

# The in Situ Effect of Phoenician Juniper Leaves on the Physicochemical and Microbiological Quality of Fermented Goat's Milk Stored at Room Temperature

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**Abstract.** This study aims to estimate the *in situ* effect of Phoenician juniper leaves on the physicochemical and microbiological quality of fermented goat's milk stored under ambient conditions. In this study, we used two samples. The first sample was goat's milk flavored with juniper leaves, compared to the control sample without juniper leaves. We analyzed three physicochemical parameters: pH, titratable acidity, and temperature. Furthermore, the bacteriological analysis focused on the following parameters: Total aerobic mesophilic flora (TAMF), total coliforms (TC), fecal coliforms (FC), coagulase-positive Staphylococci (CoPS), and pathogenic bacteria (*Salmonella* spp and *Listeria monocytogenes*). The physicochemical results showed a change in the pH and titratable acidity of both samples with a higher acidity for the test sample compared to the control sample. This change was marked by an increase in a load of total aerobic mesophilic flora and lactic acid bacteria, a decrease in the total coliforms and thermotolerant coliforms, and the Staphylococci load in the test sample compared to the control sample, which experienced an increase in these bacterial parameters. For the test sample, juniper leaves reduced bacterial contaminants in fermented goat's milk by 38% for total coliforms, 41% for fecal coliforms, and 89% for staphylococci. Both samples were free of *Salmonella* spp and *L. monocytogenes*. These results are probably due to the effect of bioactive compounds of *Juniperus phoenicea* L leaves known for their antimicrobial effect. This traditional practice of our ancestors can improve the quality of fermented goat's milk stored even at ambient temperature.

**Keywords.** Goat's milk, Fermentation, Leaves of Phoenician juniper (*J. phoenicea* L.), Quality, Antimicrobial effect, Storage, Southwest of Algeria.

## 1. Introduction

The consumption of dairy products in Algeria is an ancient tradition linked to livestock breeding [1]. At the Maghreb level, Algerians are the largest consumers of milk and dairy products [2]. The development of the country's resources receives great attention [3], including livestock through government programs and significant financial support for livestock breeders to produce a sufficient

amount of milk and meat [4, 5]. Known for their activities mainly related to agriculture and livestock, rural communities depend on milk, traditional dairy products, and artisanal activities, for their nutrition and as family income for livelihood. Additionally, these products are sold in local markets and are very popular products among urban communities [6].

Goat's milk is considered one of the most complete foods for humans due to its nutritional and functional properties, which contain various components such as proteins, lipids, sugars, mineral salts, vitamins, enzymes, and water [7, 8]. These properties make goat's milk a favorable environment for the growth of microorganisms [6, 9]. However, the people of Southwestern Algeria have an interesting traditional practice of preserving and flavoring milk.

Accordingly, this study aimed to evaluate the *in situ* effect of Phoenician juniper leaves on some physicochemical and microbiological properties of fermented goat's milk as a traditional product of the southwestern region of Algeria, against a control sample (without juniper leaves).

## 2. Materials and Methods

All experiments (preparation of Phoenician juniper leaves, and fermentation of goat's milk) were conducted at Mohammed Tahri University of Bechar (Algeria), for three months (April to June 2023). As for the leaves of the Phoenician juniper (*J. phoenicea* L.), they were collected from the Mecheria region (Naâma province-West of Algeria) in March (2023).

### 2.1. Sampling of Raw Goat's Milk

Raw goat's milk samples of the Arabian goat breed were obtained from a farm located in Bechar (Southwestern Algeria). According to the traditional practice described by Benyagoub [6], Benyagoub [10], and Benyagoub [11], a sufficient amount of goat's milk was evenly distributed in two sterilized glass containers with a sealed opening and filled at a rate of 3L per container (Figure 1-a), which we added to the test sample a mass of 50g of Phoenician juniper leaves (Figure 1-b), against a control sample without juniper leaves (raw goat's milk).



