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Effect of Foeniculum vulgare Seed Phenolic Extract as a Natural Additive on The Physicochemical, Microbiological, Antioxidant, and Organoleptic Quality of Traditional Algerian J'ben Cheese During Storage

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Research Article

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Abstract

Chemical additives widely used in the agri-food industry have raised increasing concerns because of their potential adverse effects on human health. Thus, there is growing interest in replacing synthetic additives with natural alternatives capable of preserving food quality and safety. In this context, the present study aimed to evaluate the effect of incorporating a phenolic extract from fennel (*Foeniculum vulgare*) seeds as a natural food additive on the quality of J'ben, a traditional Algerian cheese, during 15 days of refrigerated storage at 4°C. J'ben cheese, produced by mixed coagulation, was supplemented with a phenolic extract of fennel seeds obtained by hydroethanolic maceration (80:20, ethanol/water, v/v). The extract was incorporated into the cheese at concentrations of 0% (control), 1%, 2%, and 3% (w/w), and the resulting products were evaluated during 15 days of refrigerated storage at 4°C. Physicochemical, microbiological, and sensory analyses were performed on both the experimental cheeses and the fennel seed extract. The results demonstrated that the hydroethanolic extract of *Foeniculum vulgare* exhibited strong antioxidant activity, attributable to its high content of total phenolic compounds (9.2 mg GAE/mL extract) and flavonoids (0.68 mg QE/mL extract). Microbiological analyses further revealed that the extract possessed antimicrobial activity against several pathogenic and spoilage microorganisms while simultaneously promoting the growth of beneficial lactic acid bacteria, highlighting its potential prebiotic effect. Physicochemical parameters, including moisture content, fat content, dry matter, pH, and acidity, exhibited variations among the experimental cheeses during storage. However, the most significant finding of this study was the substantial reduction in malondialdehyde (MDA) levels observed in cheeses supplemented with 3% fennel extract compared with the control. This decrease can be attributed to the strong antioxidant activity of the extract, as demonstrated by the DPPH and FRAP assays. Furthermore, sensory evaluation revealed that the incorporation of fennel seed phenolic compounds positively influenced the freshness, aroma, and colour of the cheeses.

Introduction

Over the past decades, the food industry has increasingly relied on chemical additives, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitrates, and nitrites, to extend shelf life, maintain product quality, and ensure the microbiological safety of processed foods [1], despite growing concerns regarding their potential adverse effects on human health, including an increased risk of asthma, cardiovascular diseases, obesity, and certain types of cancer [2].

Several authors have proposed the exploration of plant-derived secondary metabolites as a promising approach for discovering alternative bioactive compounds [3–5]. In this regard, aromatic plants have emerged as a valuable source of natural bioactive compounds, particularly phenolic compounds and other secondary metabolites, exhibiting antioxidant and antibacterial activities [6], making them excellent natural food additives for improving the quality, shelf life, and technological characteristics of processed food products of animal and plant origin [7].

Fennel (*Foeniculum vulgare*), or "besbes" in Arabic, is among the medicinal plants recognized for its antioxidant and antimicrobial properties [3], as well as for its health benefits, which are attributed to its richness in essential bioactive compounds, including phenolic acids, hydroxycinnamic acids, coumarins, tannins, among others [8, 9].

The antioxidant properties of primary phenolic compounds of medicinal plants are important for lipid preservation. Lipids are easily oxidized and this can lead to their modification and the formation of harmful chemicals that can affect the flavor and quality of food [9]. Polyphenols scavenge free radicals and inhibit lipid oxidation, so they prevent lipid peroxidation and maintain the integrity of lipids. This process helps to increase the shelf-life of food and maintains its quality [8–10]. The fatty acid profile of different types of cheese with or without fennel enrichment showed that saturated fatty acids are the most abundant followed by monounsaturated fatty acids and polyunsaturated fatty acids [3]. Palmitic and oleic acids are the main fatty acids in all cheese samples. In the presence of fennel powder and decoction, capric acid became predominant. The results show that the addition of phenolics from fennel improves the preservation of the product and also change its lipid composition. The antimicrobial activity of phytochemical components can also inhibit or prevent the growth of potentially harmful and/or toxin-producing microorganisms in food, thereby prolonging its shelf life [3]. Such effects could be explained by their ability to compromise cellular integrity and increase permeability of the membrane and hence inhibit the microbial proliferation [9].

This study is particularly innovative as it explores the valorization of fennel seeds (*Foeniculum vulgare*), an extensively cultivated crop in Algeria (20–30 t/ha), as a source of phenolic compounds with potential applications as natural bioactive additives [11]. Indeed, the majority of prior research focused on fennel essential oils rather than its phenolic constituents. Furthermore, research on the antibacterial efficacy of phenolic-rich extracts against spoilage microorganisms and beneficial lactic acid bacteria within cheese matrices remains limited, particularly in the context of traditional Algerian J’ben cheese. Similarly, studies investigating the antioxidant potential of these extracts, especially their effects on lipid oxidation and lipid profiles in this type of cheese, are still scarce.

Therefore, this study aimed to investigate the effectiveness of fennel seed phenolic extracts as natural additives at different concentrations (0, 1, 2, and 3%) for enhancing the quality attributes and extending the shelf life of J’ben cheese. As a traditional Algerian fresh cheese, J’ben represents a valuable but underexplored dairy product with significant potential for wider recognition and valorization at both national and international levels.

Materials and methods

Biological material

Fennel *(F. vulgare)* seeds were harvested in March 2023 in the Ain Defla region (Algeria) located at approximately 36.0729193°N Latitude and 1.9881527 °E Longitude. The seeds were cleaned to remove

impurities, air-dried, ground into a powder, and stored in amber airtight containers at room temperature under light- and moisture-protected conditions until further analysis.

Phenolic compounds extraction

The major phenolic compounds from *F. vulgare* seeds were extracted by hydroethanolic maceration (80:20, v/v). Briefly, 10 g of ground fennel seeds were macerated in the hydroethanolic solvent for 6 h at room temperature under continuous stirring, following the method described by Sultana *et al.* [12]. The extract was filtered and concentrated under reduced pressure to a final volume of 10 mL. The resulting extract was stored in amber glass bottles at cold temperature until further use.

Preparation of lactic sourdough

The sourdough was prepared by inoculating 0.75 g of mesophilic strains into 750 mL of sterilized skimmed milk, previously warmed to 37°C. The mixture was then sealed with aluminum foil and incubated at 37° C for one hour.

J'Ben cheese manufacturing process

On April 16, 2023, 40 liters of raw cow's milk collected from a breeder in the wilaya of Mostaganem were delivered to the Laboratory of Food Technology and Nutrition at the University of Mostaganem. The milk was then pasteurized at 60°C for 30 minutes, cooled to 37–38°C, inoculated at 2% (v/v) with mesophilic ferments and renneted with 10 mL of an industrial rennet of animal origin with a coagulation strength of 1/10000. Before curdling, 20 g of $CaCl_2$ were added to the mixture to compensate for the calcium lost in the whey during draining. At the end of coagulation (coagulation time multiplied by 4), the resulting coagulum was cut horizontally, stirred manually, and drained for 12 hours at 4°C, with regular turning during this period. Finally, the curd was molded and then salted by immersion in 25% brine for 5 minutes.

