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EFFECT OF *CISTUS LADANIFERUS* L. ESSENTIAL OIL ON THE MICROBIOLOGICAL, PHYSICOCHEMICAL AND TEXTURAL QUALITY OF MINCED CHICKEN PATTIES DURING REFRIGERATION

Laabed Mohssen[1]*, Bouslimani Nouri[2], Abdelmounaim Khemmouli[3], Yassine Noui[1]
[1] Institute of Veterinary and Agronomic Sciences, University of Batna1, Food Science Laboratory, Batna, Algeria
[2] Department of Agronomy, Institute of Veterinary and Agronomic Sciences, University of Batna1, Food Science Laboratory, Batna, Algeria
[3] Department of Agronomic Sciences, Faculty of Natural and Life Sciences, Ferhat Abbas University, Laboratory of Valorisation of Natural Biological Resources, Sétif 1, Algeria

Keywords: *Cistus ladanifer essential oil, antimicrobial activity, meat preservation, minced meat, alginate encapsulation*

Abstract
This study investigated Cistus ladanifer essential oil (CLEO) as a natural preservative for minced chicken patties during 10-day storage at 4 ± 1 °C. CLEO was applied either directly or encapsulated in sodium alginate beads and compared to controls (sterile water). Encapsulated CLEO demonstrated superior efficacy, reducing total mesophilic bacteria by 2.03 log CFU/g, Enterobacteriaceae by 1.83 log CFU/g, and Pseudomonas spp. by 1.91 log CFU/g versus controls ($p < 0.05$). It also stabilized pH, maintaining values 1.6 units lower than spoilage thresholds. Color (L, a*, b*) and texture (hardness, cohesion, springiness) were significantly preserved, with encapsulated CLEO showing 25 % greater texture retention than direct application. The results highlight CLEO's dual antimicrobial-antioxidant capacity, enhanced by encapsulation and controlled release. While prior research confirmed CLEO's bioactivity, this is the first demonstration of its meat preservation potential. The findings support CLEO-alginate systems as a clean-label solution for extending poultry shelf life, aligning with industry demand for natural alternatives to synthetic additives.*

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Introduction

Minced meats are highly perishable due to their high water activity, nutrient-rich composition, and increased surface area from processing, which promote rapid microbial growth and spoilage [1]. Unlike eggs, which contain natural antimicrobial agents such as lysozymes, or citrus fruits with their naturally low pH inhibiting microbes, fresh poultry and minced meats lack inherent protective barriers, making them far more susceptible to deterioration.

Plant essential oils (EOs) have emerged as effective natural food preservatives due to their antimicrobial and antioxidant properties, derived from herbs and spices, such as oregano, thyme, and cinnamon. EOs contain bioactive compounds that inhibit bacterial growth and delay oxidative spoilage in foods. However, challenges such as strong flavor, volatility, and variable efficacy require further research on encapsulation and optimal application methods for wider industrial use [2]. The application of essential oils (EOs) in food preservation faces limitations due to their volatility and low solubility in aqueous matrices, as well as their sensitivity to environmental factors such as temperature, oxygen, and light. These factors reduce their stability and effectiveness in aqueous food systems, leading to rapid evaporation or chemical breakdown. To overcome these challenges, encapsulation techniques (e. g., nanoemulsions, liposomes, or cyclodextrins) are being explored to enhance solubility, protect bioactive compounds, and enable controlled release, thereby improving their practical use in food preservation [3]. In this perspective, encapsulation techniques, particularly those using sodium alginate, have also become a suitable approach to improve food preservation as well as the stability of bioactive molecules. Among them, encapsulation based on sodium alginate enhances stability, bioavailability, and controlled release of active molecules within food matrices. This method not only prolongs the antimicrobial and antioxidant effects of essential oils but also masks strong flavors, enabling broader application in fortified foods. By forming hydrogel beads or nanoparticles through ionotropic gelation,

alginate encapsulation ensures targeted delivery and enhanced bioavailability, making it a promising approach for advanced food preservation systems [4–6]. Moreover, sodium alginate-based encapsulation not only enhances the stability and controlled release of essential oils but also improves overall product quality by maintaining flavor, color, and nutritional integrity. By minimizing degradation and maximizing bioactive performance, alginate encapsulation offers a scalable, economical solution for sustainable food preservation [7]. Various encapsulation techniques, including spray drying, coacervation, liposome entrapment, and nanoemulsification, have been explored to overcome the limitations of essential oils in food applications. Among these, spray drying stands out as a cost-effective and scalable method, producing stable microcapsules with high encapsulation efficiency. This dual functionality makes alginate encapsulation particularly valuable for maintaining the antimicrobial and antioxidant efficacy of essential oils throughout a product's shelf life without compromising sensory qualities [8,9]. Among the most widely used microencapsulation methods are coacervation, emulsion extrusion, and supercritical fluid precipitation [10–13].

Cistus ladanifer essential oil (CLEO), extracted from the Mediterranean rockrose plant (*Cistus ladanifer*, Cistaceae family), is valued for its unique aromatic profile and bioactive properties. This drought-resistant shrub yields an oil rich in terpenes and phenolics, contributing to its reported antimicrobial, antioxidant, and anti-inflammatory effects. As a natural resource, CLEO holds potential for applications in food preservation, cosmetics, and pharmaceuticals, though further research is needed to optimize its extraction and stabilization for industrial use [14]. Renowned for its antimicrobial, anti-inflammatory, and wound-healing effects, CLEO has been employed to treat respiratory ailments, skin disorders, and digestive issues [15,16]. CLEO emerges as a promising natural alternative to synthetic preservatives due to its richness in natural bioactive compounds, such as α-pinene, camphene and borneol (monoterpenes, monoterpenols and sesquiterpenols), exhibiting strong broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, which is crucial for food safety. Additionally, its antioxidant properties help prevent lipid oxidation, extending shelf life while meeting consumer demand for clean-label ingredients [16–18]. The chemical composition of *Cistus ladanifer* essential oil shows notable regional variations, influencing its bioactive potential. In Algerian varieties, α-pinene (4.2 %), camphene (12.2 %), borneol (12.5 %), alongside 5-epi-7-epi-α-eudesmol (13.6 %), contribute to its antimicrobial efficacy [16]. In contrast, Moroccan CLEO is dominated by viridiflorol (17.64 %) and pinocarveol (11.02 %). These geographical differences highlight the need for standardized profiling to tailor CLEO for specific applications, such as food preservation or pharmaceutical/cosmetic uses [18].

Recognized by the Food and Drug Administration (FDA) [19] as a food additive and flavoring agent, CLEO has interesting applications in the food industry due to its antimicrobial and antioxidant properties, and its bioactive compounds, such as α-pinene and viridiflorol, helps preserve foods by inhibiting pathogens and preventing oxidation, while its distinct aroma enhances flavor profiles [14]. Moreover, *Cistus ladanifer* extract (CL extract) has demonstrated a strong safety profile in toxicological studies, with no adverse effects observed in rats administered doses up to 1000 mg/kg for 90 days, and this supports its potential as a safe, natural ingredient for food and pharmaceutical applications [20]. Encapsulation has been proposed as an effective solution for the stabilization of essential oils extracted from *Cistus* spp., given their high content of sesquiterpenes (48 %) and diterpenes (>18 %), while *C. ladanifer* oil is mainly composed of monoterpenes (70 %). Encapsulation methods (e. g., alginate microbeads, nanoemulsions, or cyclodextrin complexes) can protect these thermolabile compounds from degradation, mask strong flavors, and enable controlled release in food or pharmaceutical matrices [5]. Despite their potent antimicrobial and antioxidant properties, rockrose essential oils (CLEO) remain underutilized in food applications, currently limited to flavoring agents or in dietary supplements [17], rather than as natural preservatives, particularly in meat products. However, *Cistus ladanifer* leaves have been studied as a food additive to improve the quality of lamb meat, significantly reducing lipid oxidation while increasing beneficial unsaturated fatty acid content, without altering the color or organoleptic properties of meat [21].