**Figure 1.** Goat's milk and Phoenician juniper leaves (Source: Original, 2023). (a-1): Goat's milk flavored with Phoenician Juniper leaves (Test sample). (a-2): Raw goat's milk (Control sample). (b): Dried leaves of *Juniperus phoenicea* L.

Upon receipt, goat's milk underwent physicochemical analyses according to protocols described by Benyagoub et al [4], Benyagoub and Ayat [9], Benyagoub et al [12], Benyagoub et al [13], where the parameters analyzed are as follows: pH, titratable acidity (g/L), fat content (g/L), and density.

### 2.2. Physicochemical and Microbiological Analyses of Goat's Milk with Juniper Leaves

During natural fermentation under ambient temperature, the samples underwent physicochemical analyses according to national standards by measuring the following parameters: pH, temperature (°C), and titratable acidity (g/L). For microbiological analysis, a 0,1% peptone-salt solution was used as a diluent in preparing decimal dilutions of the samples [14, 15]. The physicochemical and microbiological parameters analyzed are given in Table 1.

**Table 1.** Physicochemical and microbiological analysis of fermented goat's milk.

Physicochemical parameters	Analytical method	References
pH and Temperature	pH meter, thermometer	AFNOR [16].
Titrateable acidity (g/L)	Titrimetry method (Indicator method)	NF V04-206 [17], ISO/TS 11869 [18].
Microbiological parameters	Bacterial isolation technique and temperature of incubation	References
TAMF	The pour plate technique on PCA agar medium at 30°C.	JORA n.32 [19], ISO 4833-2 [20].
Total coliforms and thermo-tolerant coliforms	The pour plate technique on MacConkey agar medium for TC and FC at 30 and 44°C, respectively.	ISO 4832 [21], NF V08-050 [22].
Coagulase-positive Staphylococci (CoPS)	The spread plate technique on Baird-Parker agar medium at 37°C.	JORA n.70 [23].
<i>Salmonella</i> spp	-Selective enrichment media: RVB, and SCB at 41,5 and 37°C, respectively. -Isolation step on Petri dishes using Hektoen agar, and SS agar medium at 37°C.	ISO 6785 [24], JORA n.44 [25].
<i>Listeria monocytogenes</i>	-Selective broth media: Half Fraser broth, and Fraser broth at 30 and 37°C, respectively. -Isolation step on Petri dishes using PALCAM agar medium at 37°C.	Corry et al [26], JORA n.3 [27], ISO 11290-1 [28].
LAB	The pour plate technique on MRS agar and M17 agar medium in microaerophilic conditions using an anaerobic gas jar pack system at 30°C.	JORA n.43 [29], Benyagoub [6].

TAMF: Total Aerobic Mesophilic Flora, LAB: Lactic acid bacteria, TC: Total coliforms, FC: Fecal coliforms, RVB: Rappaport-Vassiliadis Broth, SCB: Selenite Cystine Broth, SS agar: *Salmonella-Shigella* agar medium, MRS: deMan, Rogosa, and Sharpe, JORA: *Official Journal* of the People's Democratic Republic of Algeria.

### 2.3. Identification of Bacterial Isolates

The isolated bacteria on the selective medium, namely *Staphylococcus* spp, *Salmonella* spp, and *Listeria monocytogenes* were identified according to protocols described by Benyagoub [6], Tille [30], Benyagoub et al. [31], Benyagoub and Mammeri [32], and Benyagoub [33].

### 2.4. Interpretation of the Analysis Results

Horizontal counting methods for bacterial loads were performed in accordance with national regulations [23]. All colonies were counted and expressed in (n Log<sub>10</sub> CFU/mL). The physicochemical and microbiological results were interpreted according to Algerian standards [34-36].

### 2.5. Abatement Rate of Bacterial Contaminants

The abatement rate of bacterial contaminants was estimated by calculating the difference between the bacterial load during fermentation and its initial load in goat's milk [37].

