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Development and characterization of spirulina (*Arthrospira platensis*)-fortified cheeses from camel and bovine milk

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ABSTRACT

This study aimed to develop novel functional cheeses from camel and bovine milk, fortified with varying concentrations of Spirulina (*Arthrospira platensis*) powder at 0.1, 0.25, 0.5, and 1.0 %, and to assess their physico-chemical, textural, and bioactive properties. The antioxidant assays demonstrated that cheeses from camel and cow's milk revealed significant antioxidant activities, which were enhanced with the addition of Spirulina ($p < 0.0001$). Increasing Spirulina content led to a decrease in L* and a* values, along with an increase in b* values compared to the control samples ($p < 0.0001$). The textural properties of both camel and bovine cheeses were significantly affected, with a marked reduction in hardness as Spirulina concentration increased ($p < 0.0001$). However, the impact on texture was minimal in camel milk cheese. The composition of camel milk resulted in a softer curd structure, allowing for the even distribution of Spirulina without disrupting the protein network. In contrast, Spirulina interacted with the denser protein matrix of bovine cheese, leading to a notable decline in textural properties. Overall, the results suggest that Spirulina may serve as a natural component in camel cheeses formulations in order to enhance their nutritional profile while maintaining desirable functional properties.

1. Introduction

Spirulina (*Arthrospira platensis*) is the most popular microalgae species which is known for its high macronutrients content (proteins, vitamins, fats, minerals, and fibers) and bioactive compounds (unsaturated fatty acids and essential amino-acids), whose nutritional value and therapeutic properties can positively impact consumers' health [1]. Indeed, the presence of these nutritional compounds confers functional properties to Spirulina, leading to consider it as a promising ingredient for the development of novel formulations of food products. These products could help address malnutrition while providing a range of additional health benefits [2]. Additionally, Spirulina's therapeutic properties go beyond its nutritional value, offering significant potential for addressing various health concerns. Previous research has demonstrated its effectiveness in preventing and managing conditions including hypercholesterolemia, diabetes, allergies, cancer, COVID-19, toxicity, neuroinflammatory conditions and cardiovascular diseases. The bioactive compounds within Spirulina play a key role in these therapeutic effects, solidifying its position as a promising natural remedy for individuals seeking alternative treatments for these prevalent health issues [3,4]. Spirulina has been acknowledged as a safe food by both the U.S. Food and Drug Administration (FDA) and Brazil's National Health Surveillance Agency (ANVISA), showing no toxicological risks. It has also received GRAS (Generally Recognized as Safe) status [5,6]. Given its nutritional value, medicinal benefits, and non-toxic nature, Spirulina has been integrated into a variety of dairy products, particularly cheeses, where it not only boosts nutritional value but also contributes to functional and health-enhancing properties. Cheeses such as Ricotta cheese [2], Feta-type cheese [7], halloumi cheese [8], processed cheese [9], soft cheese [10] and mozzarella cheese [11] have successfully incorporated Spirulina, enriching their protein content and providing additional therapeutic properties.

Camel milk plays an essential role in human nutrition, across many countries worldwide, particularly in hot and arid regions. It is distinguished by its richness in nutrients, particularly proteins, lipids, minerals and vitamins [12]. Recently, camel milk has gained increasing attention due to its exceptional nutritional benefits and its remarkable therapeutic properties to human's health, which include anti-

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carcinogenic, anti-diabetic, hypo-cholesterolemic, anti-autism, and hypoallergenic effects. Additionally, it has shown potential in treating disorders such as Crohn's disease and sclerosis, as it enhances immune system function, setting it apart from other types of milk [13,14]. According to the recent statistics of Food and Agriculture Organization (FAO) in 2023 [15], the overall production of raw camel milk in the world is estimated to be approximately around 4.095 million tons representing 0.42 % of the total milk production of the world among all mammalian species making this milk the fifth most important produced milk in the world other species (bovine, goat, buffalo and sheep milk). Obviously, cow's milk leads global milk production [15], contributing around 80.86 % of the total production, with an annual output of 782.9 million tons. Camel milk has served as the raw material for the production of various dairy products that are characterized by nutritional value such as yogurts, fermented milk, ice cream and cheese [12].

Recent research has highlighted the potential of incorporating functional constituents into cheese derived from mammalian milk (goat and sheep milk) to enhance its nutritional, microbiological, and technological properties. For instance, the addition of date coproducts (date paste) to fresh goat cheese significantly improved its nutritional profile, texture, and microbial stability, while also preserving its sensory acceptability [16]. Similarly, the fortification of "Pecorino" type ovine cheese with *Moringa oleifera* leaf powder contributed to increased antioxidant activity and mineral content, reduced lipid oxidation, without compromising physicochemical or organoleptic qualities [17]. The enrichment of surface-ripened model cheeses with iron influenced the microbial community structure and metabolomic profiles, indicating promising applications for nutrient fortification strategies [18]. Additionally, the incorporation of oregano essential oil (*Origanum vulgare*) into traditional ovine "Tuma" cheese enhanced its antioxidant capacity and contributed to improved microbiological stability by reducing the main pathogens in dairy products [19]. Therefore, these findings underscore the value of fortifying mammalian milk cheeses with bioactive compounds to improve their functional and health-promoting attributes.

Despite the important position of cheese occupies in the dairy products bouquet, camel cheese processing remains a challenge under existing conditions because of some specific characteristics of camel milk including its low concentrations of κ-casein (1.67 g/L and 4.39 g/L for camel and bovine milk, respectively), the relatively fine diameter of fat globules (2.99 μm and 3.78 μm in camel and bovine milk, respectively) as well as the considerable size of camel milk casein micelles (220 nm and 120 nm for camel and bovine casein micelles, respectively) compared with those of bovine milk. [20–23].

Scientists have investigated strategies to enhance the characteristics of cheese made from camel milk by changing processing conditions (acidification to pH 5.6 and thermal treatment at 42 °C) and/or adding functional ingredients such as ultrafiltrated camel milk and herbs such as *Allium roseum* powder [21,23]. To date, no published research has investigated the incorporation of Spirulina to enhance the quality of camel milk cheese. Therefore, this study aims to formulate an innovative functional cheese from camel milk enriched with Spirulina (*Arthrospira platensis*) powder, and to assess its nutritional and sensory attributes through the evaluation of physicochemical characteristics, antioxidant activity, color parameters, and texture profile.