This study aimed to explore CLEO as a natural preservative for minced chicken meat by evaluating its effect on the microbiological, physicochemical, and textural quality of minced chicken patties during 10 days of refrigerated storage at 4 °C. Particular attention is paid to encapsulation technique, which could improve the preservation potential of CLEO and extend its shelf life. By comparing encapsulated and unencapsulated CLEO with control treatments, this approach refers to effective methods for measuring meat spoilage, which can be classified into microbiological, sensory, and physicochemical analyses [22].

Objects and methods

Chemical composition, formulation and preparation of Cistus ladanifer essential oil (CLEO)

The *Cistus ladanifer* essential oil (CLEO) used in this study was produced during the year 2024 through steam distillation of leafy branches at a plants distillation facility in Tlemcen, Algeria. The resulting oil presents as a transparent liquid ranging in color from light yellow to pale orange, with characteristic physical properties, including a refractive index of 1.44, a density of 0.95 g/cm^3 and a flash point measured at 65 °C. These specifications confirm the oil's typical physicochemical profile for this botanical source. According to GC–MS analysis, the studied oil (CLEO) contains camphene (14.2 %), borneol (13.5 %),

α-pinene (4 %) and tricylene (3 %) as major components. Based on the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) [16,18], a dilution ratio of 1:8 (5 mL (CLEO): 40 mL (CO)) was used to emulsify *Cistus ladanifer* essential oil (CLEO) with corn oil (CO). The addition of vegetable oils, especially corn oil (CO), stabilizes the essential oil, prevents its rapid decomposition, and extends its shelf life. This combination improves encapsulation efficiency, while the beads ensure a slow, controlled release and mitigate strong flavors [23].

Encapsulation method for Cistus ladanifer essential oil (CLEO)

The encapsulation of CLEO into sodium alginate was performed using the extrusion dripping method adapted from [24] and [25] with slight modification. A food grade sodium alginate (Cape Crystal Brands, USA) was dissolved in deionized water, stirred at 40 °C for 2 hours, and refrigerated for 12 hours to remove air bubbles. CLEO solution diluted in corn oil was blended to a 0.5 % (w/v) sodium alginate solution, homogenized and stirred continuously using an ULTRA-TURRAX®. The encapsulation process involved dripping the CLEO-alginate mixture through a 21-gauge syringe into a 1.5 % (w/v) calcium chloride solution from a 2 cm height. After 20 minutes of gelation, the formed beads were thoroughly washed, filtered, and air-stabilized for 15 minutes to ensure structural integrity. For comparative analysis, control beads were prepared following identical procedures but with sterile distilled water replacing CLEO in the alginate mixture.

Bead characterization

The physical characteristics of the alginate beads were quantitatively analyzed using ImageJ software based on 2D image analysis captured with a Canon G7X camera following established protocols according to [24] and [25]. The mean diameter of both CLEO-encapsulated and water-containing (W) beads measured 2.36 ± 0.15 mm. Density analysis revealed that 1 gram of alginate beads contained 156 ± 4 beads, whereas one gram of beads encapsulating sterile distilled water contained 140 ± 2 beads. These measurements demonstrate the formulation-dependent variations in bead packing density while maintaining consistent spherical morphology.

Preparing chicken minced meat patties

Pectoral fillets (m. pectoralis) were obtained from 52-day-old Cobb500 broiler chickens collected from the Batna slaughterhouse (Algeria) using industrial processing methods. The fillets were skinned, minced, and transported to the LSA laboratory in a portable refrigerator at 4 ± 1°C. The minced meat was mixed with 0.8 % (w/w) NaCl and divided into three 1 kg experimental units. Each unit was formulated as described in Table 1 with concentrations based on prior studies by [26] and [27]. Standardized patties (approximately 40 g, 6 cm in diameter, 1 cm in thickness) were prepared, coded (Table 1), and aerobically packaged in polyethylene film for refrigerated storage (4 ± 1) °C. Analyses included microbiological parameters, pH values, color attributes (CIE Lab) and texture properties evaluations at 0, 2, 4, 8 and 10-day intervals.

Table1. Experimental units and treatments for chicken minced meat patties

Treatment		Beads per patty	CLEO + CO	Sterile water concentration	NaCl concentration (w/w)
Encap-CLEO	Minced meat	305 ± 6	5 % (v/w)	—	0.8 %
Direct-CLEO	Minced meat	—	5 % (v/w)	—	0.8 %
Encap-W*	Minced meat	280 ± 4	—	5 % (v/w)	0.8 %
Direct-W*	Minced meat	—	—	5 % (v/w)	0.8 %

*** W is water-containing.**

Microbiological assessment

The antimicrobial efficacy of CLEO oil, applied either directly or in encapsulated form, was evaluated by monitoring the growth of the microbial population during refrigerated storage (4 ± 1) °C at 0, 2, 4, 8, and 10-days intervals. For analysis, 10 g meat samples were aseptically placed in a stomacher filter bag containing 90 ml of sterile buffered peptone water. After homogenizing the mixture for 2 minutes, serial dilutions were prepared. For monitoring of total mesophilic bacteria (TMB), *Enterobacteriaceae* and *Pseudomonas*), appropriate dilutions were inoculated into Petri dishes containing plate count agar (PCA, MERCK, Germany) and 0.1 % cycloheximide solution, violet red bile glucose agar (VRBGA, MERCK, Germany), *Pseudomonas* agar, supplemented with *Pseudomonas* CFC selective agar supplement (SR0103) CFC (Merck, Germany). The plates were incubated respectively, under aerobic conditions at 30 °C / 72 h for TMB, at 37 °C / 24 h for *Enterobacteriaceae*, and finally at 25 °C / 48 h for *Pseudomonas* spp. Bacterial colonies were counted from three replicates, and results were expressed as log CFU/g (colony forming units) of minced chicken meat ± standard deviation (SD).

Physicochemical analysis: pH, color parameters, and texture

The pH of the samples was measured in triplicate using a calibrated SENSION+PH31 GLP pH meter (Hach Company, USA), with results expressed as mean ± standard deviation. Color evolution was monitored throughout the 10-day refrigerated storage period using a Konica Minolta CR-10 Plus colorimeter (Konica Minolta Sensing Europe B. V., Bremen, Germany) with an Ø8 mm aperture.

The surface color of the minced meat patties was quantified and described in terms of lightness (L*), redness (a*), and yellowness (b*) in the CIE Lab* color space. The color was measured during 10 days in cold storage and presented as the mean ± SD of three random readings on each sample.