## 3. Results

### 3.1. Physicochemical Parameters of Raw Goat's Milk

The physicochemical analysis results of the collected raw goat's milk sample are shown in Table 2.

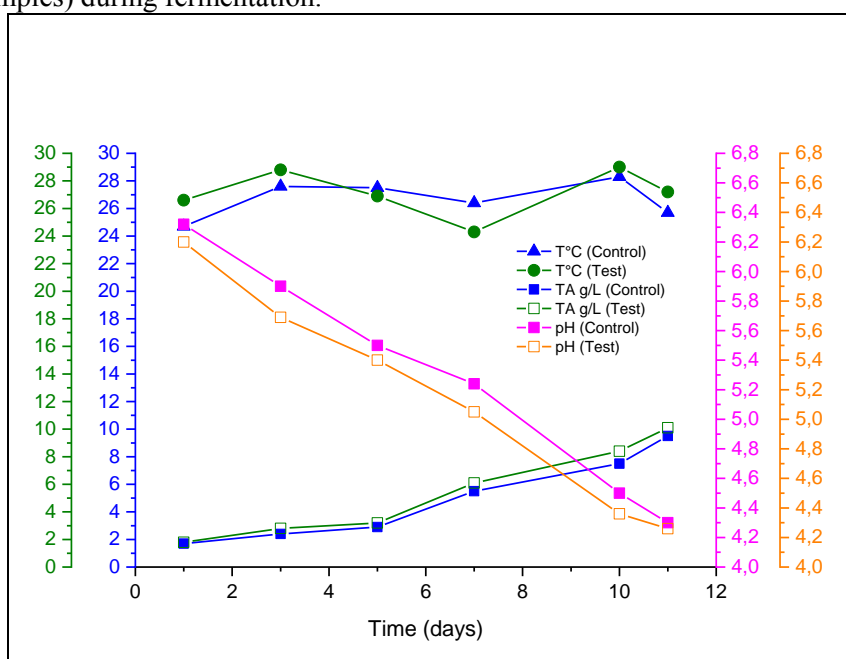
**Table 2.** Physicochemical analysis results of goat's milk sample (Source: Own study).

Sample	Physicochemical parameters			
	pH	TA (g/L)	Fat content (g/L)	Density
Raw goat's milk	6,40	1,6	3,6	1,030

TA: Titratable acidity.

### 3.2. Physicochemical Parameters of Fermented Goat's Milk with Juniper Leaves

Figure 2 shows the change in pH, temperature, and titratable acidity of fermented goat's milk (the test and control samples) during fermentation.



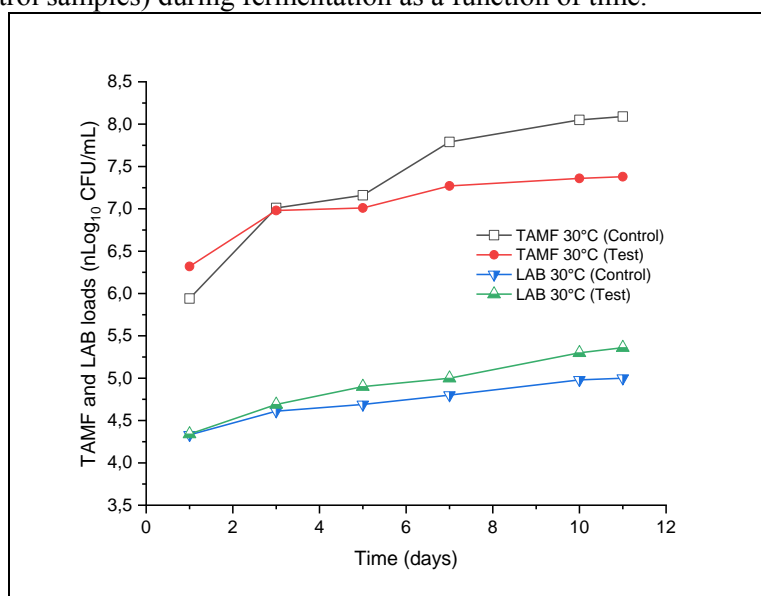
**Figure 2.** Tracking some physicochemical parameters of fermented goat's milk (Test and control samples) (Source: Own study).