2. Material and methods

2.1. Materials

Camel milk samples (from *Camelus dromedarius* specie) were sourced from a modern camel farm in the Gabes region in the south of Tunisia after being collected from various healthy Dromedary female camels (20 in total). These producing females ranged from 2 to 12 months into lactation. Additionally, fresh bovine milk (*Bos taurus*) was collected from 20 Holstein cows, provided by a local farmer in Sfax (Tunisia). Afterwards, the collected milk samples were cooled at 4 °C during 24 h before proceeding to cheese making.

Spirulina powder (*Spirulina platensis*) was purchased from the Tunisian company BIO-algues, lot No. S2.23, with an expiration date of 06/2026.

2.2. RP-HPLC analysis of camel and bovine milk

Chromatographic analyses were used in order to determine the protein composition of camel and cow's milk. In this regard, the identification of proteins in milk was carried out using RP-HPLC (Agilent 1260 Infinity quaternary LC, Germany) according to the method of Yüksel and Erdem [24] which is modified by Lajnaf et al. [25]. A Zorbax Eclipse Plus C18 column (250 mm × 4.6 mm, 5 μm) was used for milk protein separation and RP-HPLC analysis was conducted with a Shimadzu SPD6A-UV detector measuring optical density. Pure bovine caseins (κ-casein, β-casein and α_S-casein) and whey proteins (α-lactalbumin and β-lactoglobulin) from Sigma-Aldrich were used as standard to identify milk proteins. For camel milk proteins, as they are characterized by different molecular characteristics leading to different retention time (RT) than those of bovine proteins standards, each camel protein was identified using chromatograms of camel milk, caseins and whey fractions.

2.3. Preparation of camel and bovine Spirulina cheese

The overall process for preparing Spirulina-enriched cheeses from camel and bovine milk is schematically illustrated in Fig. 1. First, both of bovine and camel milk samples were pasteurized during 30s at 74 °C and then cooled down to 37 °C where microbial rennet enzymes were added (in quantities of 0.35 mL/L for bovine milk and 1.4 mL/L for camel milk) (Rennet enzyme reference: M. miehei, strength = 1:10,000, Laboratories Arrazi, Sfax Tunisia) [26]. After a coagulation period of approximately 1 h at 37 °C in a water bath for both milk samples, the resulting casein curd was cut into 1 and 2 cm cubes using a sterile knife. The curd was then left to rest for 15–30 min to allow whey separation. Following this, the resulting cheese was transferred into laboratory containers (2 cm in radius and 6 cm in length, with an approximate capacity of 50 g).

Spirulina powder was added in different concentrations (0.1, 0.25, 0.50, and 1 % *w*/w) and mixed with the curd in all cheese samples in a blender until a homogeneous mixture is obtained [27,28]. The different samples of cheese were kept at 4 °C for further analysis.

The cheese-making procedure was repeated three independent times for both camel and bovine milk, using separate batches of milk. All physicochemical and textural analyses were conducted in triplicate for each batch.

2.4. Physicochemical measurements

Following cheese production, the pH of both camel and bovine cheese samples was measured by directly immersing the electrode of a digital pH meter (HI99161, Hanna Inc., United Kingdom) into cheese solutions prepared at a 25 % (*w*/*v*) dilution. Titratable acidity (%) was assessed by titrating 10 g of each cheese sample, suspended in 20 mL of deionized water, with 0.1 N NaOH using phenolphthalein as an indicator. The results were expressed as the percentage of lactic acid [29,30].

Moisture content was determined by drying the cheese samples at 105 °C until a constant weight was achieved, following AOAC Official Method 935.29. Subsequently, ash content was measured by incinerating the samples at 550 °C for 8 h in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany), in accordance with AOAC Official Method 942.05. Ash content was expressed as a percentage of the sample's dry weight [30,31]. Water activity was assessed at 25 °C using a calibrated thermoconstanter aw-meter (Novasina Aw Sprint TH-500, Axair Ltd., Pfäffikon, Switzerland).

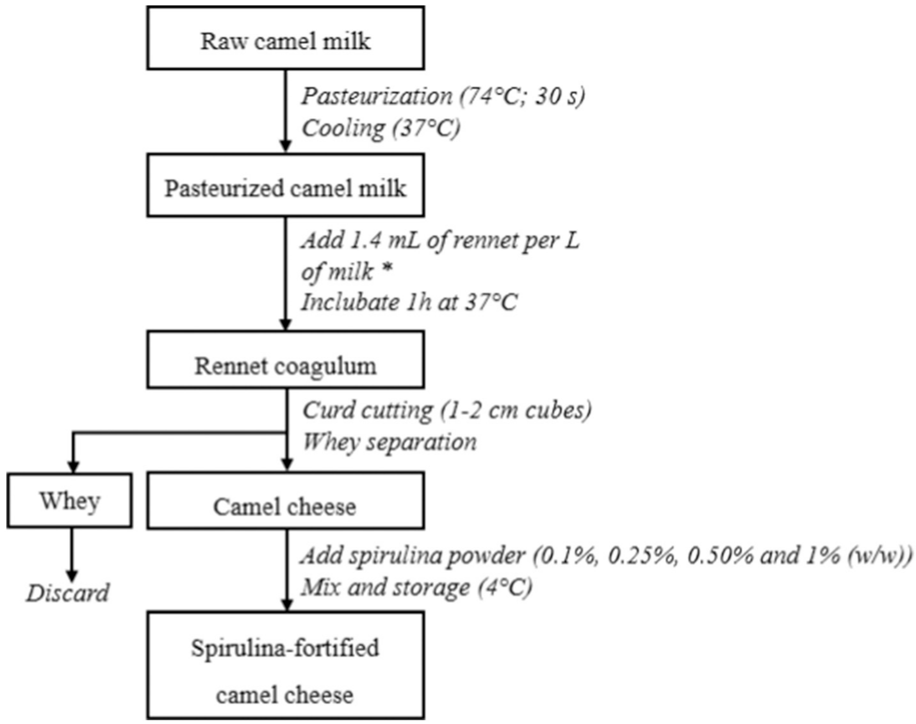

Fig. 1. Schematic representation of the processing steps for the preparation of spirulina-fortified camel cheese, including pasteurization, renneting, whey separation, and spirulina powder incorporation at varying concentrations (0.1 %–1 %, *w*/w). * For bovine milk, 0.35 mL of rennet per liter of milk was used.