Texture attributes of the samples were analyzed on days 0, 2, 4, 8, and 10 using a texture analyzer (EZ-LX Shimadzu, Kyoto, Japan) based on Witte et al. [28]. Texture profile

analysis (TPA) measured hardness (maximum force to rupture), springiness (ability to return to shape), and cohesiveness (ratio of compression area). Standardized sample molds were utilized to standardize (6 cm diameter, 1 cm height, 40 g weight). The tests were conducted at 30 % compression depth, 0.01 N preload, 50 N maximum force, and 50 mm/min speed.

Statistical analysis

Statistical analysis of all experimental data was carried out using R Studio statistical software (version 4.3.1), with data expressed as mean values (±SD). To evaluate significant differences between treatment groups, one-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test for post-hoc comparisons. Statistically significant differences between means were established at $p < 0.05$, with this threshold used to identify meaningful variations in measured parameters across different experimental conditions and storage periods.

Results

This study investigated the impact of different treatment methods on the microbiological and physicochemical stability of minced chicken patties during 10-day refrigerated storage (4 ± 1) °C. Four distinct treatments were compared: direct water addition (Direct-W), encapsulated water (Encaps-W), direct CLEO oil application (Direct-CLEO), and encapsulated CLEO (Encaps-CLEO). The experimental design allowed for comprehensive evaluation of how these treatments influenced microbial population dynamics (total mesophilic bacteria, and *Pseudomonas* spp.) alongside critical quality parameters, including pH evolution, color stability (L*a*b* values), and textural properties (hardness, cohesion, springiness).

Microbiological assessment

Figure 1 illustrates the growth of total mesophilic bacteria (TMB) in minced chicken patties stored at (4 ± 1) °C over 10 days. Initial TMB counts were comparable across different treatments (Direct-W, Encaps-W, Direct-CLEO, Encaps-CLEO; $p > 0.05$). By day 2, a uniform increase (2.3 log CFU/g) occurred in all groups, suggesting limited early antimicrobial action from CLEO. A critical divergence emerged on day 4, when control samples (Direct-W, Encaps-W) exceeded the spoilage threshold (7 log CFU/g), showing an increase of approximately 8.5 log CFU/g, while CLEO-treated samples showed significantly ($p < 0.05$) lower TMB levels. This antimicrobial effect became most pronounced by day 10, where controls peaked near 9 log CFU/g, whereas Direct-CLEO maintained lower counts (8 log CFU/g), demonstrating an inhibition of microbial proliferation, which is due to its antibacterial effect. Notably, Encaps-CLEO demonstrated superior inhibition throughout the study, validating both CLEO's antibacterial properties and the role of encapsulation in enhancing its sustained efficacy.

Figure 2 shows changes in the number of *Enterobacteriaceae* (log CFU/g) in chicken patties stored at 4 °C for 10 days, according to the four treatments (Direct-W, Encaps-W, Direct-CLEO, Encaps-CLEO). Initially, no significant difference was observed between the treatments ($p > 0.05$). According to [29], these indicators of fecal contamination show the initial microbiological quality of the product and can accelerate meat deterioration. On day 2, bacterial counts increased in all samples, reaching average values of approximately 6 log CFU/g. This may be explained by the absence of CLEO's bactericidal effect at this phase. This adaptation phase is common in minced meat [30,31], when bacteria adapt to inhibitory conditions [22]. From the 4th day, the values recorded for Direct-W and Encaps-W increased significantly (by 7.8 log CFU/g for day 4 and by approximately 8.2 log CFU/g for day 8) while the Direct-CLEO and Encaps-CLEO samples had significantly lower values ($p < 0.05$) compared to the controls. This can be explained by the inhibitory effect of CLEO on the bacteria. At day 10, bacterial counts in Direct-CLEO (6.13 ± 0.05 log CFU/g) and Encaps-CLEO (6.24 ± 0.06 log CFU/g)

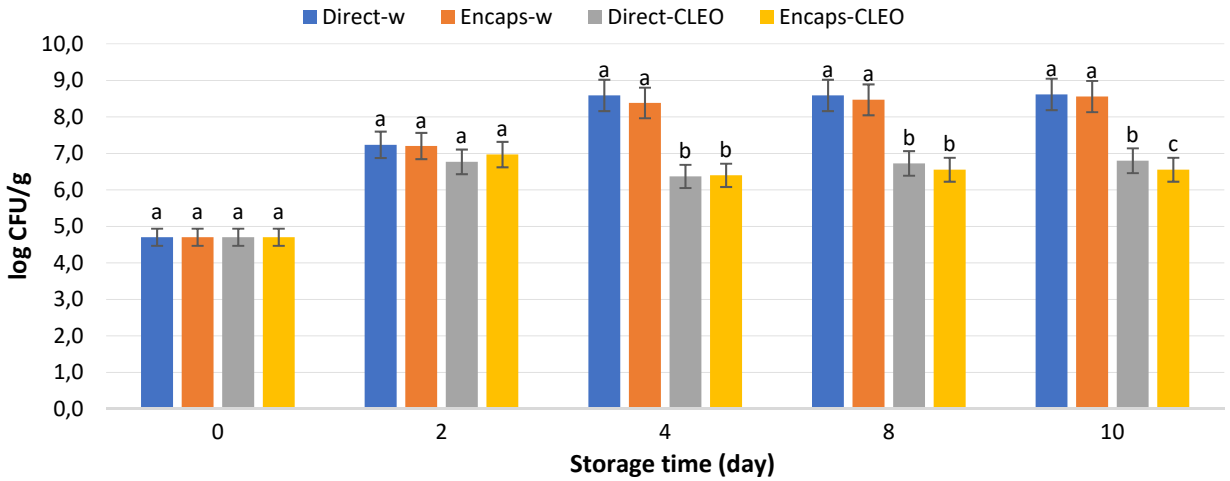

Figure 1. Evolution of total mesophilic bacteria (TMB) in chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

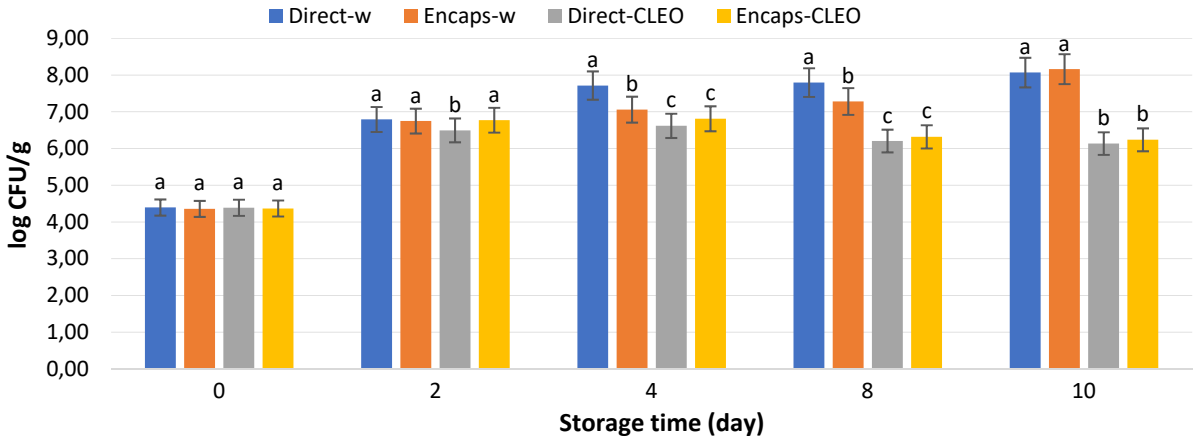

Figure 2. Evolution of *Enterobacteriaceae* in chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

were lower than those in controls (Direct-W: 8.07 ± 0.21 log CFU/g, Encaps-W: 8.16 ± 0.18 log CFU/g), with reductions of 1.83 and 1.92 log CFU/g, respectively. These results show that encapsulation improved the antimicrobial efficacy of CLEO, suggesting better stability and bioavailability during storage. This 24–25 % inhibition efficiency underscores how encapsulation optimizes CLEO's bioactive stability and gradual release, extending its antibacterial action against these critical Gram-negative spoilage organisms throughout storage.