TA: Titratable acidity, T °C: Temperature.

### 3.3. Bacterial Parameters of Fermented Goat's Milk with Juniper Leaves

#### 3.3.1. TAMF and LAB

Figure 3 shows the evolution of the total aerobic mesophilic flora and lactic acid bacteria of goat's milk (test and control samples) during fermentation as a function of time.



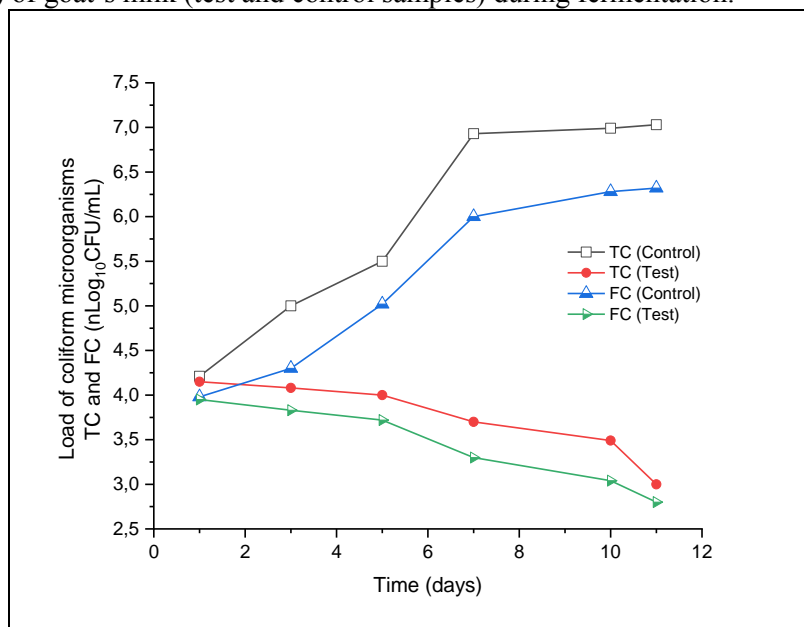
**Figure 3.** Tracking TAMF and LAB in fermented goat's milk samples (Test and control samples) (Source: Own study).

TAMF: Total aerobic mesophilic flora, LAB: Lactic acid bacteria.

### 3.3.2. Pathogenic and Contaminating Bacteria

#### 3.3.2.1. Coliforms

Figure 4 shows the evolution of coliform organisms (total coliforms) and thermotolerant coliforms (fecal coliforms) of goat's milk (test and control samples) during fermentation.

