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Research Article

Bioconservative effect of *Cotula cinerea* Delile on Klila cheese

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ABSTRACT

The Klila cheese has been consumed by Algerians for many centuries, likely fromantiquity to the presentday. It is a traditional, artisanal North African cheese known for its high nutritional value. Its high dry matter content allowsit to bestored for extended periods without the risk of microbials poilage. This study aims to investigate the bioconservation potential of *Cotula cinerea* Delile for preserving Klilacheese. To this end, physical analyses of the aqueous extract of *Cotula cinerea* Delile were conducted, including studies on its antioxidant and antibacterial activities, as well as a phytochemical screening of the plant. Additionally, physico-chemical, microbiological, and sensory analyses of both the control and enhanced Klila cheese samples were performed over the storage period.

The results show that the physical characterization of *Cotula cinerea* Delile powder revealed certain properties, such as dry matter content (96.79%), ash content (3.57%), and a plant yield of 10.2%. Preliminary tests demonstrated the plant's richness in bioactive compounds. Flavonoid content was evaluated using the AlCl method, revealing a concentration of 0.04mg EQ/g of extract. The antibacterial activity of *Cotula cinerea* Delile extract was tested on *Staphylococcus aureus* and *Escherichia coli*, with an effective concentration of 0.3g.

In the physico-chemical analysis of Klila cheese, it was observed that the pH and acidity of the Klila with 0.3g of *Cotula cinerea* Delile powder were higher than those of the control Klila. Conversely, the control cheeses howeds lightly higher levels of protein, dry matter, and ash throughout the storage period. Microbiological analyses revealed that the control Klila had higher levels of total aerobic mesophilic bacteria, lactobacilli, and enterococci compared to the Klila sample with 0.3g of *Cotula cinerea* Delile. Additionally, no *Staphylococcus aureus*, sulfite-reducing Clostridium, yeast, or mold were detected in either type of Klila cheese. Sensory analysis suggests that the inclusion of *Cotula cinerea* Delile at a 0.3g

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concentration is highly acceptable as an additive for Klila cheese, as it does not introduce undesirable qualities or defects for the end consumer.

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INTRODUCTION

Algeria has a long standing dairy tradition, with milk and dairy products being central to the Algerian diet and a primary source of animal protein [1]. Dairy products play a prominent role in Algerian culture, with this tradition being passed down through generations. A wide variety of fermented dairy products are traditionally prepared in Algeria for the purpose of milk bio-preservation [2]. As national consumption of milk and dairy, valued as a source of animal protein and calcium, grows, dairyremains essential in the Algerian diet [3]. Traditionally, milk was processed to extendits shelf life. Numerous scientific studies have shown that traditional dairy products made from fresh raw milk with distinct flavor and nutritional profiles are increasingly popular among consumers [4].

To prevent milk spoilage, given its perishable nature, most of the production must be processed [5]. The simplest preservation method is transforming milk into cheese, either through acidification or coagulation [6-8]. Dairy products are part of Algerian heritage and hold cultural, medicinal, and economic importance, [9] developed over time with rural women's expertise. Common fermented dairy products include Raib, Lben, Jben, and Klila [10].

Several traditional cheeses are produced in Algeria, withJben and Klila (alsoknown as Tiklilt) being the most popular [11]. Klila, traditionallymanufactured and primarily for householdconsumption, has been named by indigenous Algerian Berbers sinceancient times [12]. Itsnamelikelyoriginatesfrom the Berber word "Ikil," meaningcurdledmilk. Among the Chaoui people in the Aurès region of Algeria, when milk curdles, they say "T'kellel." In Kabylia, spontaneously curdled milkis called "Tiklitt," similar to traditional Klila cheese, whichis the curdrecovered after milk fermentation and coagulation [13].

Klila is one of Algeria's most popular traditional cheeses, produced using methods still in practice today (e.g., using the Chekoua for churning) [14,15]. This freshcheeseistypically made fromrawcow's, sheep's, or goat'smilk or a mixture, with spontaneous fermentation [16,17]. Klila can beconsumedfresh or dried. Its high dry matter content (over 80%) enables extended preservation without microbial spoilage [18].

The traditional production method of Klila, notably in the Naama Wilaya in southwestern Algeria [19]., contributes to itssensory and nutritional attributes, explaining its growing demand among consumers [20]. Research has confirmed that Klila production and consumption occuracross various Algerian regions, such as Guelma, Souk-Ahras, Oum El Bouaghi, Batna, and Khenchela [12] and furtherstudies have documenteditspopularity in Biskra, Tbessa, M'sila, Sétif, and other provinces.

Presently, chemical preservatives are the most common food preservation method, but they may have adverse health effects on consumers [21]. As a result, scientists are seeking natural bioconservatives, such as medicinal plants [22]. Biopreservation, which uses microorganisms or natural compounds, is an alternative to traditional chemical preservatives in food industries. Like other preservation methods, it aims to control pathogenic or spoilage flora growth while maintaining the product'sorganoleptic and nutritional qualities [23]. Thus, finding a natural and safe alternative to synthetic additives is essential for consumer health. In this context, this study focuses on *Cotula cinerea* Delile, also known as Guertoufa, widely used to treatailments like colic, cough, diarrhea, and digestive disorders [24].