Pseudomonas spp. are recognized as primary spoilage organisms in poultry stored aerobically under refrigeration (0–4 °C) [32]. These psychrotrophic bacteria thrive in cold environments, contributing to protein and lipid degradation, which leads to off-odors and off-flavors [33].

As shown in Figure 3, CLEO effectively inhibited the growth of Pseudomonas spp. in refrigerated chicken patties, with encapsulated CLEO demonstrating superior efficacy. Although all treatments started with identical initial counts (3.20 ± 0.03 log CFU/g), by day 2 the samples treated with CLEO already showed a slight antimicrobial effect, but the differences were not statistically significant (all "a"). The antimicrobial action of CLEO became evident as early as day 4, when the treated samples (Direct-CLEO and Encaps-CLEO) showed a significant reduction ($p < 0.05$) compared to the controls (Direct-W, Encaps-W reaching 4.62 log CFU/g). At day 10, the controls exceeded the spoilage thresholds (7.13 ± 0.04–7.16 ± 0.05 log CFU/g), while the Direct-CLEO and Encaps-CLEO samples maintained significant reductions compared to the Direct-W control (– 1.98 ± 0.03 and — 1.91 ± 0.53 log CFU/g; $p < 0.05$, Duncan's test) or nearly 98.7 % bacterial reduction. The addition of CLEO, whether direct or encapsulated, significantly reduced the growth of mesophilic bacteria during storage, the antimicrobial efficacy is clear from the 4th day and is maintained until the 10th day, which potentially extends the shelf life of the products.

This persistent inhibition reflects the membrane-disrupting effects of bioactive terpenes (α-pinene, camphene, borneol, with encapsulation enhancing stability and

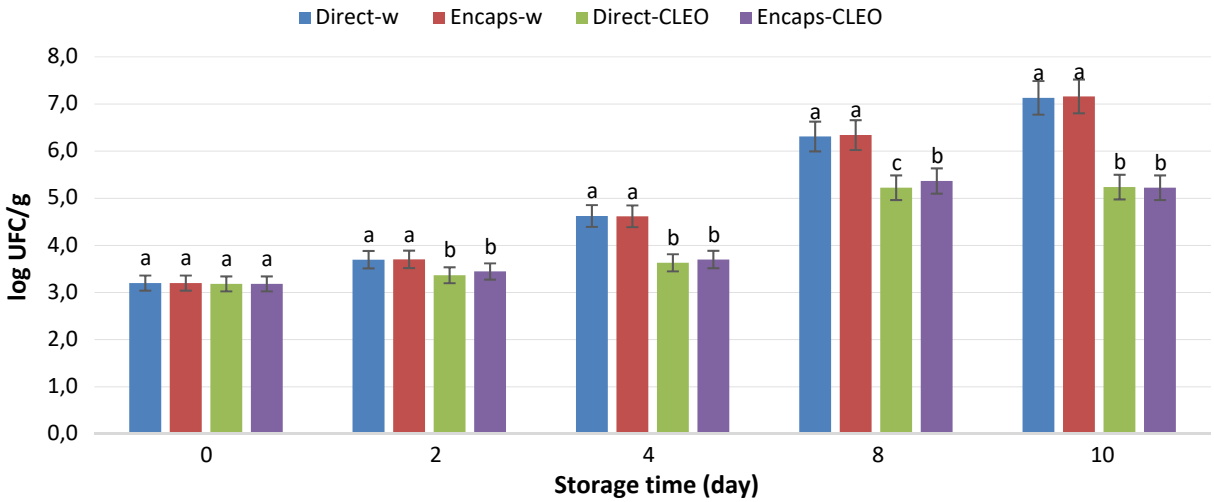

Figure 3. Evolution of *Pseudomonas* spp. in chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

controlled release for prolonged efficacy. The results confirm CLEO's potential to extend poultry shelf life by specifically targeting dominant spoilage pseudomonads [18].

Physicochemical analysis: pH, color parameters, and texture

pH determination

Table 2 demonstrates that CLEO treatments effectively stabilized pH in minced chicken patties treated during refrigerated storage at (4 ± 1) °C, with encapsulated CLEO (Encaps-CLEO) showing superior performance. While control samples (Direct-W, Encaps-W) exhibited a significant pH increase ($p < 0.05$) from day 4 onward, reaching alkaline levels by day 10 due to microbial metabolite accumulation, CLEO-treated groups maintained significantly lower pH values ($p < 0.05$). This stabilization was particularly pronounced in Encaps-CLEO samples, which showed the smallest pH fluctuation (ΔpH < 0.3), attributable to the alginate beads-controlled release of antimicrobial compounds that suppressed spoilage bacteria and their alkaline byproducts.

Table2. pH values of chicken patties during the refrigerated storage (at (4 ± 1) °C

Time (days)	Direct-W	Encaps-W	Direct-CLEO	Encaps-CLEO
0	5,60 ± 0,03^{b}	5,67 ± 0,03^{a}	5,67 ± 0,03^{a}	5,70 ± 0,02^{a}
2	5,75 ± 0,05^{a}	5,76 ± 0,05^{a}	5,40 ± 0,05^{c}	5,67 ± 0,02^{b}
4	6,48 ± 0,03^{a}	`6,56 ± 0,06^{a}	5,11 ± 0,05^{b}	5,16 ± 0,05^{b}
8	6,82 ± 0,03^{a}	6,78 ± 0,04^{a}	5,33 ± 0,04^{b}	5,27 ± 0,03^{b}
10	7,13 ± 0,03^{a}	7,06 ± 0,04^{b}	5,46 ± 0,02^{c}	5,51 ± 0,05^{c}

Data are presented as mean ± standard deviation. Statistical significance was assessed using Duncan's multiple range test at a significance level of $p \le 0.05$. The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point.

At the beginning of storage (day 0), the pH difference was not significant ($p > 0.05$) between treatments, confirming uniform starting conditions. By day 4, the pH increased significantly in the Direct-W samples (6.48 ± 0.03) and Encaps-W (6.56 ± 0.06). This is due to the presence of by-products released by the germs and the absence of a preservative. The Direct-CLEO and Encaps-CLEO samples recorded pH values of 5.11 ± 0.05 and 5.16 ± 0.05, respectively, which were significantly lower compared to the Direct-W and Encaps-W samples. This can be explained by the microbial inhibition exerted by CLEO. During day 8, the pH values of the Direct-W and Encaps-W control samples increased significantly ($p < 0.05$) recording values of 6.82 ± 0.03 and 6.78 ± 0.04, respectively, while the CLEO-treated samples remained stable with values of 5.33 ± 0.04 for Direct-CLEO and 5.27 ± 0.03 for Encaps-CLE. At the end of storage by day 10, pH values reached 7.13 ± 0.03 for Direct-W and 7.06 ± 0.04 for Encaps-W, while Direct-CLEO and Encaps-CLEO samples maintained significantly ($p < 0.05$) lower pH values of 5.46 ± 0.02 and 5.51 ± 0.05, respectively. These results show that Encaps-CLEO demonstrated better pH stability (ΔpH < 0.35 vs. ΔpH > 0.6 in controls) than direct application, suggesting a controlled release of antimicrobial compounds (α-pinene, borneol), thereby prolonging activity and maintaining meat freshness.