Cheese treatment with phenolic extract of Foeniculum vulgare:

After the cheese-making process, the J'ben was divided into four batches, each containing three 100 g portions. Each batch was then supplemented with fennel seed phenolic extract at 0, 1, 2, and 3%, respectively.

Hydro-ethanolic extract analysis

All analyses were performed in triplicate (n = 3) according to previously described methods. **Total phenolic compounds**: Total polyphenols in the phenolic extract of fennel seeds were determined by using the Folin-Ciocalteu method [13]. In a test tube, 1 ml of Folin-Ciocalteu reagent diluted 10-fold in methanol was added to 200 µl of extract diluted 50-fold in methanol.

After 5 min of incubation, 800 µl of sodium carbonate solution (7.5%) in water were added. The final solution was then homogenised and incubated in the dark at room temperature for 30 min. The absorbance was measured at 765 nm against blank in a spectrophotometer. A calibration curve was prepared in parallel under the same conditions, using gallic acid as a reference at different concentrations (0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/mL) [14]. Results were expressed in milligrams of gallic acid equivalent per milliliter of extract (mg GAE/mL extract), per gram of extract lyophilisate (mg GAE/g lyophilisate), and per gram of plant dry matter (mg GAE/g plant DM) [15]. **Total flavonoids**: A 500 µl aliquot of extract was mixed with an equivalent quantity of $AlCl_3$ solution (2% in methanol). After 10 minutes of incubation, absorbance was measured at 430 nm using a spectrophotometer. A calibration curve for quercetin was prepared over a range of concentrations (1.25 to 40 µg/mL) under the same operating conditions. Results were expressed in milligrams of quercetin equivalent per milliliter of extract (mg QE/mL extract), per gram of extract lyophilisate (mg QE/g lyophilisate), and per gram of plant dry matter (mg QE/g plant DM) [16].

Antioxidant activity of Hydroethanolic extract.

All antioxidant assays were performed in triplicate (n = 3). **DPPH free radical scavenging test**: The DPPH assay was carried out by initially preparing a series of dilutions at different concentrations (100 µg/ml) from the phenolic extract of fennel seeds. Next, 100 µl of each concentration was added to 2.9 ml of DPPH ethanolic solution (0.004%). After 30 minutes of incubation in the dark at room temperature, absorbance was read at 517 nm using a spectrophotometer. The positive control was a solution of the standard antioxidant ascorbic acid, whose absorbance was measured under the same conditions as the sample [17]. The results obtained are expressed as percent inhibition (%I) [14, 18]: I (%) = [(Abs control - Abs test)/ Abs control] x 100 (Where: **Abs control**: Absorbance of the ethanolic solution of DPPH; Abs test: Absorbance of the sample tested).The median inhibitory concentration (IC_{50}) is determined graphically by linear regression [14, 18]. **FRAP iron reduction test**: The phenolic extract of fennel seeds was diluted to prepare a series of dilutions with different concentrations (100 µg/mL). An aliquot of 1 mL of each concentration was transferred to test tubes. 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide $K_3Fe(CN)_6$ solution were added. The tubes were incubated in a water bath at 50°C for 20 min. The reaction was then stopped by adding 2.5 mL of trichloroacetic acid (10%). The tubes were centrifuged at 3000 rpm for 10 min and 2.5 ml of the supernatant from each concentration was mixed with 2.5 mL distilled water and 0.5 mL of $FeCl_3$ (0.1%). Absorbance was then measured at 700 nm against a blank. Ascorbic acid was used as a positive control and its antioxidant power was determined under the same conditions as the tested samples [14].

Physicochemical analyses of J'ben cheese:

All physicochemical analyses were performed in triplicate (n = 3) on experimental J’ben cheeses supplemented with 1, 2 and 3% hydroethanolic extract of Foeniculum vulgare and on the untreated control. To investigate the changes during storage, changes in physicochemical properties, lipid oxidation and antioxidant activity were monitored during the storage period (day 1, 5, 10, and 15) at 4°C

under refrigerated storage. **pH**: The pH of the cheese was measured by homogenising 1 g of sample with 10 mL of distilled water and measurement with a pH meter [19]. **Titratable Acidity**: To determine the titratable acidity of the cheese 10 mL of the previous suspension was titrated with 3 drops of phenolphthalein (1%). Titration was done with a sodium hydroxide solution NaOH at 1/9 N (mol.L-1) under agitation until the medium turned pink [20]. Results are given in Dornic degrees (°D) and calculated using the following formula [21]: Titratable acidity = V (NaOH) x 10. **Dry matter** (total solids content); In pre-weighed porcelain crucibles, 5 g of each cheese sample was introduced. The crucibles were then placed in an oven at 103–105°C for 3 hours. After cooling in a desiccator, the mass of residual dry matter was measured. The dry matter (DM) content achieved was expressed as a percentage of the initial sample weight and computed following the formula below [21]: DM (%) = [(M2-M1)/M0]x 100 (Where: M0: Test sample mass (g); M1: Mass of empty capsule (g); M2 = mass of crucible + residue after drying and cooling (g)). **Moisture**: The moisture content (Hm%) of the samples was determined in percentage (%) according to the following equation [22]: Mc (%) = 100 - TDE (Where: Mc: Moisture content (%); TDE: Total dry extract (%)). **Fatty matter**: Three grams of cheese were put into a Van de Gulik butyrometer. Afterwards sulphuric acid was added through the narrow opening until the acid level fully covered the weighing system. The butyrometer was placed in a water bath at 65 ± 2°C for 5 minutes. After this, the butyrometer was removed and shaken vigorously for 10 seconds. These two operations were repeated for 1 hour until the proteins were completely dissolved. After shaking, 1 mL of isoamyl alcohol was added, followed by sulfuric acid to the 35% mark on the scale. The butyrometer was inverted and shaken vigorously for 10 seconds as soon as the fat had risen into the chamber. The inversion and shaking operations were repeated twice. The butyrometer was then returned to a water bath for 5 minutes. After removing the butyrometer from the water bath, the large stopper was adjusted to bring the fat column into the graduated section. The butyrometer was centrifuged at a relative centrifugal acceleration of 350 ± 50 g for 10 minutes [23]. The fat content, expressed in grams per 100 g of cheese, was calculated as follows [23]: Fat content = B - A; (Where: **A**: reading at the lower end of the fat column; B: reading at the upper end of the fat column). **Ashes content**: The mineral content of the experimental cheeses was assessed according to a standard official method [24]. A 5 g quantity of J'ben was placed in crucibles and subjected to a muffle furnace at a temperature of 550°C for 4 hours. After cooling in a desiccator, the mass of mineral residue remaining in the crucibles was measured. The ash content was expressed as a percentage (%) using the following formula: Ash content (%) = [(M2 - M1) / M0] × 100 (Where: M_0: Mass of the test sample (g); M_1: Mass of the empty crucible (g); M_2: Mass of the crucible with incineration residue). **Organic matter**: The organic matter content of the experimental cheeses was expressed as a percentage according to the following formula [25]: OM (%) = DM (%) - MM (%) (Where: OM: Organic matter (%); DM: Dry matter (%); MM: Mineral matter (%)). **Estimation of the degree of lipid oxidation using the TBARS method**: Malondialdehyde (MDA) content in our J'ben was assessed according to the method described by Xiong *et al*. [26]. A 2 g sample of cheese was mixed with 25 mL of a 20% aqueous solution of trichloroacetic acid (TCA) and 20 mL of distilled water. The mixture was then left to stand at room temperature for 1 hour. After this, the mixture was centrifuged at 2000 rpm for 10 minutes, and the resulting filtrate was diluted to 50 mL with distilled water. A 5 mL aliquot of the filtrate thus obtained was mixed with 5 mL of a 0.02 M aqueous solution of 2-thiobarbituric acid (TBA), then