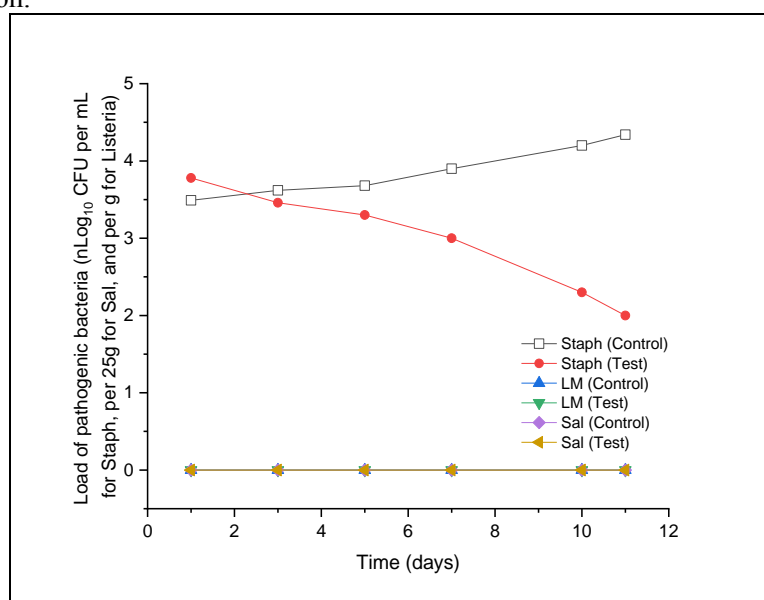


**Figure 4.** Tracking coliforms in fermented goat's milk samples (Source: Own study).

TC: Total coliforms, FC: Fecal coliforms (Thermotolerant coliforms), C: Control sample, T: Test sample.

#### 3.3.2.2. Pathogenic Bacterial Strains

Figure 5 shows the evolution of pathogenic and contaminating bacteria, namely coagulase-negative Staphylococci, *Salmonella* spp, and *Listeria monocytogenes* in goat's milk (test and control samples) during fermentation.



**Figure 5.** Tracking staphylococci and pathogenic bacteria in fermented goat's milk samples (Source: Own study).

Staph: Staphylococci, LM: *Listeria monocytogenes*, Sal: *Salmonella* spp.