This plant is commonly used in decoction, maceration, infusion, and inhalation. It is also used to filter go at butter due to its preservative properties [25]. This study is unique as itis the first to explore this topic, underscoring the importance of this research. The primary objective of this work is to study the bioconservation potential of *Cotula cinerea* Delile for traditional Klila cheese preservation.

MATERIALS AND METHODS

Biological Materials

Our work focused on studying the bioconservative properties of *Cotula cinerea* Delile on Klila cheese. For the purposes of our study, Klila cheese was prepared from traditional Lben obtained from raw milk. The Klila cheese samples studied were of cow's milk origin (Fig. 1A). The product used was the traditional Klila cheese made in the laboratory of the SNV faculty in Mascara. The manufacture of this type of cheese is based on the schemecited by [18]. The plant material used in this study corresponds to *Cotula cinerea* Delile, which was purchased in Ain Sefra (southwest Algeria) (Fig. 1B).

Cotula cinerea Delile

Determination of physical parameters of *cotula cinerea* delile

The dry matter content was determined following the NF B 51-004 standard (1985) [26]. Ash content was

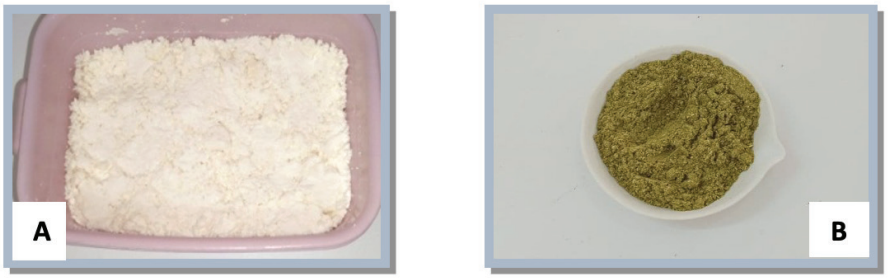

Figure 1. Biological materials used (**A.** Klilacheese, **B.** *Cotula cinerea* Delile plant '*Guertoufa*').

measured using the method described by [27]. Within cineration temperature sranging between 500–600°C. The aqueous extract preparation was performed according to the method outlined by [28], where 100 g of powdered plant leaves were macerated at room temperature in 400 mL of distilled water with agitation for 24 hours, followed by decantation. After decanting the mixture, the aqueous extract was filtered through Wattman paper and then dried at 50°C until a constant weight was achieved. A yield determination was also conducted [29].

Qualitative analysis of polyphenolic compounds in *Cotula cinerea*Delile

A phytochemical screening using a colorre action method was performed for this analysis. The characterization tests relied on precipitation and complexation reactions, forming insoluble colored complexes. The observed coloration was induced by the appropriatere agent and generally results from conjugation formation within the molecule [30]. The tests performed included: Steroid Test [31], Terpenoid Test [32], Tannin Test [33], Flavonoid Test [34], Alkaloid Test [35], Saponin Test [36], Phenol Test [37], and Coumarin Test [38].

Determination of total flavonoid contain

Flavonoid content was measured based on the method described by [39]. One milliliter of an ALCl3 solution (2%) wasadded to 1 mL of the sample or standard solution (prepared in methanol) at various concentrations. After allowing the mixture to react for 10 minutes, absorbance was measured at 430 nm. Flavonoid concentration in the extracts was calculated from a calibration curve established with quercetin (0-14 µg/mL) and expressed as micrograms of quercetin equivalents per milligram of dry matter (µg EQ/mg DM).

Evaluation of antibacterial activity

The antimicrobial activity assessment determined the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the aceticextract of *Cotula cinerea* Delile. The study used a macro dilution method in liquid media. *Escherichia coli* (Gram-) and *Staphylococcus aureus* (Gram+), strainsfrom the educational laboratory (Faculty of Natural and Life Sciences, University of Mustapha Stambouli, Mascara), wereused to evaluate the antibacterial activity of the aqueousextract of *Cotula cinerea* Delile. Following inoculum preparation and extract dilution, MIC, MBC, and the MBC/MIC ratio were determined.

Biopreservation of Traditional Klila Cheese

For the biopreservation of this traditional cheese, *Cotula cinerea* Delile (locallyknown as Guertoufa) was used. Preliminary tests led to a chosen concentration of 0.3% of the plant, homogenized with Klila cheese, while a negative control sample was prepared without the plant addition. Samples were stored at refrigeration temperature (5°C), and analyses were performed weekly (Days 0, 7, 14, 21, and 28).

Physicochemical analyses

To measure pH, Klila cheese samples were diluted 1:10 in distilled water, from which pH was measured [40]. Titrable acidity was determined by titration with 0.1 N NaOH. Protein content was measured via the formal dehyde titration method [41]. Total dry extract (TDE) was determined by drying at 103±2°C for 3 hours [42]. Ash content was determined by incinerating fresh Klila samples in a muffle furnace at 525±25°C until whitishash was obtained [43].