heated to 95°C for 20 minutes in a water bath, followed by cooling to 0°C for 5 minutes. The absorbance of the samples was measured at 532 nm using a spectrophotometer against a blank (distilled water), and the values obtained were expressed in mg/100 g of cheese according to the following formula: TBARS (mg malondialdehyde/100 g cheese) = A_{532} × 7.8 (Where: A_{532}: absorbance measured at 532 nm). **Assessment of J'ben antioxidant activity using the DPPH test**: Fifty grams of cheese were ground and mixed with 80% methanol to reach a total volume of 250 mL. After incubation for 4 hours at room temperature with stirring, the mixture was centrifuged at 3000 rpm for 15 minutes. The supernatant was filtered through a 0.45 µm filter (Whatman) into an amber glass vial. The volume was made up to 250 mL with 80% methanol to give the final extract [27]. The antioxidant activity of the obtained extract was determined by DPPH test according to the method described by Archana et al. [17].

Microbiological analysis of J'ben cheese

Microbiological analyses were conducted in triplicate on the experimental cheeses during storage at 4°C for 15 days. For the preparation of decimal dilutions, 10 g of cheese sample were aseptically weighed, homogenized with 90 mL of Tryptone Salt Water (TSW), and used as the initial dilution. From this stock solution, a series of decimal dilutions (10^{-1} to 10^{-n}), was prepared for seeding in culture media [28].

Enumeration of pathogenic and spoilage bacteria

One milliliter of each decimal dilution was transferred into sterile Petri dishes. For the enumeration of total aerobic mesophilic flora (TAMF), samples were inoculated with Plate Count Agar (PCA) and incubated at 30 ± 1°C for 72 ± 2 h, according to [29]. Total coliforms were enumerated using Violet Red Bile Lactose (VRBL) agar, followed by incubation at 30 ± 1°C for 24 ± 2 h. Fecal coliforms were enumerated after incubation at 44 ± 1°C for 24 ± 2 h [29]. For the enumeration of psychrotrophic bacteria, Petri dishes containing nutrient agar were incubated at 5–7°C for 10 days [30]. For the enumeration of yeasts and molds, plates were inoculated with Oxytetracycline Glucose Agar (OGA) and incubated at 30°C for 3–5 days, according to ISO 7954 [31] and Doukani [32]. For the enumeration of *Staphylococcus aureus*, 0.1 mL of each dilution was surface-inoculated onto Chapman agar, and the plates were incubated at 37°C for 48 h [33]. Similarly, *Pseudomonas aeruginosa* was enumerated by surface plating on King A agar, followed by incubation at 36 ± 2°C for 5 days [34].

Enumeration of lactic acid bacteria

For the enumeration of lactobacilli, 1 mL of each decimal dilution was inoculated by the pour plate method onto MRS agar adjusted to pH 5.4. The plates were then incubated under anaerobic conditions at 43°C for 48 h. Streptococci were enumerated by deep inoculation onto M17 agar, followed by incubation at 43°C for 24–48 h [35].

Organoleptic tests

Every 5 days during the 15-day storage period at 4°C, the sensory quality of the experimental cheeses was evaluated by a semi-trained panel of 10 assessors (predominantly female). The evaluation included colour, homogeneity, lumpy appearance, odour, acidity, salinity, aftertaste, and sticky texture attributes. A 10-point hedonic scale was used, with scores ranging from 1 (lowest acceptability) to 10 (highest acceptability).

Statistical processing

STAT BOX 6.4 software was used to analyze the experimental results, which were expressed as means ± standard deviations. Physicochemical and microbiological data were subjected to a randomized two-factor analysis of variance (ANOVA), followed by pairwise comparisons of means using the Newman-Keuls test. Organoleptic data were analyzed using the Friedman test. The effects of the studied factors studied were assessed at two probability thresholds: at $p < 0.05$ and $p < 0.01$.

Results

Bioactive compounds in Foeniculum vulgare extract

The results of the biochemical analysis of the extract are presented in Table 1. The DPPH and FRAP assays demonstrated that the hydroethanolic extract of *F. vulgare* possessed notable antioxidant capacity, with measured activities of 43.239 and 48.033 mg/mL, respectively. This antioxidant potential was associated with the high levels of total phenolic compounds (1528.27 mg GAE/100 g DM) and flavonoids (112.96 mg QE/100 g DM) detected in the extract.

Table 1
Polyphenol and flavonoid contents and antioxidant activity of the hydroethanolic extract of *F. vulgare* seeds.

Polyphénols			**Flavonoids**		
Extract		**Plant**	**Extract**		**Plant**
Mg GAE/mL extract	**Mg GAE/g lyophilisate of extract**	**Mg GAE/100 g DM**	**Mg QE/mL Extract**	**Mg QE/g lyophilisate of extract**	**Mg QE/100 g DM**
09.20 ± 00.05	74.80 ± 00.40	1528.27 ± 08.31	00.68 ± 00.04	05.51 ± 00.30	112.96 ± 06.00
DPPH test			**FRAP test**		
Vitamin c	**Hydroethanol extract**		**Vitamin c**	**Hydroethanol Extract**	
EC 50 (mg vitamin c/mL)	**EC 50 (mg lyophilisate/mL)**		**EC 50 (mg vitamin c/mL)**	**EC 50 (mg lyophilisate/mL)**	
15.81 ± 00.01	43.239 ± 00.04		15.81 ± 00.01	43.239 ± 00.04	

Results are expressed as mean ± standard deviation of three independent replicates (n = 3). GAE: gallic acid equivalent; QE: quercetin equivalent; EC_{50}: median effective concentration; DM: dry matter

Physicochemical quality of Cheeses

Table 2 and Fig. 1 show the impact of the addition of phenolic compounds from fennel seeds on the physicochemical characteristics of J'ben. An inverse correlation between cheese acidity and pH values ($P < 0.01$) was observed over the storage periods, with respective values of 14.94 to 17.24 °D versus 4.59 to 4.38 (pH units). This trend appears to be confirmed by the increase in plant extract levels from 0 to 3% in the cheeses ($P < 0.05$), with successive values of 17.22 to 15.49 °D compared with 4.45 to 4.49 (pH units). On the other hand, increasing the levels of extract added as an additive led to significant changes in dry matter levels ($P < 0.01$), decreasing notably from 42.18 to 36.15% RM. Moisture values ($P < 0.001$) increased from 57.82 to 63.85% RM, and organic matter values ($P < 0.001$) also decreased from 39.91 to 33.71% RM in the cheeses. Fat content increased significantly with storage time, rising from 31.58 to

51.5% RM and from 35.44 to 41.97% RM depending on the rate of addition of *F. vulgare* extracts ($P < 0.05$).