Evaluation of color parameters

Figure 4 represents the color values lightness (L*), and redness (a*), along with increased yellowness (b*) observed in minced chicken patties treated with *Cistus ladanifer* essential oil (CLEO) and sterile distilled water (W) directly or encapsulated in sodium alginate, during 10 days of storage at 4 ± 1 °C. These color parameters are indicators of meat quality [34], as high L* values improve visual appeal, while a decrease in a* values may indicate oxidation or spoilage [31].

As shown in Figure 4a, the initial L* values were highest in Direct-CLEO samples (55.95 ± 0.07), followed by Direct-W (55.85 ± 0.05), Encaps-W (55.62 ± 0.4) and Encaps-CLEO (55.69 ± 0.07). The slight improvement in brightness with CLEO treatments is probably due to the presence of natural pigments in the essential oil. From day 4 onwards, the brightness (L*) of Direct-W and Direct-W samples significantly decreased ($p < 0.05$), recording mean values of 50.33 ± 0.08 and 51.24 ± 0.23, respectively. During the intermediate storage on days 4 and 8, Direct-CLEO and Encaps-CLEO were considerably brighter compared to Direct-W and Encaps-W, which was indicated by darkening of Direct-W and Encaps-W samples, most likely due to lipid oxidation or microbial spoilage. At the end of storage (day 10), Direct-W and Encaps-W had the lowest L* values with a mean value of 50.33 ± 0.08 showing a great discoloration. However, the samples Direct-CLEO with a mean value of 56.08 ± 0.03 and Encaps-CLEO with 55.95 ± 0.06 retained and even slightly improved their L* values at the end of storage.

The results presented in Figure 4b show the redness (a*) values of chicken patties over 10 days at (4 ± 1) °C under the four treatments. Initially, all groups had similar a* values (4.96–5.03, $p>0.05$). However, the progression of redness values varied considerably between treatments. Day 4 and day 8 also recorded the Direct-W and Encaps-W samples to exhibit a significant reduction ($p < 0.05$) in a* values by about 2 units of the mean a* values, thus making the minced meat patties pale and less red in color, perhaps due to oxidation. On day 8, Direct-CLEO and Encaps-CLEO samples exhibited significantly higher values, ranging from 3.74 ± 0.41 to 3.95 ± 0.07, respectively. On the 10th day, a* values decreased considerably in Direct-W and Encaps-W to 2.06 ± 0.04 and 2.10 ± 0.27, respectively. This can be attributed to microbial alteration and associated biochemical changes in pH, which generally result in a color shift towards green [26,35], as well as accelerated oxidation of oxymyoglobin to metmyoglobin. Direct-CLEO and Encaps-CLEO treatments retained significantly ($p < 0.05$) higher a* values of 3.55 ± 0.02 and 3.75 ± 0.49, respectively, probably due to the controlled release of the active compounds [31].

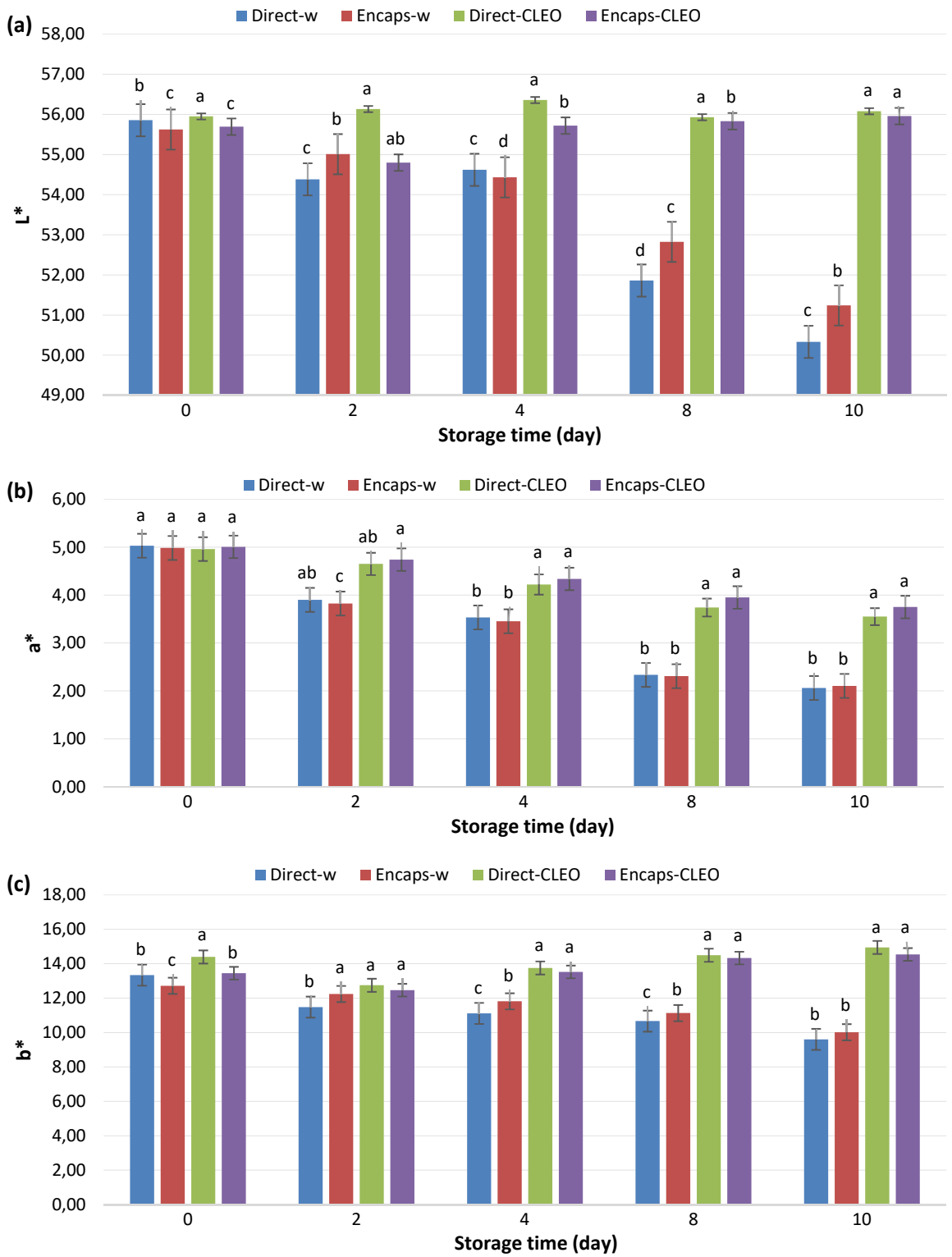

Figure 4. Color values L* lightness (a), a* redness (b), and b* yellowness (c) of chicken patties during 10 days of storage at 4 ± 1 °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). Vertical bars indicate the standard deviation of the mean. The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

As can be seen in Figure 4c, the b* (yellowing) values decreased significantly during the 10 days of storage at 4 °C. The b* value in Direct-W decreased from 13.34 ± 0.51 to 9.6 ± 0.35 a reduction by 3.74 units), while that of Encaps-W decreased from 12.72 ± 0.17 to 10.02 ± 0.27 (a reduction by 2.7 units), indicating oxidative degradation of the pigment. The value of Direct-CLEO increased from 14.39 ± 0.16 to 14.95 ± 0.16 (an increase by 0.56 units). In addition to pH-related color changes, changes in b* values in the muscle may be associated with diet-induced postmortem glycogen changes. Unlike controls, which showed a significant decrease (p>0.05) due to oxidative degradation, CLEO-treated samples not only retained but also slightly improved their yellow color.