Detemination of microbiological quality

Aerobicmesophilic flora (FMAT) enumeration was conducted on nutrient agar (30°C, 48–72 hours, aerobic conditions, Biokar) according to NF EN ISO 4833-1:10-2013 [44]. Presumptiveme sophilic lactobacilli were tested on MRS agar, [45] anaerobic incubation at 30°C for 48–72 hours. Enterococci were numerated on BEA (Biokar) with aerobic incubation at 37°C for 24 hours. Total and fecal coliform counts were performed on bright green lactose broth (VBL) using three tubes per dilution with incubation at 37°C for 24–48 hours [46]. Using the most probable number method. Yeasts and molds were detected on oxytetra cycline glucose agar (OGA) [47]. *Staphylococcusspp*. Weretested on Baird Parker agar and incubated at 37°C for 24 hours [48]. Clostridium Sulfito-reductor spores were numerated on liver agar (VF) [49].

Sensory analysis

A triangular test was conducted for sensory analysis [50].

Statistical Study

All statistical analyses were performed using the R software (R Development Core Team, 2008). In this study, two types of statistical analyses were conducted: ANOVA and Tukey's test.

RESULTS AND DISCUSSION

Results of Cotula Cinerea Delile Analyses

Physical parameters

The results of somephysicalparameters of *Cotula cinerea* Delile powder showthat the ash content is 3.57%, the moisture content is 96.79% and the dry matter content is 3.21%. The aqueousextract of the *Cotula cinerea* Delile plant is brown in colour and viscous in appearance, with a yield of: 10.2%.

Table 1. Results of phytochemical screening of plant extracts

	Metabolitestested	Results
1	Steroid test	++
2	Terpenoid test	+++
3	Tannin test	+++
4	Flavonoid test	++
5	Alkaloid test	+
6	Saponin test	++
7	Phenol test	+
8	Coumarin Test	++

(+): Weakpresence; (++): Averagepresence; (+++): Strongpresence.

Qualitative analysis of polyphenolic compounds

Concerning the qualitative analysis of polyphenolic compounds and more specifically the phyto chemical analysis of *Cotula cinerea* Delile extract. Our phyto chemical study of *Cotula cinerea* Delile extractindicates a high presence of terpenoids and tannins, a moderate presence of steroids, coumarins, flavonoids and saponins, and a low presence of phenols and alkaloids. The results of this phytochemical screening are presented in Table 1 below.

Antioxydant activity

For antioxidant activity, flavonoids were measured using a colorimetric method based on the use of a standard, Quercetin. Our results indicate that our plant contains a low quantity of flavonoids of the order of 0.04 mg EQ/g extract.

Evaluation of antibacterialactivity

The antibacterial activity of *Cotula cinerea* Delile was studied in vitro using the macro-dilution method against two bacterial strains: *Staphylococcus aureus* and *Escherchia coli*. The results are shown in Table 2.

It can be seen that the MIC value was 0.312 g/ml for *Escherichia coli*, while *Staphylococcus aureus*was more sensitive with a MIC of 0.039 g/ml and the same BMC value = 0.312 for bothstrains.

In order to study the effect of the *Cotula cinerea* Delile plant on the preservation of traditional Klila, we compared

Table 2. Antimicrobialactivity of *Cotula cinerea* Delile

	MIC	BMC	CMB/CMI	Type of activity
Staphylococcus aureus	0.039	0.312	1.25	Bactericide
Escherchia coli	0.312	0.312	1	Bactericide

Table 3. Results of physico-chemical analyses

		pH	Acidity °D	Protein content (%)	Ash content (%)	TDE Total dry extract (%)	Moisture (%)
J0	**Control**	4.38±0.05	10.67±0.58	3.47±0.20	1.53±0.12	44.47±0.20	55.53±0.20
	0.30%	4.19±0.01	12.67±0.58	2.11±0.26	1.27±0.12	36.42±0.20	63.58±0.20
J7	**Control**	5.02±0.01	9.67±0.58	1.47±0.10	2.20±0.20	55.67±0.41	44.33±0.41
	0.30%	5.46±0.01	5.67±0.58	1.13±0.20	1.40±0.20	42.72±0.20	57.28±0.20
J14	**Control**	4.91±0.02	7.67±0.58	1.42±0.10	2.87±0.31	58.83±0.49	41.17±0.49
	0.30%	5.08±0.02	13.67±0.58	1.59±0.10	1.80±0.20	44.56±0.31	55.44±0.31
J21	**Control**	5.40±0.01	11±1.00	3.48±0.10	3.47±0.12	60.28±0.20	39.72±0.20
	0.30%	5.52±0.03	53.33±0.58	1.71±0.18	2.20±0.20	48.56±0.30	51.44±0.30
J28	**Control**	5.41±0.02	16±1.00	3.36±0.10	3.87±0.12	64.63±0.20	35.37±0.20
	0.30%	5.54±0.02	57±1.00	2.16±0.10	2.60±0.20	49.41±0.20	50.59±0.20

two Klila samples, control and Klila with 0.3g of *Cotula cinerea* Delileadded.