Table 2
Effect of adding phenolic extract of *F. vulgare* on the physicochemical quality of J'ben fresh cheeses during 15 days of storage at 4°C.

	Extract incorporation rate (%) (F1; n = 12)				**Effect of doses**
	0%	**1%**	**2%**	**3%**	
PH	4.45^{b} ±0.051	4.51^{a}±0.008	4.49^{ab}±0.038	4.49^{ab}±0.017	$P < 0.05$
Acidity (°D)	17.22 ± 5.09	15.39 ± 2.32	16.32 ± 2.80	15.49 ± 1.65	$P > 0.05$
Dry matter (%RM)	42.18^{a}±0.93	38.74^{b}±1.64	36.15^{c}±1.63	38.93^{b}±2.07	$P < 0.01$
Humidity (%RM)	57.82^{c}±0.93	61.26^{b}±1.64	63.85^{a}±1.63	61.07^{b}±2.07	$P < 0.01$
Organic matter (%RM)	39.91^{a}±1.02	36.89^{b}±1.82	33.71^{c}±1.67	36.67^{b}±2.16	$P < 0.01$
Mineral matter (%RM)	2.27 ± 0.21	1.85 ± 0.88	2.44 ± 0.39	2.26 ± 0.74	$P > 0.05$
Fat matter (%RM)	37.17^{b}±4.24	37.5^{b} ± 3.29	35.44^{b} ±4.31	41.97^{a}±3.15	$P < 0.01$
FM/ DM	0.88^{b}±0.11	0.96^{ab}±0.09	0.98^{ab}±0.12	1.09^{a}±0.12	$P < 0.01$

Results are expressed as means ± standard deviations. n: number of replicates; F1: Factor 1; RM: raw matter; FM: fat matter; DM: Dry matter; $p < 0.01$: highly significant effect of the studied factor; $p < 0.05$: significant effect of the studied factor; $p > 0.05$: non-significant effect of the studied factor; a,b,c,…: homogeneous groups according to pairwise comparison of means using the Newman-Keuls test.

Malondialdehyde levels and antioxidant activity of cheeses:

Table 3 and Fig. 2 illustrate the impact of adding phenolic extract from *F. vulgare* seeds on lipid oxidation and antioxidant activity of experimental cheeses during a 15-day storage period. A significant reduction ($p < 0.05$) in malondialdehyde levels was observed in cheese treated with 3% extract compared to the control (0.24 vs. 0.29 mg/100 g). This decrease can be attributed to the high antioxidant activity demonstrated, notably with the DPPH test (197.21 mg/mL vs. 266.53 mg/mL) in the respective products. A clear increase in malondialdehyde levels was recorded throughout the storage period, and a decrease in antioxidant activity was revealed by the DPPH test in the respective products (Fig. 2).

Table 3
Effect of adding phenolic extract of *F. vulgare* as a natural additive on malondialdehydes levels and DPPH antioxidant activity of cheeses during storage.

	Extract incorporation rate (%) of *F. vulgare* seed (F1, n = 12)				**Effect of doses**
	0%	**1%**	**2%**	**3%**	
MDA rate (mg MDA /100 g)	00.29 ± 00.017	0.29 ± 0.1	00.25 ± 00.09	00.24 ± 00.04	**P > 0.05**
	0%		**3%**		
EC 50 of cheese (mg/mL)	266.53^{a}**±**13.15		197.21^{b}**±**46.33		**P < 0.01**

Results are expressed as means ± standard deviations. n: number of replicates; F1: Factor 1; MDA: malondialdehyde; EC_{50}: median effective concentration; p < 0.01: highly significant effect of the studied factor; p < 0.05: significant effect of the studied factor; p > 0.05: non-significant effect of the studied factor; a,b,c,…: homogeneous groups according to pairwise comparison of means using the Newman-Keuls test.

Microbiological quality of cheeses

The results of the microbiological analyses are presented in Table 4 and Fig. 3. The hydroethanol extract of *F. vulgare* demonstrated antimicrobial and prebiotic properties beneficial for the preservation of J'ben. It offered protection against pathogens such as *Psychrotrophs*, Faecal coliforms, and *Pseudomonas aeruginosa*, reducing the development of undesirable spoilage bacteria (TAMF, total coliforms, yeasts and molds) while promoting the growth of beneficial lactic acid bacteria. During the cheese storage period, no significant changes were observed for TAMF, total coliforms, *Staphylococcus aureus*, yeasts and olds, as well as *Streptococcus*. However, a decrease was noted over time for *Lactobacillus*. *Psychrotrophs*, feal coliforms and *Pseudomonas aeruginosa* were consistently absent in cheeses supplemented with fennel extract throughout the entire storage period.

Table 4
Effect of adding phenolic extract of *F. vulgare* on the microbiological quality (CFU/g) of experimental cheeses during storage.

	Extract incorporation rate (%) (F1, n = 12)				Effect of doses
	0%	1%	2%	3%	
TAMF	$175x10^{4a}$	$133x10^{3b}$	$111x10^{3b}$	$72x10^{3b}$	P > 0.05
Psychrotrophs	ABS	ABS	ABS	ABS	ABS
Fecal coliforms	ABS	ABS	ABS	ABS	ABS
Pseudomonas auerogenosa	ABS	ABS	ABS	ABS	ABS
Total coliforms	$79x10^3$	$52x10^3$	$50x10^3$	$29x10^3$	P > 0.05
Staphylococcus aureus	$138x10^3$	$143x10^3$	$59x10^3$	$68x10^3$	P > 0.05
Yeasts and molds	$30x10^3$	$46x10^3$	$131x10^2$	$153x10^2$	P > 0.05
Lactobacillus spp.	$54x10^4$	$61x10^4$	$86x10^4$	$92x10^4$	P > 0.05
Streptococcus spp.	$46x10^3$	$46x10^3$	$33x10^3$	$279x10^2$	P > 0.05

Results are expressed as means ± standard deviations. n: number of replicates; F1: Factor 1; CFU: colony-forming unit; TAMF: total aerobic mesophilic flora; ABS: absence; p < 0.01: highly significant effect of the studied factor; p < 0.05: significant effect of the studied factor; p > 0.05: non-significant effect of the studied factor; a, b, c, ...: homogeneous groups according to pairwise comparison of means using the Newman-Keuls

test.

Organoleptic quality of cheeses

Figure 4 shows the results of the sensory evaluation carried out throughout the 15-day storage period on the experimental J'ben cheeses. The sensory analysis revealed that the cheese prepared with 3% fennel phenolic extract was highly appreciated by the panelists over time, at least as much as the control, if not more, for certain organoleptic criteria such as odour and colour.