Texture analysis

According to the data on the texture evolution of minced meat patties over a 10-day storage period at 4 °C, presented in Figure 5, the textural properties (hardness, cohesion, and springiness) were significantly influenced ($p < 0.05$) by CLEO treatment, both in direct (Direct-CLEO) and encapsulated (Encaps-CLEO) forms, depending on the storage duration.

Figure 5a shows that the Encaps-W samples, which recorded a mean value of 16.56 ± 0.07 N, and Encaps-CLEO with a value of 15.98 ± 0.36 N initially presented significantly ($p < 0.05$) higher hardness values compared to the Direct-W treatments with a value of 11.89 N and Direct-CLEO with a value of 10.61 N. This is probably due to the firm and elastic structure of the alginate beads. After day 4, Direct-W and Encaps-W samples showed a significant ($p < 0.05$) reduction in hardness, with a 33 % decrease for Direct-W (from 11.89 ± 0.18 N to 7.95 ± 0.07 N) and a 41 % change for Encaps-W (from 16.56 ± 0.07 N to 9.82 ± 0.04 N), which is likely due to protein degradation and moisture loss due to

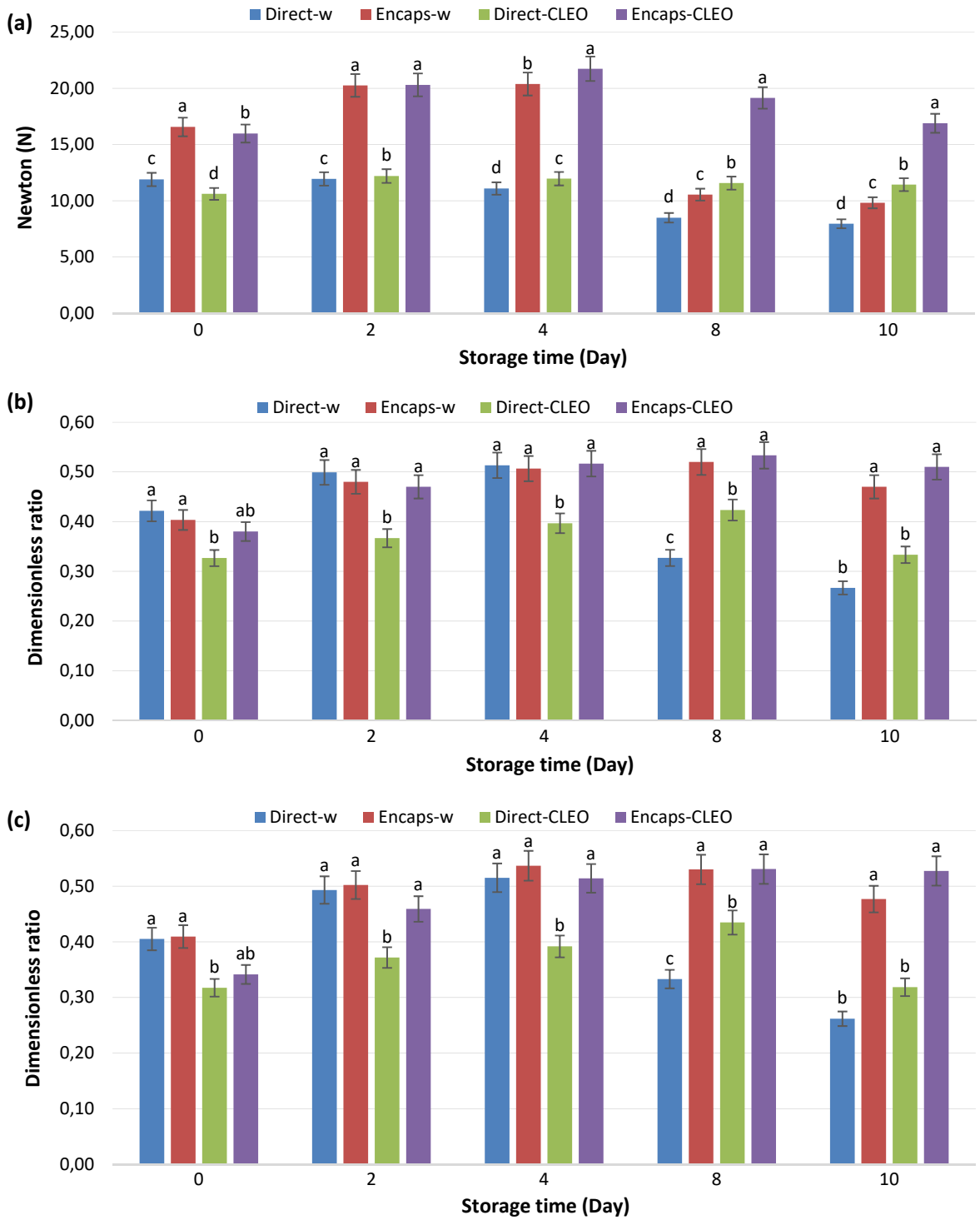

Figure 5. Textural properties (a) hardness, (b) springiness and (c) cohesiveness of chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). Vertical bars indicate the standard deviation of the mean. The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

microbial activity. On day 4, the hardness of Direct-CLEO increased from 10.61 ± 0.15 N to 11.96 ± 0.07 N and then stabilized at 11.43 ± 0.06 N on day 10, which can be explained by CLEO's bioactive compounds interacting with muscle proteins, forming cross-links that improve firmness and delay proteolysis. Encaps-CLEO recorded the highest hardness value throughout storage, increasing from 15.98 ± 0.36 N to 21.74 ± 0.52 N on day 4 and stabilizing at 16.89 ± 0.21 N on day 10. This increase suggests a controlled release of CLEO's bioactive compounds, improving protein stability and water retention.

Figures 5b and 5c show that springiness and cohesion followed similar trends over the 10 days of the experiment. Indeed, samples treated with CLEO addition, either directly or by encapsulation, maintained better structural integrity than controls. Springiness decreased significantly ($p < 0.05$) in Direct-W samples, from 0.42 ± 0.04 at baseline to 0.27 ± 0.01 at the end of storage, due to protein degradation, while in the Encaps-W samples it decreased to an average value of 0.47 ± 0.05 on day 10, maintaining higher elasticity. CLEO treatments helped preserve springiness. In Encaps-CLEO, it increased from 0.38 ± 0.04 to 0.51 ± 0.05, suggesting that encapsulation delays proteolysis and improves water retention. Similarly, cohesion remained stable in Encaps-CLEO, increasing from 0.34 ± 0.07 to 0.53 ± 0.03, while it decreased in Direct-W by 37 % (from 0.41 ± 0.00 to 0.26 ± 0.02). These results confirm that CLEO, especially in encapsulated form, improves textural stability during refrigerated storage. The encapsulation technique prolongs the antioxidant and antimicrobial activity of CLEO, thus reducing biochemical degradation and preserving patties texture.