2.5. Antioxidant activities

Antioxidant activity assays (Scavenging activity) of *Arthrospira platensis* fortified camel and bovine cheese samples were performed according to the method of Bersuder et al. [32].

Ethanolic extracts of cheese samples were first made by mixing 5 g of cheese sample in 15 mL of a solution that contains ethanol and HCl (0.1 N) (*v*/v) and then macerated at 5 °C during 24 h. Afterwards, the obtained mixture was centrifuged at 3000 rpm during 30 min at 15 °C (centrifuge Beckman CO-LE80K, Coulter, Fullerton, CA) and the supernatant was recovered, filtered and stored at 4 °C [21,33]. A volume of 500 μL of ethanolic extract was added to ethanol (99 %) (375 μL) and the DPPH (1,1-diphenyl-2-picrylhydrazyl) reagent solution (0.02 % DPPH in 95 % ethanol *w/v*) (125 μL) in glass test tubes before incubation for 1 h in dark and at room temperature. The absorbance of the samples was subsequently measured at 517 nm, and the DPPH radical scavenging activity (%) was calculated using the following equation (Eq. (1)):

$$DPPH\ radical\ scavenging\ activity = \frac{C + B - S}{C} \times 100 \quad (1)$$

In this context, C (Control) refers to the absorbance at 517 nm of a mixture containing 500 μL of deionized water, 125 μL of DPPH solution, and 375 μL of ethanol. B (Blank) represents the absorbance of a mixture composed of 500 μL of the sample's ethanolic extract and 500 μL of pure ethanol. S (Sample) denotes the absorbance at 517 nm of 500 μL of the ethanolic extract combined with 375 μL of ethanol and 125 μL of DPPH solution.

2.6. Color analysis

Color analysis was instrumentally performed using Solid-phase colorimetric test (Lab Scan II, Hunter Associate Laboratory Inc., Reston, VA, USA). Different parameters of CIELAB color coordinate system (L*, a*, b*, ΔE*, C*, h°) were determined to characterize and quantify the total color differences between the camel and bovine cheese samples as a function of the contents of the added Spirulina The cheese samples were placed in transparent Petri dishes to maximum capacity, and their color characteristics were evaluated using the L*, a*, and b* coordinates.

2.7. Textural profile analysis

Textural properties of camel and bovine Spirulina cheeses were determined using an instrumental texture profile analysis (TPA) at was performed at room temperature (25 °C). A texture profile analyzer (Lloyd Instruments, Fareham, UK) was employed in order to obtain the force-time curve for a two-cycle compression as described previously [2]. Samples were compressed using a cylindrical probe (flat diameter: 10 mm) to 50 % deformation at a displacement speed of 15 mm/min, which corresponds to the testing velocity. The probe was then returned to its original position.

Indeed, the TPA technique can quantify texture characteristics including hardness, springiness, chewiness and cohesiveness through wo-cycle compression test in which using a cylindrical probe that compresses tested samples at 50 % of deformation level. Textural parameters including firmness, springiness, adhesiveness, cohesiveness and chewiness, were subsequently calculated using an appropriate software “Nexygen plot” which is connected to the measurement instrument [34].

2.8. Statistical analysis

The significance of the main effects of milk cheese (camel and bovine cheeses) and concentrations of the incorporated Spirulina in cheese (0.1, 0.25, 0.50, and 1 % *w*/w), along with their associated interactions on physicochemical characteristics, color measurements, textural analysis was tested by three-way analysis of variance (ANOVA) followed by post hoc Duncan test to compare the means ($p < 0.05$). Statistical analyses were carried out using IBM SPSS Statistics, version 19. All experiments were conducted in triplicate or more, and the results are presented as mean values ± standard deviation. Pairwise comparisons were performed with Tukey's test at a significance level of $p \leq 0.05$.

3. Results and discussion

The cheese samples with microalgae biomass incorporation in different levels presented visually attractive and unusual appearances (Fig. 2). This may be attributed to the color of the incorporated samples which are similar to that of Roquefort cheese [2]. Innovative green tonalities of cheese prepared with camel and bovine milk varied, depending on the microalga contents (0.1, 0.25, 0.5 and 1 % *w*/w) and the origin of milk.

3.1. RP-HPLC analysis of camel and bovine milk and whey

The main proteins in bovine and camel milk were identified and quantified by RP-HPLC (Fig. 3). RP-HPLC chromatograms of bovine and camel milk, as well as their respective whey fractions (Fig. 3a and b), revealed five prominent peaks for bovine milk (RT: 18.3 ± 0.8 min, 23.0 ± 0.8 min, 24.5 ± 1.1 min, 26.8 ± 0.7 min and 28.7 ± 0.7 min) were identified as κ-casein (6.2 ± 1.6), α_S-casein (23.3 ± 2.4), β-casein (44.6 ± 2.4), α-lactalbumin (3.7 ± 0.7), and β-lactoglobulin (22.2 ± 2.8 %) *w/*w. On the other hand, four major proteins were detected and identified in camel milk (Fig. 3b) with RT of 20.0 ± 0.6 min, 22.8 ± 3.1 min, 24.8 ± 4.4 min and 25.7 ± 0.6 min as α_S-casein (28.8 ± 0.4 % of total milk proteins), β-casein (49.3 ± 1.6 % of total milk proteins), α-lactalbumin (19.3 ± 0.6 % of total milk proteins) and protein fractions F (3.1 ± 0.4 % of total milk proteins), respectively. Thus, chromatograms revealed that the β-casein is the predominant protein in camel milk in agreement with previous work carried out with camel milk and casein fraction [35–37]. Furthermore, the primary camel whey protein in is α-lactalbumin, while β-lactalbumin is completely absent [25,38,39]. No peaks were detected for κ-casein, likely because of its very low concentration, which is overshadowed by the other caseins, as previously reported by Farah et al. and Lajnaf et al. [25,40].

3.2. Physicochemical characteristics of camel and bovine Spirulina cheese

Table 1 presents the effect of enriching camel and bovine cheeses with Spirulina powder at various concentrations (0.1, 0.25, 0.50, and 1 % *w*/w) on the physicochemical composition of the cheese.