Discussion

Bioactive compounds in fennel extract

Aromatic and medicinal plants contain a wide range of bioactive molecules that have potential applications in various fields, including cosmetics, pharmaceuticals, agri-food products, phytosanitary

formulations, and other biotechnological sectors [36]. *F. vulgare*, commonly known as "fennel" or "*besbes*" in Arabic, a member of the *Umbelliferae* family (*Apiaceae*), has been known and used by humans since ancient times [37–38]. In particular, the seeds of this plant have traditionally been regarded as a rich source of diverse phytoconstituents with potential biological activities [39].

Among these bioactive compounds, the phenolic content of the hydroethanolic extract (80/20, ethanol/water, v/v) prepared from fennel seeds harvested in Ain Defla, Algeria, was very high at 1528.27 mg GAE/100 g DM. These values remain higher than those reported by Harrak *et al*. [40] in a study evaluating the physical, physicochemical, and biochemical criteria and biological activities of fennel (*F. vulgare* Mill.) seeds and seed oils, whose contents were estimated at 623.64-959.24 mg GAE/100 g DM.

Flavonoids are among the most extensively studied classes of plant compounds [41, 42]. As with phenolic compounds, the *F. vulgare* seed extract studied showed a relatively low flavonoid content of 112.96 mg QE/100 g DM. Higher values on the order of 5.57 and 4.16 mg QE/g DM have, however, been previously reported [43]. According to Bentahar *et al*. [44], the content and nature of bioactive compounds in plants can vary according to several factors, including climatic and environmental conditions, plant maturity stage, seed storage time, and the different extraction and assay methods used. These bioactive compounds, including phenolics and flavonoids, are well known for their diverse biological activities, including antioxidant activity [43]. To this end, two activity tests, namely DPPH radical scavenging capacity and reducing power (FRAP), were carried out in this study to determine the antioxidant activity of the phenolic extract from fennel seeds collected in the Ain Defla region of Algeria.

The EC_{50} values of the extract obtained by the two tests ranged from 43.239 mg extract lyophilisate/mL for the DPPH test to 48.033 mg extract lyophilisate/mL for the FRAP test. The antioxidant power of the experimental extract was thus low compared with that of vitamin C, whose EC 50 was evaluated at 15.81 mg vitamin C/mL for the DPPH test and 22.155 mg vitamin C /mL for the FRAP test. However, compared with the results reported by Bettaieb Rebey *et al*. [43], who reported EC_{50} values of 1.11 mg lyophilisate /mL extract for the DPPH test and 49 µg lyophilisate /mL extract for the FRAP test, the pure extract of 100% Algerian concentrated fennel seeds possesses moderate antioxidant activity.

Because of its antioxidant power, as demonstrated in this study, and its other properties, including antimicrobial activity against numerous pathogenic germs responsible for food poisoning (*Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhimurium, and Shigella flexneri*), as reported by several authors [38, 45], fennel seed extract can be used as a natural food additive that may replace chemical additives in preserving and improving the quality of processed agri-food products.

Physicochemical quality of cheeses

In Algeria, cheeses are still traditionally produced on a family scale [46]. J'ben, a fresh cheese, occupies a special place among the many local cheeses available throughout the country [47]. It is made from raw or pasteurized cow's, goat's, and sheep's milk on a small scale during the period of milk abundance [48].

The method of making J'ben is still in use today, with increased consumption in steppe areas due to its pleasant organoleptic and nutritional properties [46].

According to Samet-Bali *et al*. [49] and Benkerroum [50], J'ben is considered to be one of the fresh cheeses with a limited shelf life (3 to 10 days) even when stored at refrigeration temperature. This suggests the need to find alternative solutions in order to develop its production, improve its quality, and better preserve it to satisfy ever-increasing consumer demand.

In the present study, the antioxidant and antimicrobial potential of *F. vulgare* extract, which is rich in phenolic compounds [3], was used as a natural ingredient in J'ben in order to increase its shelf life and improve its chemical composition as well as its nutritional quality.

Evaluation of the chemical characteristics of J'ben during cold storage at 4°C showed that acidity and pH values varied inversely over time and with increasing levels of phenolic extract from fennel seeds incorporated into the cheese. pH values evolved during storage from 4.55 to 4.38, while acidity values increased from 14.94 to 17.24 °D. At extract concentrations of 0%, 1%, 2%, and 3%, the results fluctuated ($p < 0.05$), with pH ranging from 4.45 to 4.49 and acidity ranging from 17.22 to 15.49 °D.

Our results contradict those of Borges *et al*. [4], who studied artisanal *Minas Canastra* cheese made with *Cynara cardunculus* L. These discrepancies are undoubtedly related to the probiotic or antimicrobial effects exerted by the bioactive compounds contained in the various extracts, as well as their ability to stimulate or inhibit the fermentative activity of the bacteria in the cheese matrix. This in turn, modulates lactate production, which is responsible for the observed decreases in pH. Despite these variations in acidity as a function of storage time and extract concentration, the values remain close to those reported by numerous studies on this J'ben-type fresh local cheese [46, 51, 52].

The cheese studied also presented dry extract levels that, on the one hand, decreased remarkably from 42.18 to 38.93% with increasing concentrations of hydroethanolic extract added, and, on the other hand, increased from 34.84 to 41.34% from the beginning to the end of cold storage at 4°C after 15 days. Moisture levels, conversely, increased from 57.82 to 61.07% with the addition of extracts and decreased from 65.16 to 58.77% during storage. These dry extract levels for experimental J'ben with or without added fennel extract were higher than those obtained for the same cheese by Dahou *et al*. [46], while moisture content levels were well below those obtained by Benkerroum and Tamime [51].

Fat content varied significantly in the experimental cheeses according to the concentration of fennel seed phenolic extract added (37.17 to 41.97% RM) and during the storage period (31.58 to 51.5% RM on average). These levels are much higher than those reported by some authors [46, 53], who found lower and variable values ranging from 16.5 to 42% in the same fresh cheese. Moreover, the ratios between fat and dry matter observed were very high compared with the results obtained by Dahou *et al*. [46]. Regarding mineral matter, no change was observed during storage regardless of the concentration of phenolic fennel seed extract added, whereas organic matter decreased with the addition of hydroethanolic fennel seed extract. These responses are undoubtedly the result of several factors,

including the quality of the milk used, the type of coagulation performed, the draining and the diversity of technological parameters applied during milk processing [46].

Regarding the antioxidant activity of J'ben with hydroethanolic extract of *Foenuculum vulgare* seeds rich in phenolic compounds, as revealed by the DPPH test, it appears that cheeses with added extract, particularly at a concentration of 3%, exhibit a more favorable EC_{50} than the control: 197.21 vs. 266.53 mg/mL. Our results corroborate those of Caleja *et al*. [3], who conducted an experiment in the same study context. These findings were further confirmed by the TBARS test carried out on the experimental cheeses during storage, in which malondialdehyde levels decreased remarkably from 0.29 to 0.24 mg/100 g with an increase in extract concentration from 0 to 3%. In this regard, Rather *et al*. [37] state with certainty that the phenolic extract of fennel seeds, regardless of the extraction solvent used, possesses an extraordinary capacity to reduce malondialdehyde levels in food products over a long shelf life.