The effect of this plant was observed by monitoring the following parameters:

- The physico-chemicaleffect
- The microbiologicaleffect
- Sensoryeffect

Results of the Bioconservation of Traditional Klila Cheese

Physico-chemical analyses

The results of physico-chemical quality analyses of two types of Klila cheese analysed from D_0 to D_{28} (Klila control, Klila with 0.3% *Cotula cinerea* Delile) are shown in Table 3.

pH variation and titratable acidity in Klila cheese

The pH variation results for the two types of Klila cheese analyzed from Day 0 to Day 28 are shown in Figure 2A. As observed in Figure 2A, the average pH of the control Klila ranged from 4.38±0.05 to 5.41±0.02. For the Klila with the addition of 0.3 g/100 g *Cotula cinerea Delile*, the pH rangedfrom 4.19±0.01 to 5.54±0.02. These values are relatively similar, with a marked increase in pH levels over the preservation period. Initially, the pH of the enhanced Klila was lower than the control, but a slight increase in pH for the enhanced Klila compared to the control was observed during storage. According to the statistical analysis (ANOVA and Tukey tests), significant differences were noted between days and doses. For interaction results (Days x Doses), the highest pH was observed in Klila with 0.3% *Cotula cinerea Delile* on Day 28 at 5.54, while the lowest was in the enhanced Klila on Day 0 at 4.19.

The titratableacidity results for different Klila samples are presented in Figure 2B. Titratableacidity values for the control Klila varied from 7.6±0.58 °D to 16±1.00 °D, while for Klila enhanced with 0.3% Cotula cinerea Delile, acidity ranged from 5.67±0.58 °D to 57±1.00 °D. The results were similar from Day 0 to Day 7, with an increase in acidity for the enhanced Klila from Day 14 to Day 28. Statistical analysis (ANOVA and Tukey tests) showed significant differences between days and doses. For interaction (Days x Doses), the highest acidity (57 °D) was observed in the Klila with 0.3% Cotula cinerea Delile on Day 28, while the lowest (5.67 °D) was in the enhanced Klila on Day 7.

Protein and Ash Content in Klila Cheese

The protein content for different Klila samplesis shown in Figure 2C. In the control Klila, protein content ranged from 1.42±0.10% to 3.48±0.10%, while in Klila with 0.3 g of Cotula cinerea Delile, it ranged from 1.13±0.20% to 2.16±0.10% from Day 0 to Day 28. The control Klila exhibited higher protein content than the enhanced sample, except on Day 14. According to statistical analysis (ANOVA and Tukey tests), significant differences were noted between days and doses. For interaction (Days x Doses), the highest protein level was observed in the control Klila on Days 0, 21, and 28 (3.36 to 3.47%), while the lowest (1.13%) was recorded in the enhanced Klila on Day 7.

Ash content results are presented in Figure 2D. Ash content in the control Klila ranged from 1.53±0.12% to 3.87±0.12% from Day 0 to Day 28, while in Klila with 0.3% *Cotula cinerea* Delile, it ranged from 1.27±0.12% to 2.60±0.20%. The ash content was higher in the control Klila than in the enhanced sample, with a gradual increase over the storage period. Statistical analysis (ANOVA and Tukey tests) indicated significant differences between days and doses. For interaction (Days x Doses), the highestash content (3.47% to 3.87%) was observed in the control Klila on Days 21 and 28, while the lowest values (1.27%, 1.40%, and 1.53%) were found in the enhanced Klila on Days 0 and 7, and in the control on Day 0, respectively.

Total dry extract in Klila cheese

Total dry extractresults are presented in Figure 2. In the control Klila, the total dry matter ranged from 44.47±0.20% to 64.63±0.20% between Days 0 and 28. For Klilaenhancedwith 0.3 g Cotula cinerea Delile, itranged from 36.42±0.20% to 49.41±0.20%. The dry extract content showed an inverse relationship with moisture content. A notable increase in total dry extract was observed in the control Klila throughout the storage period, compared to the enhanced Klila. Statistical analysis (ANOVA and Tukey tests) for moisture content revealed significant differences between days and doses. For interaction (Days x Doses), the highest dry extract was found in the enhanced Klila with 0.3% *Cotula cinerea Delile* on Day 0 (63.58%), while the lowest was in the control Klila on Day 28 (35.37%).

Microbiological analysis results

Microbiological analysis results for both control and enhanced Klila samples with 0.3 g Cotula cinerea Delile are shown in Figures 3 and 4. For total aerobic mesophilic flora (FMAT), count sranged from 7.8 x Log UFC/g to 26.8 Log CFU/g for the control and from 7.4 Log CFU/g to 13 Log CFU/g for the enhanced Klila. Microbial loads increased in both samples over the storage period. For *Lactobacillus* counts, the control ranged between 2.78 Log CFU/g and 7.80 Log CFU/g, while the enhanced Klila with 0.3 g Cotula cinerea Delile ranged between 2.30 Log CFU/g and 6.59 Log CFU/g. Higher microbial loads were observed in the control Klila compared to the enhanced sample from Day 0 to Day 28. By Day 28, the control Klila sample showed uncountable bacterial levels, likely due to spoilage. Environmental factors, such as temperature, may influence bacterial growth. *Enterococci* counts for the control Klila varied from 3.0×10^2 CFU/g to 2.0×10^2 CFU/g, and for the enhanced sample from 1.1×10^2 CFU/g to 0.8×10^2 CFU/g. Lower counts were observed in the enhanced sample from Day 0 to Day 28.