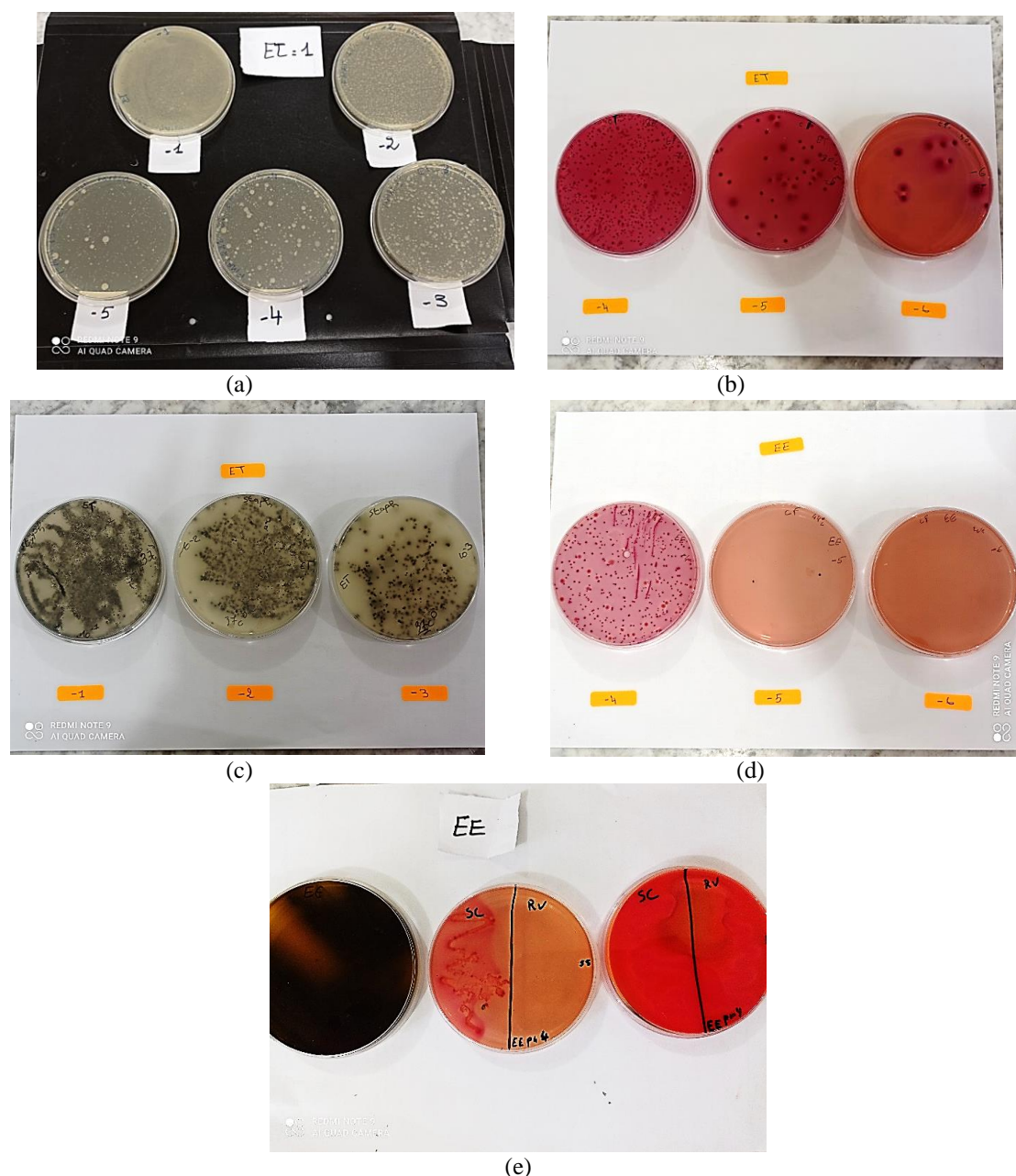
### 3.4. Bacterial Identification

The identification results of bacteria isolated from fermented goat's milk samples are shown in Table 3 and Figure 6.

**Table 3.** Identification of pathogenic bacterial species isolated from fermented goat's milk (test and control samples).

Samples	<i>Salmonella</i> spp	<i>L. monocytogenes</i>	Coagulase-positive staphylococci (CoPS)
Test sample	-ve	-ve	-ve CoPS
Control sample	-ve	-ve	+v CoNS, VP+ ( <i>S. saprophyticus</i> , <i>S. epidermidis</i> )

CoPS: Coagulase-Positive Staphylococci, CoNS: Coagulase-Negative Staphylococci, -ve: Negative culture of presumed isolate on selective medium, +ve: Positive culture of presumed isolate on selective medium, VP+: Voges-Proskauer test, Source: Own study.



**Figure 6.** Isolation of microbial species from fermented goat's milk flavored with leaves of Phoenician juniper (Original, 2023) (Ph. E. Benyagoub). (a): TAMF on PCA medium (Control

sample), (b): Total coliforms on MacConkey agar medium (Control sample), (c): Staphylococci on Baird-Parker agar medium (Control sample), (d): Fecal coliforms on MacConkey agar medium (Test sample), (e): Suspected presence of *Salmonella* spp on SS agar medium, and *Listeria monocytogenes* on Palcam agar medium (Test sample).

### 3.5. Abatement Rate of Bacterial Contaminants

The results of the abatement rate of bacterial contaminants from fermented goat's milk (test sample) are shown in Table 4.

**Table 4.** Abatement rate of bacterial contaminants from fermented goat's milk through the antimicrobial effect of Phoenician juniper leaves.

Sample	Abatement rate (%)		
	TC	FC	CoNS
Test sample (fermented goat's milk flavored with juniper leaves)	1,71-38,33	3,13-41,07	9,25-89,00

TC: Total coliforms, FC: Fecal coliforms (Thermotolerant coliforms), CoNS: Coagulase-Negative Staphylococci.

## 4. Discussion

The results of the physicochemical parameters of the raw goat's milk intended for fermentation were close to the data reported by Boumendjel et al [3], and were similar to our previous studies, except for the fat content [6, 11]. The breed, season (environmental factors), breeding techniques, diet (feeding management practices), and time of lactation are the main factors that can affect milk composition [3, 7, 9, 38, 39].

The samples under study were stored at ambient temperature (24-29°C). For this, the analyzed samples showed an average temperature of 26,7 and 27,13°C for the control and test samples, respectively. In this temperature range, the microbial growth rate was favored by the high biochemical properties of goat's milk rich in proteins, calcium, and vitamins [40].