First, findings demonstrated that camel cheese exhibited significantly higher water activities (a_w) values when compared to bovine cheese with values of 0.95 ± 0.00 and 0.96 ± 0.00 for bovine and camel control sample cheeses, respectively. As indicated in Table 1, the water activity (aw) values of Spirulina-enriched cheeses were comparable to those of the control. Statistical analysis revealed no significant differences between any of the samples ($p = 0.674$). These findings are in great consistence with those of Bchir et al. [41] carried out with Spirulina-enriched yogurts. The water activity values (a_w) of camel cheese samples was observed to be similar to that of bovine cheese in previous studies which achieved a value of 0.94 regardless of Spirulina's contents in manufactured cheeses (0.5, 1, 2 and 3 %, *w*/w) [9].

Results showed that pH and titrable acidity of camel and bovine cheeses did not change significantly upon addition of microalgae at different concentrations (0.1, 0.25, 0.5 and 1 % w/w) ($p = 0.149$). These findings are in agreement with those of Bchir et al. [41] carried out with enriched yogurt with fresh and dried Spirulina.

Table 1 shows also that camel cheese exhibited a lower pH values when compared to bovine cheese regardless of the Spirulina content: pH ~6.46 and ~6.39 for cheeses made from bovine and camel milk, respectively ($p < 0.0001$). Additionally, the pH values of camel and bovine cheeses did not show a statistically significant difference based on the amount of Spirulina used ($p = 0.469$). The lower pH value of camel cheese is not only attributed to the naturally lower pH of camel milk but also to its weaker response to rennet, which affects curd formation and whey expulsion, further contributing to the pH difference [42,43].

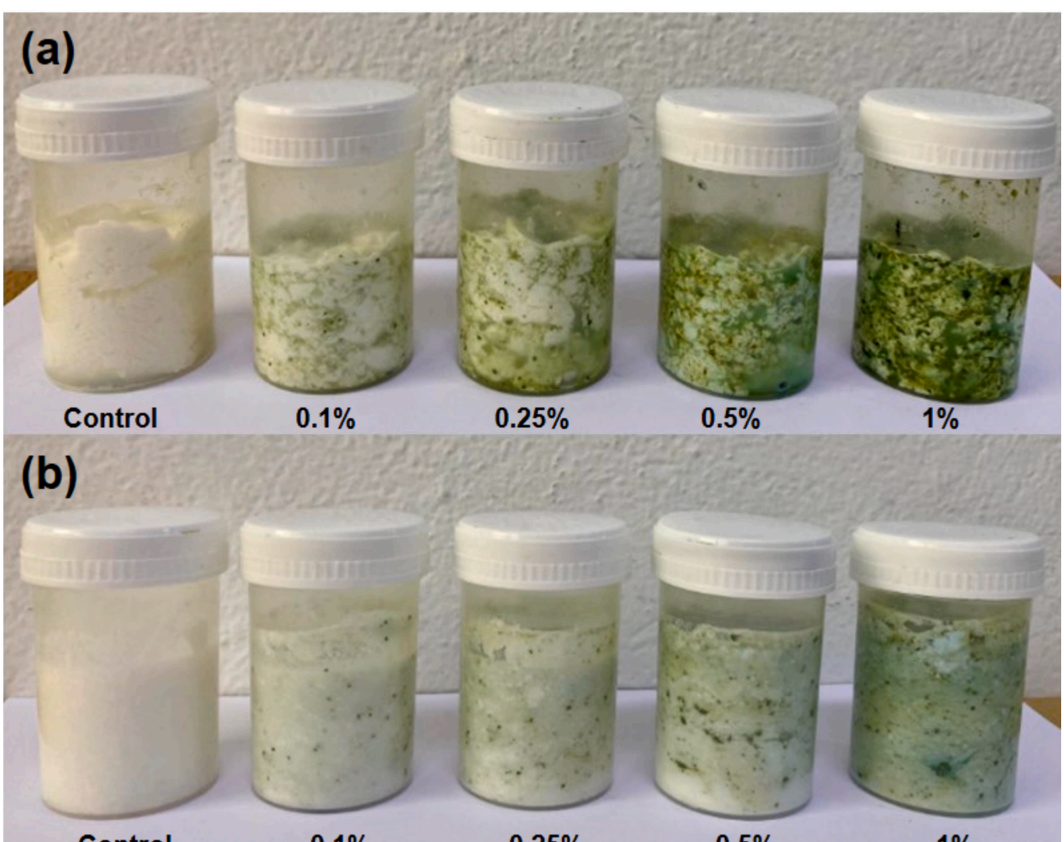

Fig. 2. Photographed images of Bovine (a) and Camel (b) milk cheeses with varying Spirulina levels (0.1, 0.25, 0.5 and 1 %, w/w).

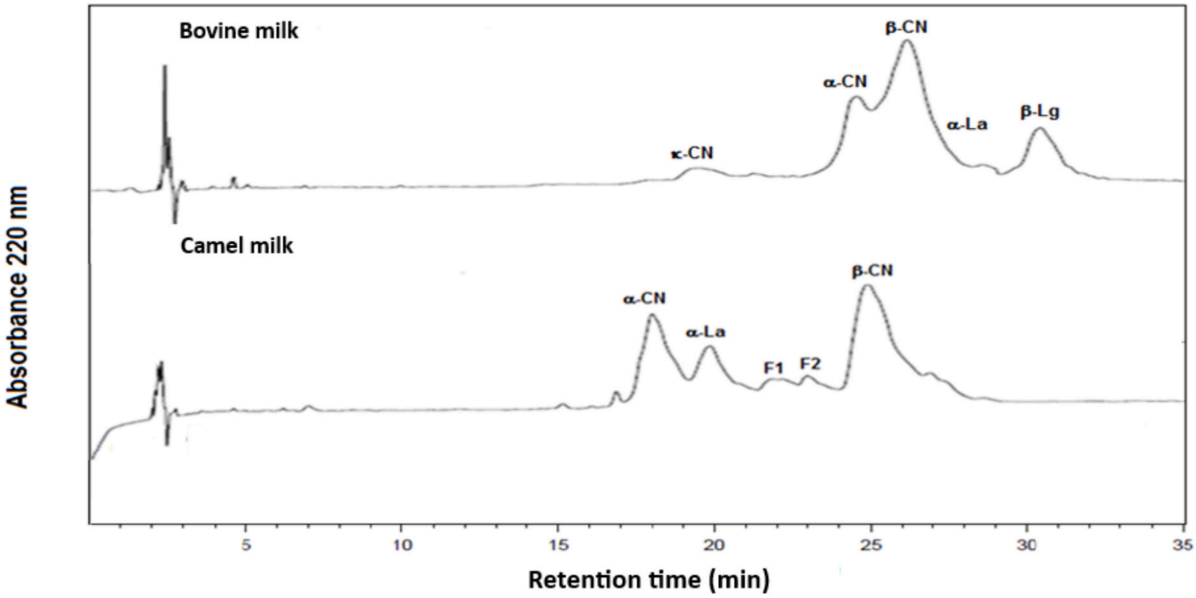

Fig. 3. RP-HPLC chromatograms at 220 nm for protein fractions of bovine (a) and camel (b) milk, respectively. Abbreviations used: β-CN (beta-casein), α-CN (alpha-casein), α-La (alpha-lactalbumin), β-Lg (beta-lactoglobulin), F (protein fraction).