Fecal coliforms were present only in the first week, with counts of 0.4×10^2 CFU/g for the control and 1.4×10^2 CFU/g for the enhanced Klila on Day 7. From Day 7 to Day 28, coliforms

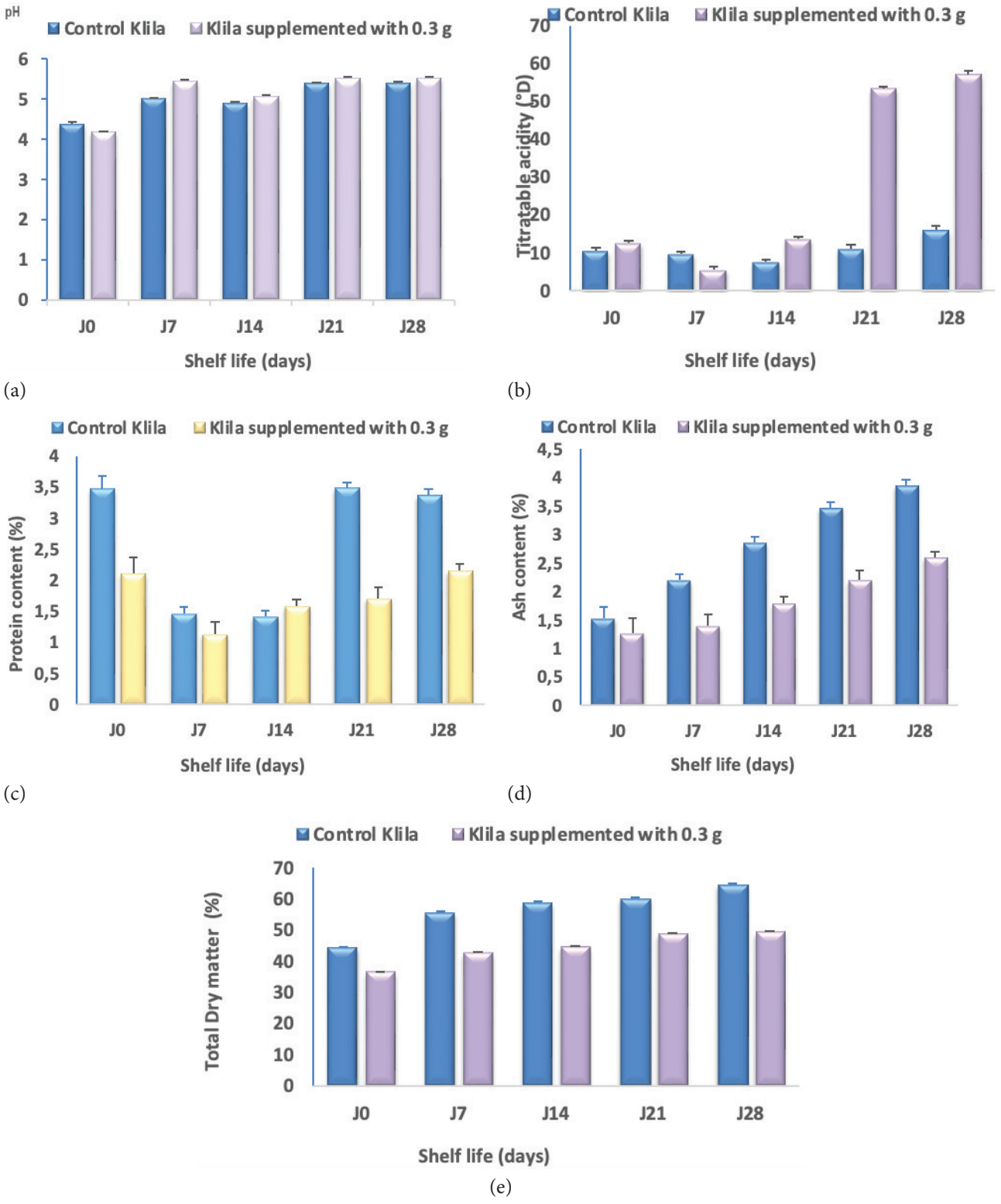

Figure 2. Variation of the different physicochemical parameters in the various Klila cheeses studied from Day 0 to Day 28 (a) pH, b) Titratable acidity, c) Proteins, d) Ash, e) Total Dry Matter).

were absent in both samples, indicating good hygiene practices during production. Yeasts, molds, *Staphylococcus aureus*, and Clostridium sulfite-reducerswere absent in both samples through out storage. Statistical analysis (ANOVA) showed no significant differences, indicating no variation between doses, days, and interactions (Dose x Day).

