebili matrix'. The model's adequacy was verified by Analysis of Variance (ANOVA).

3 Results and discussions

3.1 Characterization of fresh goat milk and fig latex

The success of the coagulation optimization process relies heavily on the physicochemical quality of the milk substrate and the enzymatic efficiency of the fig latex. Table 1 provides a comprehensive overview of the analytical data collected for the Saharan goat milk (Arab breed) and the latex-based biomass.

Table 1. Physicochemical characteristics of raw materials

Parameter	Value	FAO Reference Range
Goat Milk (Kebili Region)		
pH	6.62	6.3 – 6.8
Density	1.051	1.027 – 1.035
Titrateable Acidity (°D)	20	14 – 21
Total Solids (%)	16.3	13.6 – 15.0
Ash Content (g/L)	8	7.7
Color	Creamy White	—
<i>Ficus carica</i> (Fig Latex) Coagulant		
pH	5.5	—
Coagulating Strength	422.5	—
Milk-clotting activity (UP/mL)	2.33	—
Coagulation Time (s)	72	—
Color	Brilliant White	—

The physicochemical analysis of Arab breed goat milk from Kebili (Table 1) reveals a substrate of exceptional quality, significantly exceeding international standards. Key findings include a total solids content of 16.3% and a density of 1.051, reflecting the breed's physiological adaptation to the Tunisian arid environment. This high-density profile is further supported by a sugar content of 9.3 °Brix and mineral ash of 8 g/L, which contribute to the milk's robust structure. Regarding the coagulant, the *Ficus carica* latex exhibits high catalytic potency, with a coagulating strength of 422.5 and a rapid reaction time (72s). This kinetics suggests a high affinity between the ficin enzyme and the dense protein network of the milk. However, the milk's high buffering capacity and nutrient density make its coagulation complex.

While the potency of ficin is essential to destabilize this robust matrix, precise control of temperature (40–60 °C), dosage (2–4 mL/50mL), and latex dilution (0–75%) is critical to prevent over-proteolysis, which can weaken curd structure and reduce efficiency. These unique characteristics justify the use of Response Surface Methodology (RSM) to identify the technical optimum for artisanal production.

3.2 Response Surface Methodology (RSM) analysis and optimization

The experimental data obtained from the Box-Behnken design were processed to establish a mathematical relationship between the processing factors and the cheese yield. The selection of a quadratic model proved to be the most appropriate for capturing the non-linear interactions occurring during the enzymatic coagulation of the high-solids milk.

Model accuracy and fit summary

The model's reliability was confirmed by an *Adjusted R*² of 0.9850, indicating that 98.5% of the variance in cheese yield is explained by the factors studied. The high correlation between experimental and predicted values, along with the strong agreement with the *Predicted R*², demonstrates the model's robustness for optimizing the coagulation of high-solids goat milk.

Response surface analysis and interactions dynamics

The 3D response surfaces generated by the model (Fig.1) provide a visual representation of the combined influence of temperature and enzyme dosage on cheese yield while highlighting the modulating effect of latex dilution. As defined in the methodology, these plots represent 'slices' of the global quadratic model at three discrete levels of factor C (0%, 37.5%, and 75%). This highlights how latex dilution shifts the interaction dynamics between thermal conditions and enzymatic dosage, particularly within the high-solids 'Kebili matrix' (16.3% TS).

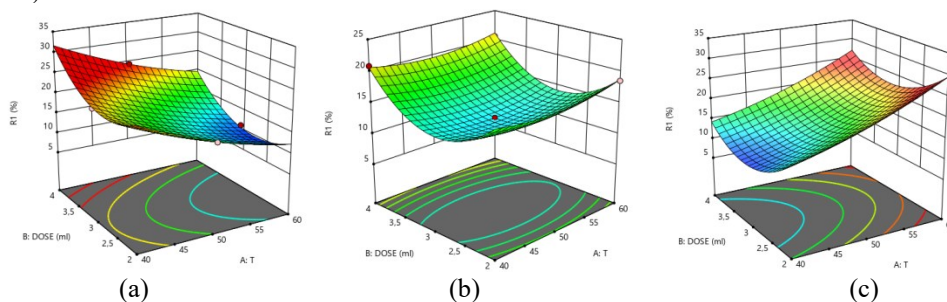


Fig. 1: 3D response surface plots showing the effect of temperature and dosage on cheese yield (R) at different dilution levels: (a) 0%, (b) 37.5%, and (c) 75%.

The topography of the response surfaces reveals several key insights into the coagulation dynamics of the Arab breed milk:

- **Yield "Valley" phenomenon and Kinetic Coordination**

All surfaces exhibit a convex (U-shaped) profile, identifying a critical minimum yield zone at intermediate factor levels. This phenomenon can be explained by a lack of coordination between enzymatic action and protein aggregation. In the nutrient-rich milk of Kebili, the high protein and mineral content create a buffering effect that slows down the structural formation of the curd. At these intermediate levels of dosage and dilution, although the ficin enzyme is sufficient to initiate proteolysis (cleavage of κ -casein), it is insufficient to overcome electrostatic repulsions quickly enough to ensure a synchronized aggregation of the micelles. This results in a weak and porous curd that loses fat and solids into the whey. The comparison between the 0% and 75% dilution plots (Fig. 1a and 1c) further supports this kinetic coordination theory. While the 'Yield Valley' persists in both scenarios, the shift of the optimal zone from 60°C (at 0% dilution) to 40°C (at 75% dilution) demonstrates that the hydration of the coagulant directly modulates the enzyme's thermal requirement to overcome the milk's buffering capacity. However, when the dosage is increased to 4 mL/50mL, the reaction becomes fast enough to overcome this kinetic barrier, creating a firm curd and restoring a high yield (R).

- **Crude coagulant vs. diluted Latex**

The 3D plot for crude latex shows maximum amplitude (with yields reaching approximately 35%) but reveals higher process instability. The steep slopes indicate that the reaction is highly sensitive to minor temperature fluctuations.

In contrast, the 37.5% dilution plot (the central point) exhibits a smoothing effect. Here, dilution acts as a distribution agent, ensuring a more homogeneous enzymatic action within the viscous Arab breed milk and preventing localized over-proteolysis, which can otherwise lead to bitter peptides and curd shattering.

- **Optimization Synergy**

The elliptical contour plots confirm a strong interaction between temperature and coagulant dosage. High yields are achieved at the boundaries of the experimental design, where thermal activation and enzyme load are precisely balanced to maximize the entrapment of solids ($R^2=0.9850$).

4 Conclusion

This study successfully optimized the coagulation of high-solids Kebili milk (16.3% TS) using local *Ficus carica* latex. The 3D response surface analysis revealed a complex convex yield dynamic ($R^2=0.9850$), driven by a three-way interaction between temperature, coagulant dosage and dilution. The results prove that balancing thermal activation with precise dosage, alongside strategic dilution (37.5%–75%), is essential to ensure homogeneous enzymatic distribution and maximize the entrapment of solids within the dense Arab breed milk. This research provides a precise scientific framework to valorise the synergy between the Saharan biodiversity (Arab breed) and indigenous plant coagulants.

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