Table 1
Physiochemical composition of camel and bovine cheeses fortified with Spirulina powder in different concentrations (0.1, 0.25, 0.5 and 1 % w/w). Values are expressed as mean ± standard deviation (SD). Standard error of the mean (SEM) is reported in a separate row. Different superscript letters within the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Cheese sample	Bovine cheese					Camel cheese					*p*-Value
Parameter	Control	0.10 %	0.25 %	0.50 %	1 %	Control	0.10 %	0.25 %	0.50 %	1 %	
pH	6.470 ± 0.014^{a}	6.480 ± 0.028 ^{a}	6.443 ± 0.04^{a}	6.465 ± 0.021^{a}	6.465 ± 0.035^{a}	6.415 ± 0.021^{a}	6.39 ± 0.028^{a}	6.388 ± 0.011^{a}	6.395 ± 0.014^{a}	6.383 ± 0.032^{a}	p = 0.469
SEM	0.010	0.020	0.003	0.015	0.025	0.015	0.020	0.014	0.010	0.023	
Titrable acidity	0.256 ± 0.007^{a}	0.266 ± 0.007^{a}	0.279 ± 0.013 ^{a}	0.297 ± 0.013^{b}	0.298 ± 0.039^{a}	0.256 ± 0.005^{a}	0.257 ± 0.007^{a}	0.266 ± 0.019^{a}	0.284 ± 0.020^{a}	0.275 ± 0.007^{a}	p = 0.149
SEM	0.005	0.005	0.009	0.009	0.028	0.004	0.005	0.013	0.014	0.005	
Water activity (aw)	0.944 ± 0.003^{a}	0.946 ± 0.0028^{a}	0.945 ± 0.0028^{a}	0.949 ± 0.0028^{a}	0.947 ± 0.002^{a}	0.956 ± 0.0057^{a}	0.958 ± 0.0021^{a}	0.956 ± 0.0028^{a}	0.956 ± 0.002^{a}	0.958 ± 0.0021^{a}	p = 0.674
SEM	0.0025	0.0020	0.0020	0.0020	0.0015	0.004	0.0015	0.0020	0.0020	0.0015	
Total solids (%)	29.56 ± 0.95^{a}	32.31 ± 0.82^{b}	34.42 ± 0.70^{b}	32.61 ± 1.21^{b}	32.07 ± 1.77^{a}	18.99 ± 1.15 ^{a}	20.44 ± 1.05 ^{a}	22.34 ± 1.77 ^{a}	22.44 ± 2.22 ^{a}	22.50 ± 1.31^{a}	p = 0.018
SEM	0.676	0.592	0.500	0.873	1.247	0.816	0.740	1.244	1.422	0.873	
Ash (%)	10.420 ± 1.107^{a}	8.295 ± 0.777^{a}	8.095 ± 0.596^{a}	7.905 ± 0.404^{a}	7.075 ± 0.695^{b}	6.155 ± 0.597^{a}	5.905 ± 0.2975^{a}	5.525 ± 0.404^{a}	5.335 ± 0.606^{a}	5.770 ± 0.303^{a}	*p* = 0.013
SEM	0.783	0.550	0.421	0.286	0.491	0.422	0.210	0.266	0.428	0.214	

The results indicated that the solid and ash contents were highest in Spirulina-fortified bovine cheeses. Specifically, the solid content of the control and Spirulina-enriched cow's milk cheeses were approximately 29 and 32 %, respectively. These findings are in great consistence with those of Ismail et al. [2] who reported that the increased levels total solids, ash, fats and proteins content in Spirulina fortified cheeses can be explained by the higher concentrations of these parameters in Spirulina. In the same way, Lee and Lucey [44] showed that the incorporation of Spirulina in yogurt samples increases their solid contents levels. Additionally, Atik et al. [28] reported a significant increase in the total solid content of samples upon the addition of Spirulina powder for vegan kefir samples manufactured with soy milk and almond milk.

Meanwhile, a significant increase was observed for both solids content of camel cheese before and after the supplementation of Spirulina as shown in Table 1 ($p = 0.0018$). These findings can be attributed to the weak curd structure of camel milk, which likely led to increased whey losses, thereby minimizing the impact of Spirulina on total solids and ash content [23]. Due to its inherently poor coagulation properties, camel milk forms a fragile curd, resulting in a greater proportion of solids being lost in the whey and subsequently reducing the measurable effect of Spirulina incorporation.

3.3. *Antioxidant activities of camel and bovine Spirulina cheese*

Antioxidant capacity of Spirulina cheeses from bovine and camel milk was determined via DPPH radical-scavenging activity (Fig. 4). First, rennet cheese samples from camel milk showed higher antioxidant activity than in bovine cheese based on DPPH assays regardless of the Spirulina level.

The antioxidant properties of dairy products derived from camel milk have been investigated by various authors; whereas, no studies have specifically addressed these properties in cheese made from camel milk. The antioxidant activity of camel milk is known to be strongly influenced by the processing conditions used. For instance, Ayyash et al. [45] studied the effect of fermentation process on the antioxidant activities of dairy products produced from camel and bovine milk. These