Microbiological quality of cheeses

Milk and the cheese derived from it are very rich in proteins, lipids and sugars, providing an ideal ecological niche for the development and proliferation of numerous micro-organisms [54]. The microbiological quality of J'ben fresh cheese may vary from one region to another and from one producer to another. As a fermented product, J'ben is often characterized by the presence of very high levels of total aerobic mesophilic flora (TAMF), which can exceed10^8 CFU/g at the end of the manufacturing process [55]. During storage, experimental samples of cheese prepared with and without fennel seed extract showed TAMF counts ranging from 84.103 to 63.10^5 CFU/g. Superior microbial proliferation during storage of J'ben-type cheeses have has been reported by many authors [48, 56].

As fermentation promotes the active multiplication of all microorganisms (including beneficial lactic acid bacteria) present in the manufacturing milk, the enumeration of TAMF alone in this case cannot reflect the hygienic quality of processed J'ben cheese [54]. The fermentation process that can occur in milk and cheese during storage may also promote the multiplication of certain contaminating bacteria, particularly microorganisms of fecal origin such as coliforms. Indeed, studies carried out on Moroccan J'ben have shown high levels of faecal microflora exceeding of 10 germs/g [55]. This may constitute a potential hazard for consumers [54]. In the present study, no presence of these microorganisms, nor of *psychrotrophic* bacteria or *Pseudomenas aeruginosa*, was detected in the products. Regarding total coliforms, yeasts, molds and *Staphylococcus aureus*, the values recorded in the experimental cheeses appear to exceed accepted standards [57], but are comparable to those found by Salmerón *et al*. [56] and Tadjine *et al*. [48].

Overall, the addition of *F. vulgare* seed extracts as natural additive rich in phenolic compounds, particularly at a concentration of 3%, led to a drastic reduction in the microbial load, both common and pathogenic, of the J'ben-type cheeses studied during storage. This included a 57.52% reduction in TAMF, a 58.97% reduction in total coliforms, and 81.99% reduction in *Staphylococcus aureus* and a 15.90% reduction in the proliferation of yeasts and molds. According to Rather *et al*. [37], and Manju Nag and

Shahi [38], these results are linked to the strong antimicrobial activity of the bioactive compounds contained in fennel extract against certain pathogenic and common food-borne bacterial strains. Indeed, several chemical constituents of *F. vulgare* have been identified as active antimicrobial principles, such as the phenylpropanoid derivative dillapional and scopoletin, a coumarin derivative with lesser antimicrobial activity [58]. However, as pointed out by Achemchem [54] and Benyagoub *et al*. [59], good hygienic manufacturing and storage conditions must remain a priority in order to better control and preserve the microbiological quality of J'ben-type fresh cheeses produced in Algeria.

Moreover, the addition of fennel seed extract does not appear to affect the beneficial lactic flora of the cheese during the entire 15-day cold storage period at 4°C, including *Lactobacillus*, whose growth was slightly stimulated, likely due to the content of the main bioactive compounds with a prebiotic effect [56]. This flora, native or added in the form of selected strains to milk during the manufacturing process, plays a key role in the biopreservation of J'ben through the lactate produced by lactose fermentation [54]. In addition, *Lactobacillus* and certain intestinal species of the *Enterococcus* genus, are considered probiotics capable of preventing colonization of the gastrointestinal tract by various pathogens, owing to their ability to adhere to *enterocytes* and hinder pathogen adhesion to the intestinal mucosa through the production of bacteriocin-type inhibitory substances via a competitive exclusion mechanism [54].

Organoleptic quality of cheeses

Regarding the organoleptic assessment, the panelists who took part in the sensory evaluation indicated that the bitterness, lumpiness, and sticky texture of the cheeses were not altered by the addition of phenolic fennel seed extract. Panelists even noted a clear improvement in the perceived freshness of the experimental cheeses compared with the control. The acidity of the cheeses remained constant throughout the storage period, even with the addition of fennel seed phenolic extract, except that on day 5, the cheese with 2% extract showed better scores than the other experimental trials. The normal development of lactic acid bacteria in cheeses [5], including *Lactobacillus*, which was unaffected by the antibacterial effect of the phenolic compounds contained in the extract, is the likely explanation for these findings.

The salinity of J'ben cheeses was well accepted by all panelists involved in the study, who perceived no difference except on the first day of storage in cheeses prepared with a concentration of 3% hydroethanolic extract of *F.vulgare*, whose taste was very pronounced. This was not due to the dose of plant extract added to the cheeses, but rather to poor control of the curd salting time, which varied among the experimental cheeses. Furthermore, a clear improvement in colour and pleasant characteristic odour of the cheeses was observed with increasing concentrations of *F. vulgare* phenolic extract added to the products as a food additive. In this regard, it has long been known that fennel seeds are rich in aromas and aromatic compounds such as trans-anethole, cineole, fenchone, α-pinene, anisaldehyde, and limonene [40], which have traditionally been used as an ingredient to improve the odour of processed products [38] and as a natural food coloring that is highly appreciated by consumers [60].

Conclusion

The hydroethanolic extract of *F. vulgare* demonstrated strong antioxidant activity due to its high phenolic and flavonoid content, significantly reducing malondialdehyde levels in preserved cheeses. In addition, this extract showed promising antimicrobial properties against certain pathogenic microorganisms, as well as favorable prebiotic potential for lactic bacteria. The addition of this extract to J'ben at a concentration of 3% extended the microbiological shelf life to over 15 days, and promoted the growth of probiotic lactic acid bacteria beneficial to health. The physicochemical parameters of the experimental cheeses were also influenced, while organoleptic tests demonstrated an improvement in taste, odour, and colour attributable to the phenolic compounds of fennel. These results suggest that the phenolic extract of *F. vulgare* seeds represents a promising natural alternative to chemical additives in the food industry.

Declarations

Author Contribution

Author Contributions: Conceptualization: Ait Saada D.; Methodology: Ait Saada D., Ait Chabane O., Abbassene F., Moghtit K., Tabet Aoul F.; Investigation: Ait Chabane O., Abbassene F., Moghtit K., Ait Chabane F.; Data Curation: Ait Chabane O., Abbassene F., Ait Saada D.; Formal Analysis: Ait Saada D., Ait Chabane O., Abbassene F.; Resources: Ait Saada D., Ait Chabane O.; Writing – Original Draft Preparation: Ait Chabane O., Ait Saada D.; Writing – Review and Editing: Ait Saada D., Drider D., Abbassene F.; Visualization: Ait Chabane O., Moghtit K.; Supervision: Ait Saada D.; Project Administration: Ait Saada D.; Funding Acquisition: Ait Saada D.