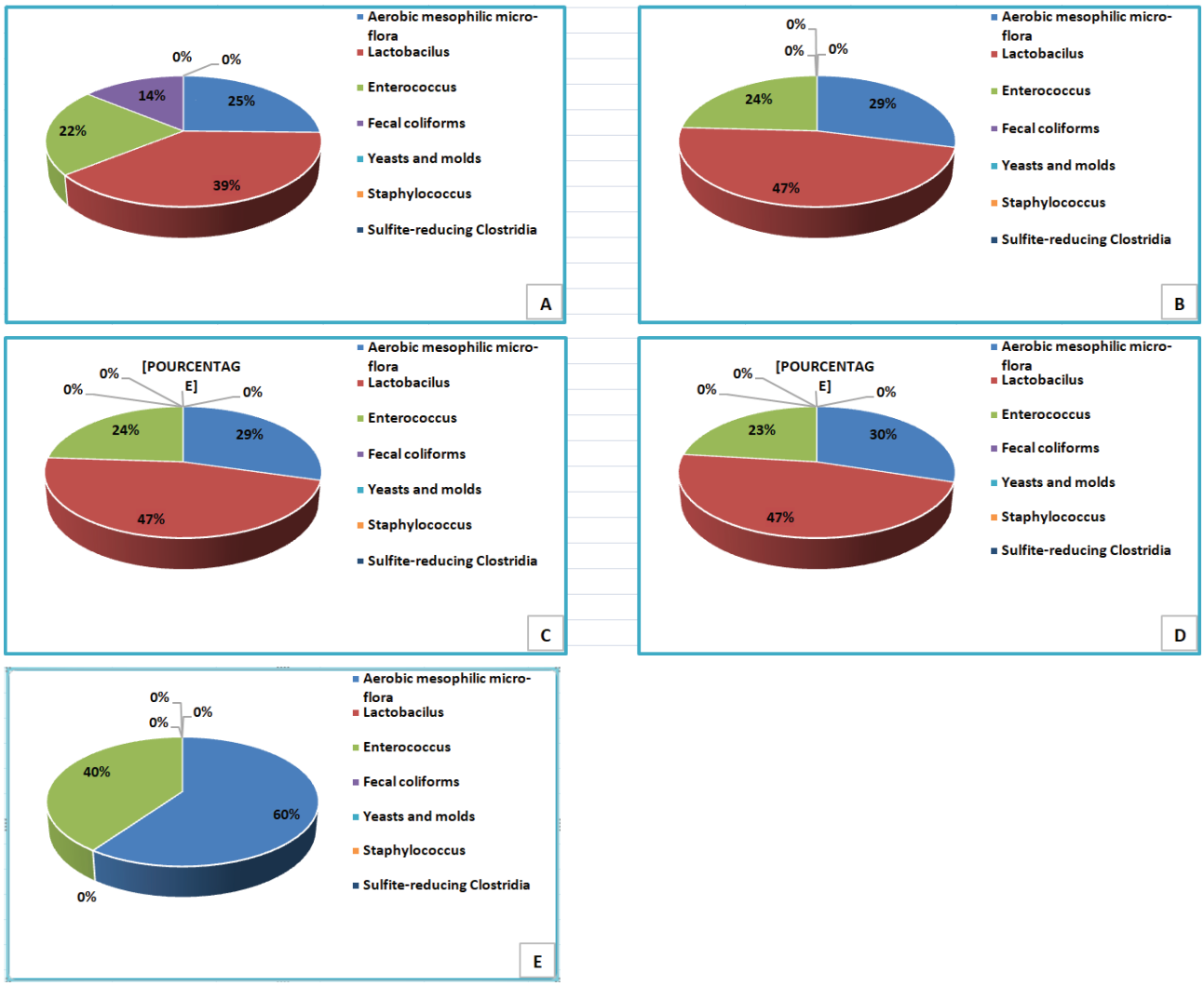

Figure 3. Distribution of the different microorganisms isolated from control Klila throughout the entire storage period.

Sensory analysis results

In this test, assessors were presented with three samples, two of which were identical. These samples were arranged in six possible combinations, with results summarized in the Table 4 below.

Half of the tasters found it easy to differentiate between the control Klila cheese and the enhanced Klila cheese. The reasons for the observed differences between the cheeses were not systematically recorded. Generally, color and odor are the primary factors for differentiation, while aroma and

Table 4. Results of the triangular test

Jury	Order of presentation	Sampleselection	Results
1	AAB	B	1
2	ABA	A	0
3	BAA	A	0
4	BBA	A	1
5	BAB	A	1
6	ABB	A	0

A: Klilawith the addition of *Cotula cinereaC* Delile; **B:** Control Klila (Witness).