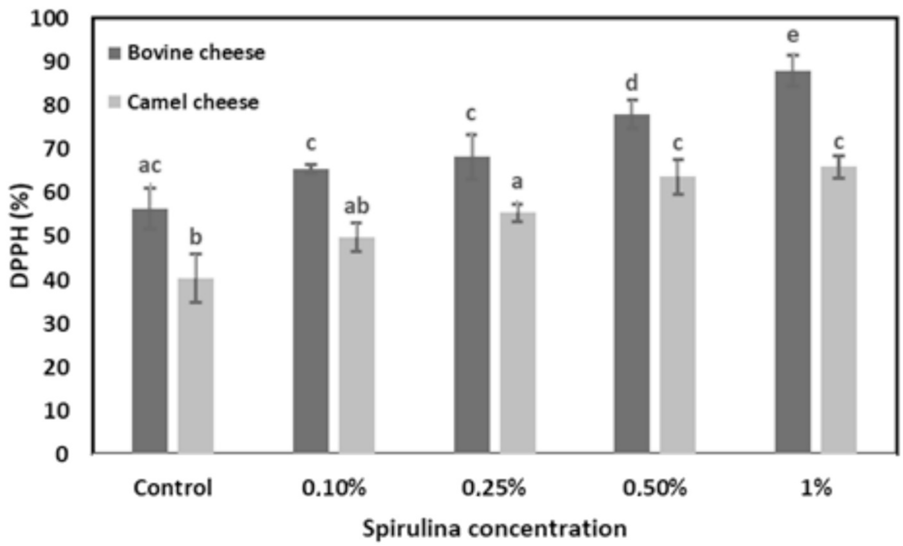

Fig. 4. Antioxidant activities of camel and bovine milk cheeses enriched with Spirulina at different levels (0.1, 0.25, 0.5 and 1 %, w/w). Samples labeled with different letters (a–e) indicate statistically significant differences ($p < 0.05$). Error bars represent the standard deviation of the mean DPPH scavenging activity values (%).

authors revealed that fermented camel milk fermented by *Lactobacillus acidophilus* showed lower DPPH antioxidant activities when compared with fermented bovine milk. Meanwhile, Soleymanzadeh et al. [46] found that fermented camel milk products showed with *Leuconostoc lactis* showed higher antioxidant activity than fermented bovine milk.

The results show that the addition of Spirulina powder had a significant effect on the antioxidant activities of both camel and bovine cheeses compared to the control samples ($p < 0.0001$). Specifically, as the Spirulina concentration increased, a marked rise in DPPH radical-scavenging activity (in %) was observed (Fig. 4).

Fig. 4 also shows that, an incorporation of 0.25, 0.5 or 1 % to bovine cheese resulted in a significant increase of the DPPH rates from 56.3 ± 4.7 % (control) to 68.1 ± 5.1 %, 78.0 ± 3.2 % and then to 87.9 ± 3.5 %, respectively. No significant raise was noted in DPPH rates between cheese samples with 0.1 and 0.25 % (*w*/w) of Spirulina. For the camel cheese, statistical analysis showed that the incorporation of Spirulina significantly enhanced the antioxidant activities in comparison with that of native cheese, with better DPPH rates achieved after an incorporation of 0.25 and 0.5 %. These findings align with those reported by Ismail et al. [2] who found that Ricotta cheese had significantly higher antioxidant capacity when fortified with Spirulina microalgae as compared to that in control cheese ($p \leq 0.05$). Additionally, yogurts fortified with *Chlorella vulgaris* and *Dunaliella* sp. powders exhibited increased free radical scavenging activity, which may be attributed to the higher chlorophyll content [47]. For camel milk, no studies have been reported on cheese with Spirulina to date. However, a study conducted by Shori [48] demonstrated that the antioxidant activity of the soybean-supplemented yogurt from both cow and camel milk was higher than that of the control suggesting that the incorporation of dairy products derived from camel milk could enhance their properties, including antioxidant properties.

3.4. Color of camel and bovine Spirulina cheeses

Table 2 presents the color changes of the camel and bovine Spirulina cheese samples. To determine color properties, CIELab parameters—L*, a*, and b*—were measured for the Spirulina -enriched samples. Here, L* indicates the brightness, a* represents the greenness, and b* denotes the

Table 2
Color parameters, L*, a*, b*, C*, h° and ΔE* of camel and bovine cheeses with 0.1, 0.25, 0.5 and 1 % (w/w) Spirulina biomass incorporation. Values are expressed as mean ± standard deviation (SD). Standard error of the mean (SEM) is reported in a separate row. Different superscript letters within the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Cheese sample	Bovine cheese					Camel cheese					p-Value
Parameter	Control	0.10 %	0.25 %	0.50 %	1 %	Control	0.10 %	0.25 %	0.50 %	1 %	
L*	88.99 ± 0.18^{a}	78.40 ± 0.40^{b}	73.89 ± 0.52^{c}	65.63 ± 0.07^{d}	58.12 ± 0.27^{e}	90.73 ± 0.04^{a}	83.35 ± 0.01^{b}	75.24 ± 0.03^{c}	69.25 ± 0.03^{d}	63.65 ± 0.06^{e}	p < 0.0001
SEM	0.250	0.286	0.364	0.050	0.190	0.025	0.010	0.020	0.020	0.045	
a*	−0.60 ± 0.04^{a}	−4.14 ± 0.01^{b}	−5.93 ± 0.08^{c}	−5.98 ± 0.14^{c}	−5.17 ± 0.01^{d}	−1.14 ± 0.01^{a}	−3.52 ± 0.02^{b}	−5.38 ± 0.01^{c}	−7.05 ± 0.10^{d}	−7.34 ± 0.20^{d}	p < 0.0001
SEM	0.030	0.005	0.050	0.100	0.000	0.010	0.010	0.007	0.070	0.012	
b*	11.96 ± 0.09^{a}	11.91 ± 0.09^{a}	14.34 ± 0.01^{b}	16.26 ± 0.12^{c}	14.50 ± 0.13^{b}	5.64 ± 0.04^{a}	6.63 ± 0.01^{b}	9.55 ± 0.12^{c}	7.72 ± 0.10^{b}	8.64 ± 0.07^{b,c}	p < 0.0001
SEM	0.064	0.065	0.005	0.056	0.090	0.025	0.005	0.080	0.070	0.050	
C*	11.97 ± 0.08^{a}	12.61 ± 0.09^{b}	15.52 ± 0.04^{c}	17.33 ± 0.06^{d}	15.44 ± 0.06^{c,d}	5.72 ± 0.01^{a}	7.50 ± 0.01^{b}	10.96 ± 0.11^{c}	10.46 ± 0.01^{c,d}	11.34 ± 0.05^{d}	p < 0.0001
SEM	0.060	0.065	0.020	0.040	0.001	0.002	0.001	0.075	0.005	0.030	
h°	92.88 ± 0.22^{a}	109.17 ± 0.06^{b}	112.49 ± 0.28^{c}	110.21 ± 0.58^{b,c}	109.64 ± 0.16^{b}	101.55 ± 0.0^{a}	117.96 ± 0.16^{b}	119.42 ± 0.25^{b,c}	134.39 ± 2.06^{d}	130.35 ± 0.25^{c,d}	p < 0.0001
SEM	0.155	0.040	0.180	0.400	0.115	0.000	0.115	0.176	1.475	0.176	
ΔE*	–	11.17	16.19	24.35	31.30	–	7.81	16.52	22.372	27.94	–

yellowness. Additionally, the color difference (ΔE*, using the control as a reference), Chroma (C*, saturation), and hue angle (h°) were also calculated.