Exploration of complex relationships

The PCA biplot (Figure 6A) and correlation matrix (Figure 6B) illustrate the relationships between microbiological (*TMB, Enterobacteriaceae, Pseudomonas* spp.), physicochemical (pH, color attributes), and textural (springiness, cohesiveness, hardness) properties during storage.

Multivariate analysis of quality parameters

Principal Component Analysis (PCA) (Figure 6A) demonstrates the relationships between quality parameters, with Dimension 1 (57.43 %) separating microbiological parameters (TMB, *Enterobacteriaceae*, *Pseudomonas* spp.), pH, and color attributes (L*, a*, b*), while Dimension 2 (25.91 %) distinguishes textural properties (cohesion, springiness, hardness). The arrangement in the diagram shows that microbial proliferation and pH are negatively correlated with the color parameters lightness (L*) and redness (a*) but are positively correlated with yellowness (b*), demonstrating a link between oxidative degradation, pH increase, and yellowing. Finally, textural attributes (hardness, cohesion, and springiness) are distinct from microbiological and physicochemical properties, implying that they are influenced by independent factors, likely related to protein denaturation or water retention.

Correlation matrix

The correlation matrix (Figure 6B) reveals significant relationships between quality parameters, with pH demonstrating strong positive correlation with microbial growth (*Pseudomonas* spp. (0.70), TMB (0.71) and *Enterobacteriaceae* (0.59)), which shows that microbial growth influences pH variations during storage. On the other hand, the color parameter b* is strongly correlated with *Pseudomonas* spp. (0.94) and moderately correlated with *Enterobacteriaceae* (0.60) and TMB (0.62). This indicates that the proliferation of *Pseudomonas* spp. contributes significantly to the yellow coloration. It is also noted that the parameter L* shows a negative correlation with *Pseudomonas* spp. (–0.77) and *Enterobacteriaceae* (–0.68), which indicates that microbial growth causes meat darkening. Hardness shows a weak

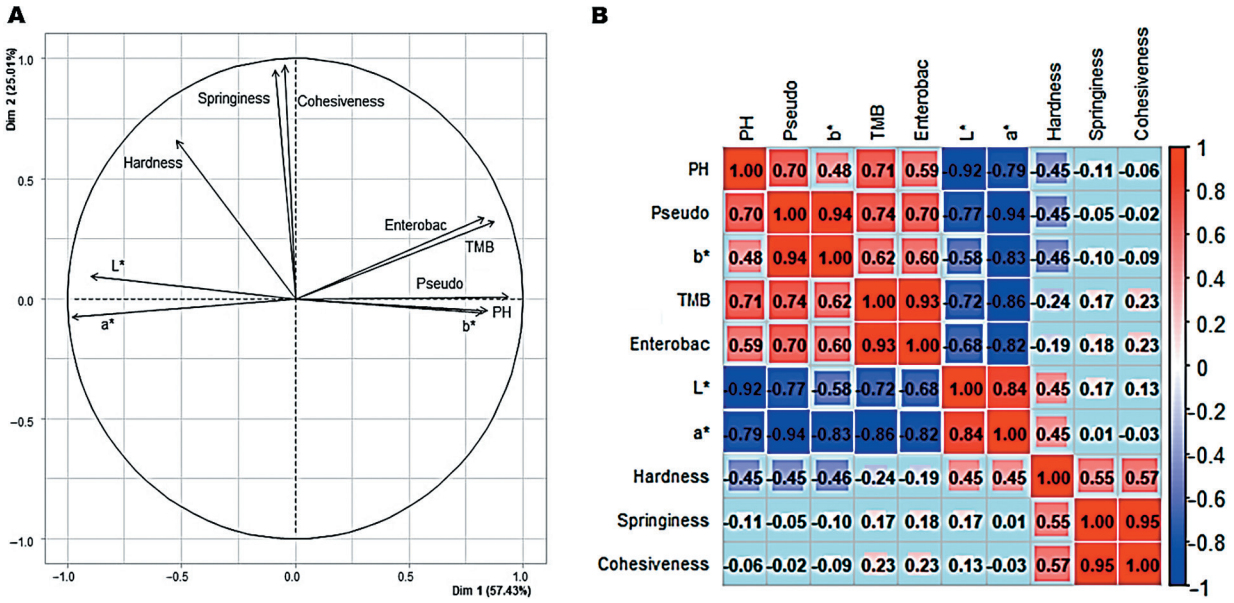

Figure 6. Graphical Representation of PCA (Figure 6A) and Correlation Matrix (Figure 6B) for the studied variables: microbiological parameters (*TMB, Enterobacteriaceae* = Enterobac, *Pseudomonas* spp.= Pseudo), physicochemical attributes (pH, color attributes a*, b*, L*), and texture properties (hardness, springiness, cohesiveness) over the storage period

negative correlation with *Pseudomonas* spp. (–0.45) and b* parameter (–0.46), indicating that its growth activates oxidative processes and contributes to softening. Elasticity shows a weak correlation with *Enterobacteriaceae* (0.18), suggesting a minor impact on elasticity. These patterns collectively demonstrate that *Pseudomonas* spp. serves as the primary driver of both color changes (yellowing/darkening) and textural degradation in stored meat products.

Discussion

The chemical composition of the essential oil used in this study is close to that reported by [16], who identified significant levels of borneol (12.5 %), camphene (12.2 %) along with a lower proportion of α-pinene (4.2 %). This compositional pattern indicates that the essential oil derived from this particular geographic region contains relatively reduced levels of monoterpene hydrocarbons, potentially influencing its physicochemical properties and biological activity.

The results revealed that samples treated with CLEO and in particular Encaps-CLEO, which contained CLEO encapsulated in alginate beads, were more stable in terms of microbial control, pH, color and texture than the Direct-W controls based on sterile distilled water. The results showed that the populations of total mesophilic bacteria (TMB), *Enterobacteriaceae* and *Pseudomonas* spp. in both Direct-W and Encaps-W samples increased significantly at the end of the storage period, while treatment with Encaps-CLEO successfully inhibited microbial growth, validating its antimicrobial efficacy. This decrease could be due to the mode of action of essential oils on bacterial cells, as already reported by [36]. In addition, and as shown in the results, the antibacterial effect of cistus essential oil in Encaps-CLEO samples became particularly effective after the fourth day of storage. Shabkhiz et al. [3] reported that the release of bioactive compounds from encapsulated *Thymus daenensis* essential oil (Td-EO) in alginate beads occurs in two distinct phases, which are short-release and long-release. They also found that encapsulation in β-cyclodextrin, combined with the formation of an alginate hydrogel, effectively slows down the release of bioactive compounds, thus improving their stability and prolonged activity.