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Figures

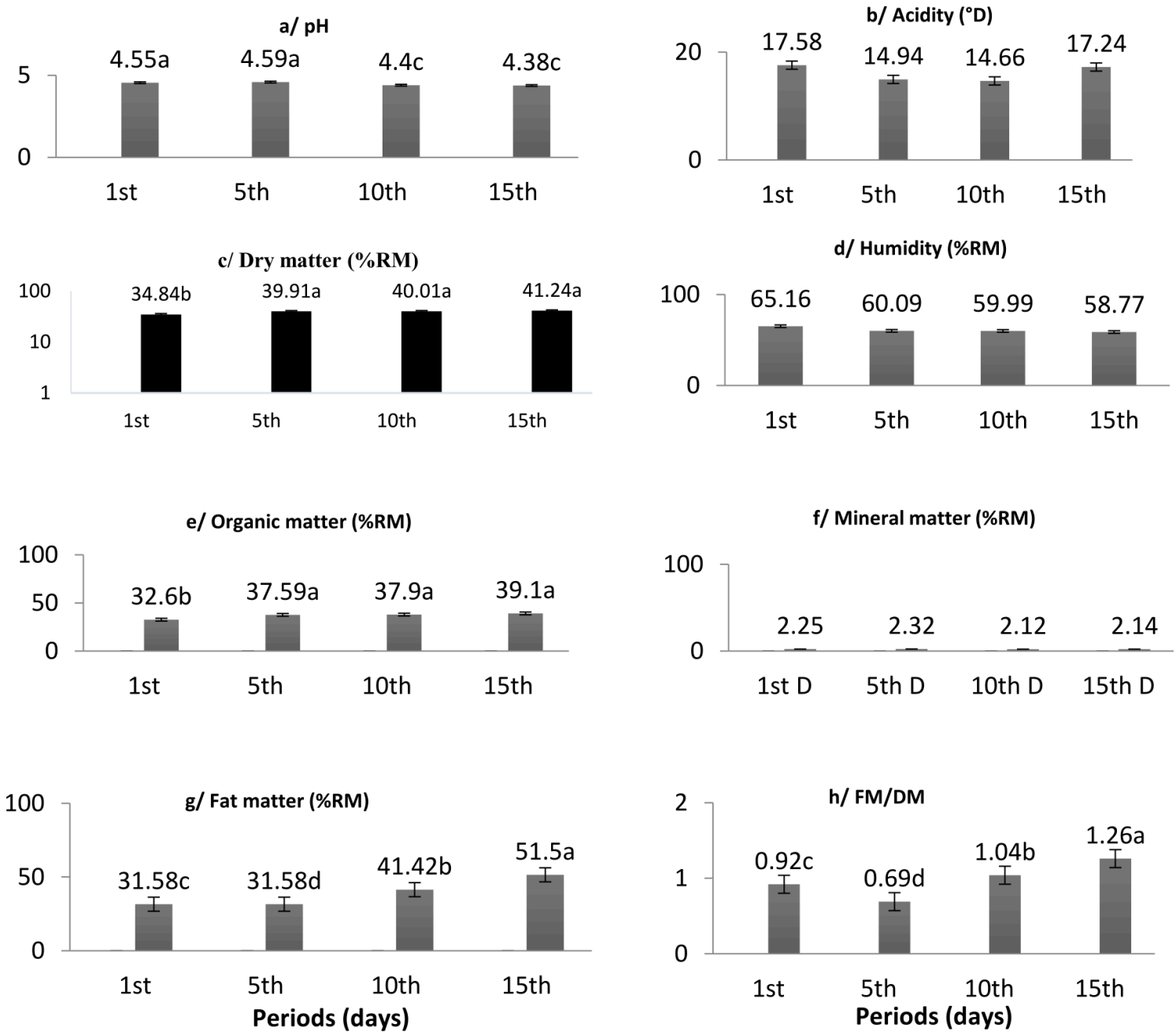

Figure 1

Overall changes in physicochemical parameters of experimental J'ben fresh cheeses during storage (DM: raw material; FM: fat matter; RM: raw matter; a,b,c...etc.: homogeneous groups according to pairwise comparison of means using the Newman-Keuls test).

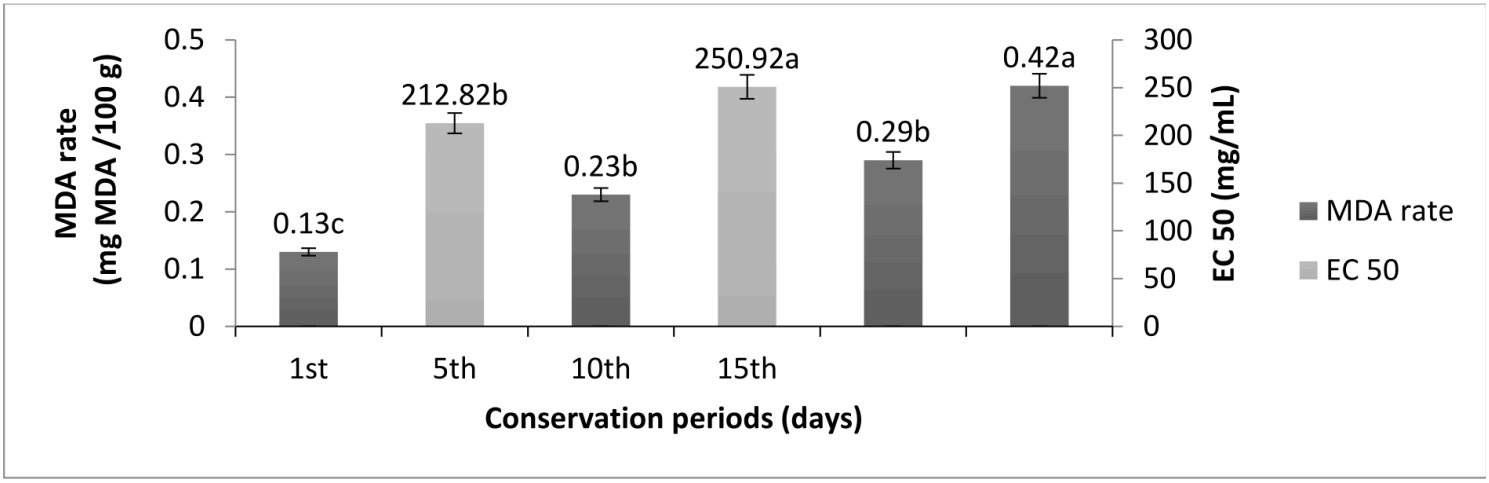

Figure 2

Evaluation of malondialdehyde (MDA) levels and DPPH antioxidant activity of experimental cheeses during storage (EC_{50}: median effective concentration; a,b,c...: homogeneous groups according to pairwise comparison of means using the Newman-Keuls test).