Tables 2 shows that Increasing the amounts of the added Spirulina, significantly decreased lightness (L*) value from 88.99 ± 0.18 for control to 73.89 ± 0.52, 65.63 ± 0.07 and 58.12 ± 0.27 for bovine cheese fortified with 0.25, 0.5 and 1 % of Spirulina, respectively ($p < 0.0001$). Furthermore, a* parameter was lower for control bovine cheese samples than Spirulina fortified cheese samples with negative values that range between −5.17 and −4.14 depending on the Spirulina level, while b* parameter increased along with increasing Spirulina contents in cheese made by cow's milk ($p < 0.0001$).

These findings are in great consistence with those of Karimy et al. [8] carried out with halloumi cheese. These authors noted that the lightness of cheese samples was reduced after the incorporation of Spirulina (0.5, 1 and 1.5 % *w/v*) due to the complex pigments in this microalgae that influenced the cheese's lightness. These authors reported that negative values in a* parameter reflect the green color of the incorporated cheese product. Spirulina cheeses showed a greater green hue than those in the control samples, likely due to the chlorophyll pigments from algae including chlorophyll *a*, beta-carotene, xanthophyll, zeaxanthin, phycobiliproteins echinenone, myxoxanthophyll, 3-hydroxyechinenone, canthaxanthin, diatoxanthin, beta-cryptoxanthin and oscillaxanthin [28,49].

Similarly to bovine cheeses, increasing the Spirulina level from 0.25 % to 1 % induced a significant decrease of the L* value from 75.24 ± 0.03 to 63.65 ± 0.06, respectively. Camel Spirulina cheeses have a less dark color as compared to bovine cheeses regardless of the added microalgae contents. Additionally, Spirulina-fortified camel cheeses displayed higher yellowness (b* values) compared to the control cheese, yet these values remained lower than those observed in bovine cheese. The color difference parameter i.e. ΔE* that takes the control cheese as reference, showed that the addition of Spirulina has a more pronounced effect on the color of bovine cheese than on camel cheese with a values of 31.30 and 27.94 for bovine and camel cheeses with 1 % (*w*/w) of Spirulina. This behavior could be attributed to the white color of camel milk it contains lower carotene content when compared to cow's milk as β-carotene is responsible for the slightly yellowish hue observed in bovine milk and its derived products [12,50].

The variation of h° parameter indicated that Spirulina-cheese samples leaned towards green with increasing the level of added Spirulina. Otherwise, a notable increase in C* values, from 11.97 ± 0.08 for bovine cheese control to 15.52 ± 0.04 and 17.33 ± 0.06, respectively for the cheese with 0.25 and 0.5 % of dried Spirulina ($p < 0.0001$). In the same way, C* values increased significantly in camel cheeses from 5.72 ± 0.01 for cheese control to 11.34 ± 0.05 for camel cheese with 1 % of Spirulina ($p < 0.0001$). The low C* values of camel cheeses reflects that a higher interaction of bovine cheese matrix with Spirulina pigments, enhancing the intensity of color as the concentration of Spirulina increases. Therefore, camel milk is different in its composition (in term of fat globule size, protein content, and casein micelle structure) compared to cow's milk which may affect the way the Spirulina pigments disperse and interact within the cheese matrix.

3.5. Textural properties of camel and bovine Spirulina cheeses

Textural parameter values such as hardness, cohesiveness adhesiveness, springiness, gumminess and chewiness of prepared cheese samples using different levels of Spirulina (0.1, 0.25, 0.5 and 1 % *w*/w) are shown in Table 3. First, control samples bovine cheese is characterized by higher hardness values (~2.29 N) than those of camel cheese (~0.53 N) ($p < 0.0001$) suggesting that in these conditions the structural network of bovine cheese is more compact and rigid, likely due to differences in protein composition, micelle structure, and calcium bonding compared to camel cheese. These results are in agreement with those of Mbye et al. [51] who reported that the camel milk cheeses are characterized by lower hardness and rheological properties when compared to bovine cheeses This phenomenon may be attributed to the distinct protein profiles of camel and bovine milk—specifically, the lower κ-casein content and higher β-casein proportion in camel milk—which contribute to a weaker gel structure and lower firmness in camel cheese. Our results on camel milk cheese are supported by the relative concentrations of α_S- and β-caseins in this milk reported being approximately total 28.8 and 49.3 % of total camel casein while no peaks were detected for the κ-casein because of its extremely low concentration (Section 3.1), in contrast to 6.2, 23.3 and 44.6 % for κ-, α_S- and β-caseins in total casein of cow's milk, respectively (Section 3.1). Therefore, the poor coagulation of camel milk is primarily linked to its low κ-casein concentration. However, its high β-casein content may also influence the differences in cheese quality, as β-casein exhibits chaperone-like activities that can inhibit milk protein aggregation [52].

On the other hand, the incorporation of Spirulina significantly reduced the hardness and springiness of both camel and bovine cheeses, resulting in a softer texture compared to the control. However, no significant differences in hardness were observed between the 0.5 and 1.0 % Spirulina levels in bovine cheese, nor among the 0.25 %, 0.5 %, and