Cistus ladanifer essential oils exhibit significant antimicrobial activity tested against both Gram-positive and Gram-negative bacteria [17]. This antimicrobial effect of CLEO is due to the presence of bicyclic organic compounds belonging to the terpene family. In most articles, the chemical profile of cistus essential oils revealed that monoterpene hydrocarbons are represented mainly by high percentages of α-pinene, with values ranging from 19.46 to 47.1 % [17]. The Algerian essential oil contains minor levels of monoterpene hydrocarbons of 0.7 to 5.2 %, but major levels of 5-epi-7-epi-α-eudesmol (13.6 %) and borneol (12.5 %) [16]. In this study, camphene (14.5 %), a monoterpene ($C_{10}H_{16}$), and borneol (13.5 %), which is a monoterpenol ($C_{10}H_{18}O$), are both antimicrobials and antioxidants, possessing broad-spectrum antibacterial properties through a membrane-rupturing mechanism [37]. The antioxidant activity of *Cistus ladanifer* essential oil stems partly from its chemical structure, which allows it to interact with reactive oxygen species [37]. Essential oils cause bacterial membrane rupture, leading to leakage of intracellular components, loss of bacterial viability and ATP depletion, disrupting energy functions and lowering intracellular pH (pHin), thus influencing metabolic reactions [38]. Parafati et al. [26] and Mehaya et al. [39] also reported the same results showing that encapsulation increases the antimicrobial activity of bioactive compounds due to stability, controlled release, and prolonged activity against meat spoilage bacteria. The inhibition of *Pseudomonas* spp. in Encaps-CLEO samples is particularly interesting because these bacteria are considered dominant in the spoilage flora of poultry in cold storage and are responsible for the emission of unpleasant odors and discoloration [40].

The pH of the samples Direct-W and Encaps-W showed a significant increase ($p < 0.05$), which can be explained by bacterial metabolism that produces alkaline compounds such as protein metabolites and essential amines [41]. The Direct-CLEO and Encaps-CLEO samples demonstrated a non-significant pH variation. This stability is due to the antimicrobial effects of CLEO in addition to the prolonged release of the latter by the alginate beads, which decrease bacterial growth and the production of by-products during storage. Similar results were reported by Lu et al. [40] and Yu et al. [42], where thyme and eucalyptus oil microcapsules delayed the increase in pH of refrigerated meat by inhibiting bacterial enzymatic activity.

Samples treated with sterile distilled water alone showed a decrease in color throughout the storage period, while a strong correlation existed between color parameters (L*, a*, b*) and the presence of CLEO upon both encapsulated and direct addition into minced meat patties. However, the Encaps-CLEO intervention preserved a* values better and showed better b* scores than Direct-CLEO. This is attributed to the controlled release of bioactive compounds present in CLEO, such as α-pinene, camphene, and borneol, which participate in the capture of free radicals, slowing down the oxidation of lipids and pigments and degradation of myoglobin [43]. Previous studies corroborate these findings, demonstrating that oregano essential oil maintained a* values in chicken meat [44], encapsulated beetroot extract improved color stability [27], and iron additives improved a* values but accelerated oxidation [45].

The results showed that the texture parameters (hardness, cohesion, and springiness) of the control samples were significantly reduced, causing meat softening. The most pronounced decline was recorded after day 4 of storage, coinciding with an increase in microbial growth and proteolysis. Texture deterioration during storage is linked to protein degradation, microbial growth, and lipid oxidation. At the same time, Encaps-CLEO also maintained

texture better than Direct-CLEO, indicating a synergistic effect of encapsulation on protecting structural integrity.

Multivariate analysis revealed significant relationships between microbial growth and quality parameters. *Pseudomonas* spp. proliferation exhibited a strong positive correlation with pH and yellowness (b), while inversely correlating with lightness (L) and redness (a*) parameters. However, the statistical analysis did not reveal a direct correlation with the texture parameters, which predicts a direct impact of yellowing, loss of redness, and blackening associated with an alkaline pH of a product, and an indirect impact of texture loss during storage of the minced chicken patties. Furthermore, according to the experimental results of the studied parameters and the statistical interpretation of the correlations, the addition of *Cistus ladanifer* essential oil (CLEO), either directly or encapsulated in alginate beads, controlled bacterial growth even of *Pseudomonas* spp., which are Gram-negative species, and delayed the modification of the physicochemical and texture parameters of the product, which validates its antimicrobial and consequently, antioxidant activity.

Conclusion

This study demonstrates that encapsulated *Cistus ladanifer* essential oil (Encaps-CLEO) significantly enhances the shelf life and quality of minced chicken patties by effectively controlling microbial growth, stabilizing pH, preserving color, and maintaining texture attributes of refrigerated samples during 10 days of storage. The antimicrobial efficacy of CLEO, particularly against spoilage-related bacteria such as *Pseudomonas* spp., is attributed to its bioactive compounds (camphene, borneol, and α-pinene), which disrupt bacterial membranes and inhibit metabolic activity. Encapsulation in alginate beads further optimizes CLEO's performance by ensuring controlled release, prolonged activity, and improved stability, thereby overcoming limitations associated with direct application.

The preservation of color (especially redness and lightness) and texture in Encaps-CLEO-treated samples underscores its antioxidant potential, preventing oxidative degradation of lipids and proteins. Notably, the delayed pH increase in treated samples confirms reduced bacterial metabolism, further validating CLEO's role in inhibiting spoilage. Statistical analysis (PCA) reinforced the strong correlation between microbial inhibition and maintained physicochemical quality, with encapsulated CLEO outperforming both direct CLEO and control treatments (Direct-W and Encaps-W).

These findings highlight CLEO, particularly in encapsulated form, as a promising natural alternative to synthetic preservatives in meat products. Its dual antimicrobial and antioxidant effects, combined with the technological advantages of encapsulation, offer a sustainable solution for improving food safety and extending shelf life while meeting consumer demand for clean-label ingredients. Future research should focus on scaling up encapsulation techniques and exploring synergies with other natural preservatives for broader industrial applications.

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AUTHORS INFORMATION

Laabed Mohssen, Assistant Professor, Food Science Laboratory, Institute of Veterinary and Agronomic Sciences, University of Batna1. Batna, 05000, Algeria. Tel.: +21–366–996–43–93, E-mail: mohssen.laabed@univ-batna.dz
ORCID: https://doi.org/0000-0002-8280-7865
* corresponding author

Bouslimani Nouri, Associate Professor, Food Science Laboratory, Department of Agronomy, Institute of Veterinary and Agronomic Sciences, University of Batna1. Batna, 050000 Algeria. Tel.: +21–356–012–34–98, E-mail: noubou77@gmail.com
ORCID: https://doi.org/0000-0003-2815-0038

Abdelmounaim Khemmouli, PhD Student, Laboratory of Valorisation of Natural Biological Resources, Department of Agronomic Sciences, Faculty of Natural and Life Sciences, Ferhat Abbas University. Sétif 1, 19000, Algeria. +21–369–870–97–30, E-mail: cochnille1@gmail.com
ORCID: https://doi.org/0000-0001-7569-5976

Yassine Noui, Associate Professor, Food Science Laboratory, Institute of Veterinary and Agronomic Sciences, University of Batna1. Batna, 05000, Algeria. Tel.: +21–366–022–78–57, E-mail: noui.yassine@gmail.com
ORCID: https://doi.org/0000-0002-8085-1715

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