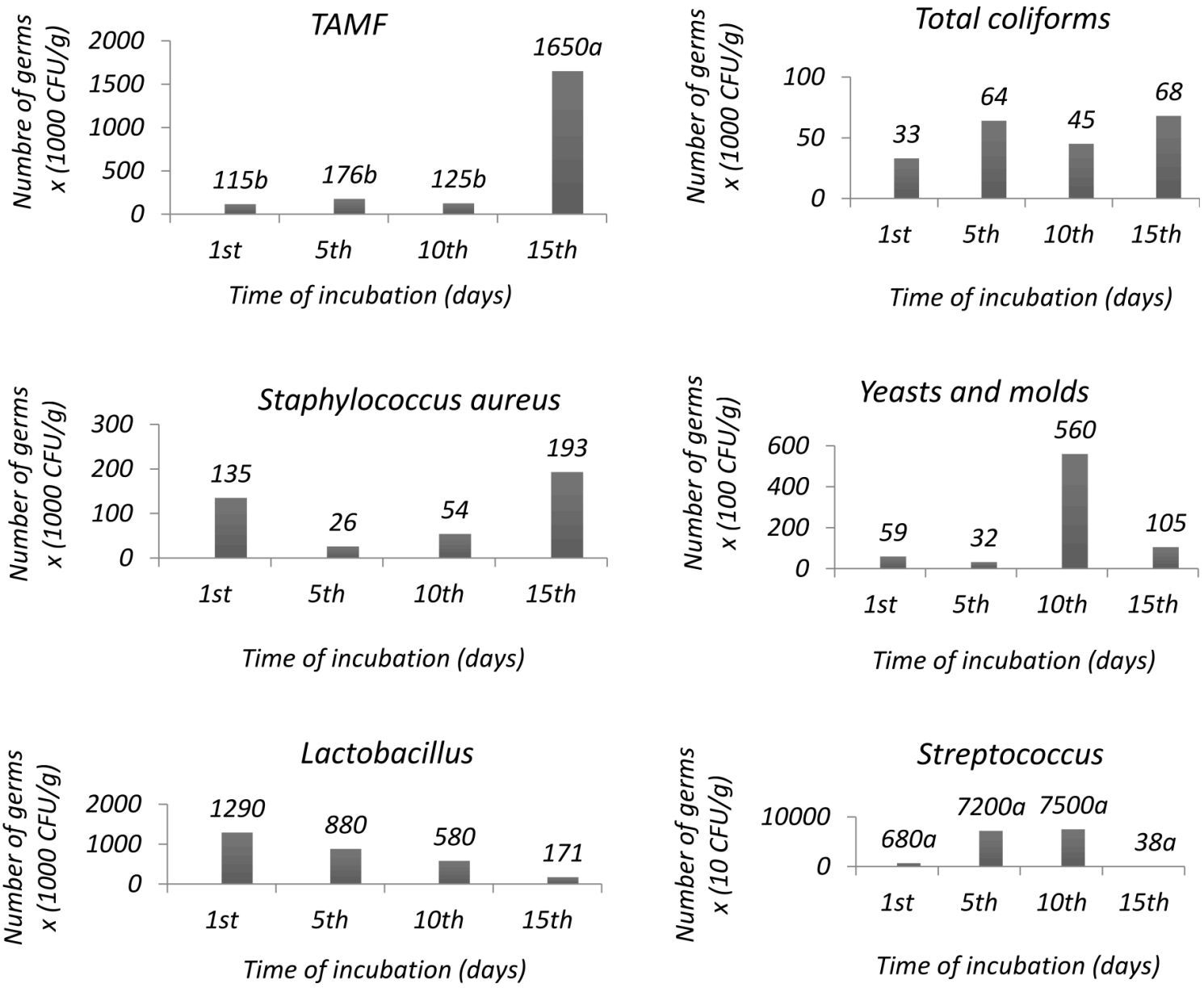

Figure 3

Evolution of microbiological quality of experimental cheeses during storage (D: days; CFU: olony-forming unit; TAMF: total aerobic mesophilic flora; a,b,c,…: homogeneous groups according to pairwise comparison of means using the Newman-Keuls test).

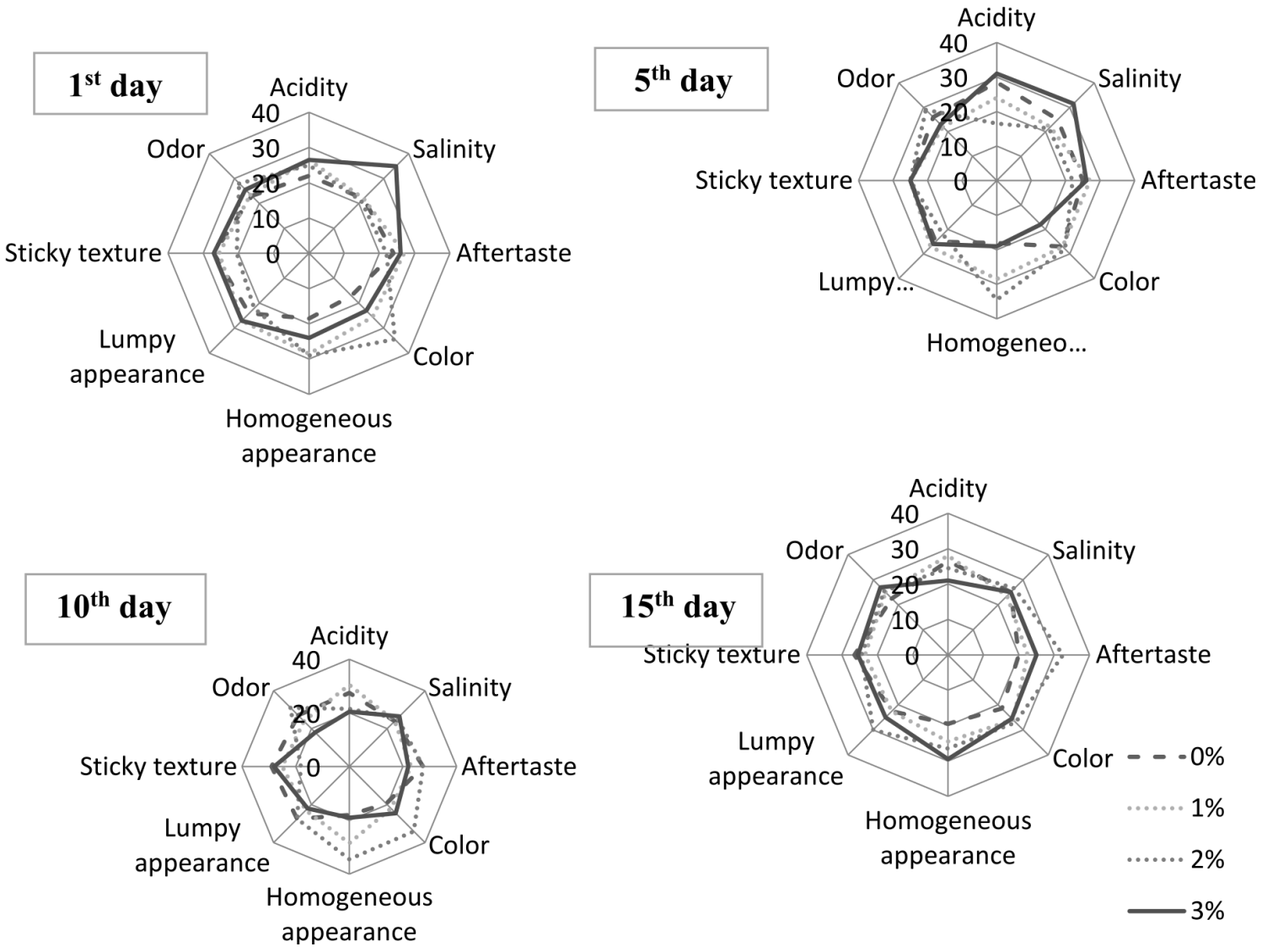

Figure 4

Effect of *F. vulgare* extract on the sensory quality of cheeses during 20 days of cold storage (0, 1; 2 et 3% ; Percentage of hydroethanolic extract of *F. vulgare* incorporated into Jben cheese).