Changes in pH and titratable acidity are mainly related to the development of the microbial load in the product, where lactose transformed into lactic acid by microorganisms creates an acidic environment [6]. According to Benyagoub [6], Benyagoub [10], and Benyagoub [11], juniper leaves flavor milk, improving the taste of milk by reducing the sour taste resulting from fermentation.

For microbiological analysis, the control sample had a higher load of TAMF (5,94-8,09 Log<sub>10</sub> CFU/mL) with a low LAB load compared to the test sample. The load of thermotolerant coliforms in collected goat's milk does not comply with national regulations set at m=3,17 Log<sub>10</sub> CFU/mL and M=4,17 Log<sub>10</sub> CFU/mL. This was probably due to non-compliance with the rules of good hygiene practices [6, 41]. However, the test sample saw a decrease in TC and FC load going from 4,15 to 3 Log<sub>10</sub> CFU/mL, and from 3,95 to 2,8 Log<sub>10</sub> CFU/mL, respectively.

The control sample showed an increase in staphylococcal load going from 3,49 to 4,34 Log<sub>10</sub> CFU/mL. The test sample revealed a decrease in staphylococcal load going from 3,78 to 2 Log<sub>10</sub> CFU/mL.

The identification results of staphylococci revealed contamination of the analyzed samples with *S. saprophyticus*, and *S. epidermidis* as coagulase-negative staphylococci (CoNS). This is similar to the results reported in the previous studies [6, 11].

The obtained results were very promising compared to those reported in the previous study [6], despite the relatively high bacterial load recorded in this present study at t=0 (reception of goat's milk) which was similar to the bacterial load of goat's milk placed in a goatskin churn (Chekoua) conducted by Benyagoub [11] probably resulting from the higher ambient temperature T°C (24 to 29°C vs. T <20°C) as well as the type of containers used for milk storage.

The amount of juniper leaves added (50g vs. 26g) resulted in a higher removal rate of bacterial contaminants load compared to that reported in the previous study [6, 11]. No contamination with *Salmonella* spp and *Listeria monocytogenes* was detected. This is similar to the results obtained by Benyagoub [6], and Benyagoub [11], and is consistent with the limits of microbial parameters set by national regulations [36].

For the test sample, juniper leaves reduced bacterial contaminants in fermented goat's milk by 38% for total coliforms, 41% for fecal coliforms, and 89% for staphylococci. Abatement of bacterial contaminants loads is mainly due to the effect of bioactive compounds found in the leaves of Phoenician juniper [11]. Phytochemical screening of leaves of Phoenician juniper conducted by Benyagoub [6], and Benyagoub [11] showed that they are rich in coumarins, tannins, terpenoids, free quinones, alkaloids salts, flavonoids with a total phenolic content (TPC) of  $322,8 \pm 38,33 \mu\text{g GAE/mg DW}$  (Dry weight). In addition, Benyagoub [11] reported that *J. phoenicea* L. aqueous leaves macerate had a pH value of 5,29. This acidic environment and the diffusion of secondary metabolites from Phoenician juniper leaves into milk could have a selective effect on microbial growth, preventing the growth of bacterial contaminants, or even pathogenic bacteria. The antimicrobial effect of *J. phoenicea* species attributed to the presence of essential oils composed of several bioactive compounds has been widely studied [42-45].

However, the antimicrobial effect is not limited to the action of juniper leaves but could be a synergistic result of bioactive substances released by LAB during the fermentation that inhibit the growth of bacterial contaminants [10, 46, 47]. With these characteristics, fermented milk is a healthy dairy product comparable to commercially available fermented milks [48].

### Conclusion

The results of this study suggest that adding leaves of Phoenician juniper to goat's milk can play the role of a barrier against microbial contaminants and pathogenic bacteria, promoting the growth of lactic acid bacteria, and thus improving the sensory quality and extending the shelf life of the fermented goat's milk stored at ambient temperature.

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### Conflict of Interest

The authors have no conflicts of interest to declare.

### Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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