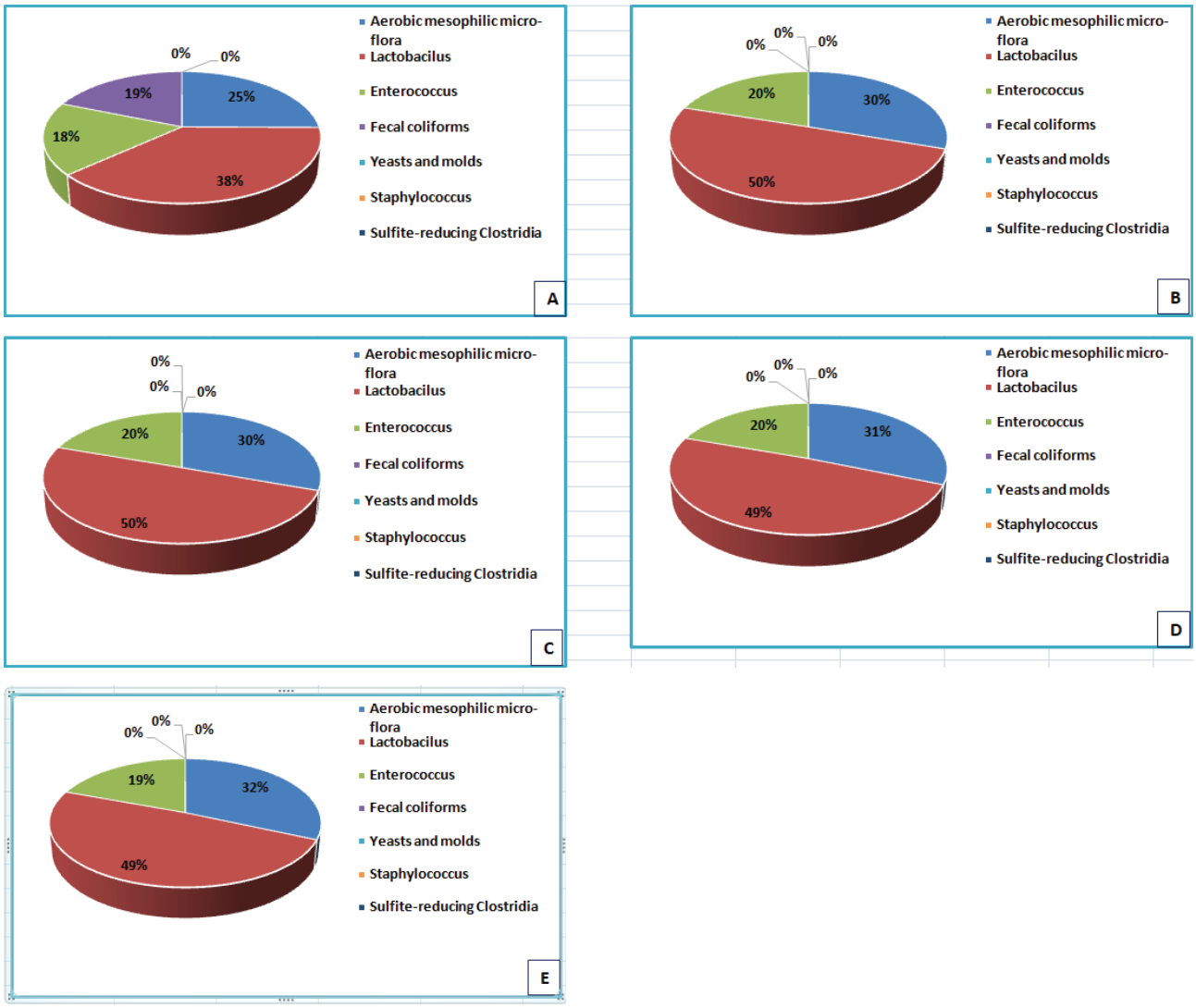

Figure 4. Distribution of the different microorganisms isolated from Klila containing 0.3% *Cotula cinerea* Delile throughout the entire storage period.

appearance are less frequently cited. In some rare instances, rancid sensations (for the enhanced Klila) and a bitter taste were also reported.

Our pH measurements on day J0 are close to those reported by [12] which indicate a pH of 4.6, as well as those of [16], who reported a pH ranging from 3.8 to 4.8, with an average of 4.2, in their studies on dried Klila. These values are comparable to those obtained by [51]; who report a pH of 4.71. Subsequently, pH values gradually increase during storage, from day J7 to day J28, varying from sample to sample due to several factors, including the method and duration of cheese preparation, the type of milk used, and the animals' diet [52].

The acidity levels measured in this study are lower than those reported by [53] at 27.71 °D, and by [51]; at 42.24 °D. Our results also fall below those of [16], who reported an average acidity of 79.4 °D for dried Klila, and those of [54], who found a value of 99 °D for fresh Klila. According to the study by [20], the titratable acidity of Klila varies between 38 °D and 42 °D. Boubekri and Ohta [55] reported titratableacidity values (39 and 42.25 °D) for two Klila samples, which are higher than our results.

The pH and acidity depend on the contents of casein, mineralsalts, and ions [56]; the hygiene conditions during milking, the total microbial flora, and its metabolic activity [57]. This increase in pH may also be attributed to the effect of the plant *Cotula cinerea* Delile.

Regarding protein content, the values obtained in our study are lower than those reported by [58], which indicated values for fresh Klila ranging from 7.11% to 13.48%. They are also lower than the values reported by [51]; who provided a rate of 53.86 g/100 g. Protein content varies based on factors such as breed, age, lactation stage of the

cow, milking frequency, diet, climate, season, and genetic criteria.

Our ash content results are slightly higher than those indicated by [59], who found an ash content of 1% in fresh Klila, and those reported by [58], which were 0.34% for dried Klila. It is observed that the control Klila is richer in minerals than the Klila supplemented with 0.3% of *Cotula cinerea* Delile. The variability in ash content may depend on several factors, including lactation stage, health status of the animal, climatic conditions, and feeding system.

Our findings regarding dry matter content are consistent with those of [53], who reported a dry matter content of 35.03% in dried Klila. However, this rate remain slower than the values reported by [51], who indicated a moisture content of 12.53% and consequently a total dry extract content of 87.47%. Moreover, in the study by [59], the dry matter content of freshcow Klila was found to be 36.6 g/100 g. The dry extract corresponds to the complement of water content up to 100%, influenced by the dry matter of milk and the degree of drainage, as the removal of whey leads to a significant increase in the dry matter content of the cheese [59]. This observation maybe explained by the addition of water to the milk, which reduces the total dry extract content, as well as by climatic, dietary factors and the lactation stage [60].