Table 3
Textural parameters in TPA assay of camel and bovine cheeses fortified with Spirulina powder in different concentrations (0.1, 0.25, 0.5 and 1 % w/w). Values are expressed as mean ± standard deviation (SD). Standard error of the mean (SEM) is reported in a separate row. Different superscript letters within the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Cheese sample	Bovine cheese					Camel cheese					p value
Parameter	Control	0.10 %	0.25 %	0.50 %	1 %	Control	0.10 %	0.25 %	0.50 %	1 %	
Hardness (N)	2.29 ± 0.28^{a}	2.28 ± 0.07^{a}	1.88 ± 0.18^{b}	1.48 ± 0.01^{c}	1.58 ± 0.09^{b,c}	0.53 ± 0.10^{a}	0.31 ± 0.05^{b}	0.21 ± 0.09^{c}	0.19 ± 0.06^{c}	0.17 ± 0.04^{c}	$p < 0.0001$
SEM	0.203	0.040	0.127	0.006	0.060	0.071	0.030	0.062	0.040	0.020	
Cohesiveness	0.41 ± 0.01^{a}	0.43 ± 0.01^{a}	0.40 ± 0.02^{a}	0.44 ± 0.02^{a}	0.38 ± 0.01^{a}	0.16 ± 0.02^{b}	0.25 ± 0.03^{b}	0.22 ± 0.01^{b}	0.15 ± 0.01^{b}	0.15 ± 0.06^{b}	$p = 0.0189$
SEM	0.004	0.008	0.010	0.015	0.006	0.014	0.020	0.007	0.009	0.042	
Springiness	0.81 ± 0.05^{a}	0.76 ± 0.08^{a}	0.69 ± 0.16^{a}	0.64 ± 0.03^{a}	0.59 ± 0.07^{a}	0.76 ± 0.01^{a}	0.70 ± 0.02^{a}	0.68 ± 0.02^{a}	0.65 ± 0.03^{a}	0.67 ± 0.01^{b}	$p = 0.056$
SEM	0.030	0.060	0.080	0.005	0.005	0.004	0.010	0.015	0.015	0.004	
Adhesiveness (N)	0.94 ± 0.07^{a}	0.98 ± 0.06^{a}	0.76 ± 0.11^{b}	0.65 ± 0.03^{b}	0.60 ± 0.03^{b}	0.19 ± 0.01^{a}	0.19 ± 0.01^{b}	0.17 ± 0.02^{b}	0.16 ± 0.01^{b}	0.04 ± 0.01^{b}	$p < 0.0001$
SEM	0.025	0.038	0.082	0.021	0.016	0.008	0.007	0.015	0.006	0.006	
Gumminess (N)	0.94 ± 0.13^{a}	0.98 ± 0.06^{a}	0.76 ± 0.11^{b}	0.65 ± 0.03^{c}	0.60 ± 0.05^{c}	0.09 ± 0.03^{d}	0.08 ± 0.03^{d}	0.05 ± 0.02^{d}	0.03 ± 0.01^{d}	0.03 ± 0.01^{d}	$p < 0.0001$
SEM	0.093	0.030	0.080	0.019	0.035	0.010	0.021	0.014	0.007	0.007	
Chewiness (Nmm)	12.13 ± 1.03^{a}	11.90 ± 2.05^{a}	8.33 ± 0.03^{a,b}	6.66 ± 0.04^{b}	5.65 ± 0.1.18^{b}	0.80 ± 0.02^{a}	0.39 ± 0.01^{a}	0.50 ± 0.01^{b}	0.30 ± 0.01^{b}	0.39 ± 0.01^{b}	$p < 0.0001$
SEM	0.700	1.450	0.010	0.013	0.083	0.010	0.001	0.001	0.004	0.004	

1.0 % levels in camel cheese. Meanwhile, for camel cheese, no significant difference was observed between Springiness values of camel Spirulina cheese samples.

These findings are in great consistence with those of Lousada Falcão et al. [53] carried out with microalgae *Chorella vulgaris*. These authors reported that the reduction in hardness of fortified cheeses may be explained by the water-holding capacity of the compounds found in Spirulina, especially proteins and polysaccharides. As a result, the presence of Spirulina macromolecules may enhance water retention within the casein gel network during curd cutting and draining, contributing to the softer texture observed in fortified cheeses [53,54]. Additionally, added Spirulina may potentially influence high-energy bonds, such as the calcium-phosphate cross-links. These bonds are responsible for the crosslinking and formation of a solid gel structure. These modifications, which occur upon Spirulina addition, contribute to a softer and less compact texture which is reflected in lower values for hardness and elasticity.

Table 3 shows also that incorporation of Spirulina into camel and bovine cheeses significantly reduced the cohesiveness of samples with similar values to that of control (~0.4 and ~0.2 for cheeses derived from bovine and camel milk, respectively) ($p = 0.0189$) which is in great consistence with the findings of Bchir et al. [41]. Bovine cheese samples enriched with Spirulina demonstrated reduced adhesiveness, gumminess, and chewiness in comparison to the control, especially at elevated Spirulina concentrations (0.5 and 1 % *w*/w). In contrast, the incorporation of Spirulina had no remarkable effect on these parameters in camel cheese ($p = 0.32$, 0.71, 0.83 for adhesiveness, gumminess, and chewiness tests, respectively) in agreement with the findings of Ismail et al. [2] who reported that Ricotta cheese samples containing Spirulina at levels of 0.25, 0.5, 0.75, and 1 % (*w/v*) exhibited a softer texture with lower gumminess, cohesiveness, and chewiness values both immediately after preparation and after 21 days of storage at 5 °C, making the cheese easier to chew. For camel cheese, the effect of Spirulina on the adhesiveness, chewiness, and gumminess can be attributed to its distribution and incorporation within the protein network. Indeed, Spirulina might be more evenly distributed in camel cheese without disrupting the protein network due to the weak curd structure. In contrast, in bovine cheese, this microalga could interfere more with the formation of a tight protein matrix, leading to the reduction of textural parameters. The structural resilience of camel cheese, due to its distinct protein profile and higher moisture content, likely explains why Spirulina has a negligible effect on its texture compared to the noticeable softening effect observed in bovine cheese.

4. Conclusion

Incorporating Spirulina powder into both cheese types significantly enhanced their antioxidant activity and resulted in a smoother, softer texture compared to the non-enriched counterparts. The addition of Spirulina significantly influenced the color of both camel and bovine milk cheeses, producing noticeable differences among the samples. In camel milk cheese, the impact on texture was minimal, allowing the product to retain acceptable structural properties. Overall, Spirulina incorporation enhanced the nutritional and bioactive profile of the cheeses, providing valuable proteins, vitamins, and antioxidants. These findings demonstrate that Spirulina can be used as a functional ingredient to develop innovative dairy products that combine improved health benefits with appealing sensory characteristics. The study highlights the potential of Spirulina-enriched cheeses as functional foods that meet consumer demand for nutrient-rich and novel dairy products, offering opportunities for product diversification in both camel and bovine milk cheese markets.

CRediT authorship contribution statement

Roua Lajnaf: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Conceptualization. **Hamadi Attia:** Supervision. **Mohamed Ali Ayadi:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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