The count of total viable microorganisms (TVM) reflects the general microbiological quality of a natural product and allows for monitoring its evolution. The total germ count can indicate the state of freshness or decomposition (spoilage) of the product [61]. The values obtained during our study are similar to those found by [17], ranging from 1.43×10^5 CFU/g to 1.01×10^6 CFU/g. The TVM count by [53] yields an average value of 1.24×10^3 CFU/g, while [16] report an average value of 2.22 CFU/g. Furthermore, [62] noted an average TVM value of 1.2×10^6 CFU/g for traditional Klila. The failure to adhere to good hygiene practices during milking, collection of raw milk, or its processing into Klila is likely responsible for this observation.

*Lacticbacteria*are widely distributed in nature and are involved in numerous transformations, not only of milk but also of plant and meat products [17] noted an average Lactobacillus count for fresh Klila ranging from 2.6×10^2 to 4.0×10^3 CFU/g, while in the study by [18], a value of 6.15 log CFU/g isfound, corresponding to 1.4×10^4 CFU/g in dry Klila cheese. The obtained rate ishigherthan the indicated values.

Enterococci are lactic bacteriaused for centuries in food processing. These microorganisms play an essential role in preservation (extendingstorage time) and in the bacteriological quality of foods, while respecting their nutritional and organoleptic properties. However, they are also markers of fecal contamination (*Enterococcus faecalis and Enterococcus faecium*) and are involved in the emergence of nosocomial diseases [63]. Our results differ from those reported by [53], who indicated a value of 4×10^5CFU/g, and are also lower than the results of [10], who reported a value of 5.5×10^5 CFU/g in fresh Klila.

Coliforms are microorganisms associated with spoilage. Our results are consistent with those obtained by [17], which indicate the absence of this germ in this type of traditional cheese. According to [64], the presence of coliforms reflects a lack of hygiene in the animals' environment, during milking, and during the storage and preservation of milk. The absence of coliforms in the later days of storageis likely due to the hygienic quality of the cheese and may also depend on the intrinsic properties of *Cotula cinerea* Delile, which has antimicrobial effects that inhibit *E. coli*.

Our results show also the absence of yeasts and molds in both samples; on the other hand, [16] report yeast and mold counts ranging from 1.5×10^2 CFU/g to 2.2×10^2 CFU/g, while [18] found very high values for this type of count exceeding 5.7 log CFU/g in dry Klila cheese, likely due to poor drying conditions. The presence of yeasts and molds in the analyzed products could be attributed to external contamination, such as that from the stable or laboratory environment (walls, floor, air).

In the other hand; Our results show the absence of Clostridium sulfito-reductans in the two samples analyzed during their preservation. This finding aligns with the results for fresh Klila obtained by [53], who explained the absence of this bacterium in the analyzed samples by the lack of contamination.

Also the results obtained show a complete absence of this pathogenic germ in both types of Klila. Our findings are consistent with those obtained by [53], who indicated a total absence of this germ in all dairy product samples, revealing good hygiene practices from sampling through laboratory handling, as well as the good health of the animals (udder health).

CONCLUSION

The use of biopreservation techniques appears to be a promising alternative to methods involving "chemical" molecules and should meet consumer demands for "more natural" products. Consequently, the evaluation of such properties remains an interesting and useful task, particularly for identifying new sources of natural antioxidant and antimicrobial agents. In this context, we aimed to assess the bioconservation potential of *Cotulacinerea* Delile on the traditional cheese known as Klila. To this end, our study focused on analyzing the physical parameters, antioxidant and antibacterial activity of the aqueous extract of *Cotula cinerea* Delile, as well as the physico chemical, microbiological, and sensory analysis of this type of traditional cheese.

The results of our study allowed us to identify some properties of *Cotula cinerea* Delile powder, such as the dry matter content (3.21%), ash content (3.57%), and plant yield (10.2%). Furthermore, the phyto chemical study indicates a high presence of terpenoids, moderate presence of steroids, coumarins, flavonoids, and saponins, and low presence of

phenols and alkaloids. Additionally, regarding antibacterial activity, the aqueous extract obtained by maceration of *Cotula cinerea* Delile showed strong activity against the tested bacterial strains at a concentration of 0.3g. The physico chemical analysis revealed that the pH and acidity of Klila supplemented with 0.3 g of *Cotula cinerea* Delileare elevated compared to the control Klila. Moreover, for protein content, electrical conductivity, and ash content, the control Klila exhibited slightly higher values than the Klila supplemented with 0.3g during the entire preservation period. The results of the microbiological analyses indicate that the total viable microbial count (TVMC), lactobacilli, and enterococci were present at significant levels in the control Klila compared to the improved Klila with 0.3 g of *Cotula cinerea* Delile. Furthermore, there was a complete absence of *Staphylococcus aureus*, Clostridium sulfito-reductans, and yeasts and molds in both types of analyzed Klila cheese. Based on these results, we can deduce that the extract of *Cotula cinerea* Delile appears to be suitable as a natural agent for the preservation of food products.

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AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was not used in the preparation of the article.

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