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RESEARCH ARTICLE

Conception of an environmental friendly O/W cosmetic emulsion from microalgae

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Abstract

The development of eco-friendly cosmetic such as those from microalgae for skin regeneration and collagen synthesis has gained a great interest worldwide. Accordingly, the potential of microalgae biomass as source of anti-aging cosmetic cream with high antioxidant activity has been investigated. Stabilities and sensory characteristics of cosmetic creams supplemented with *Spirulina*, *Tetraselmis* sp. and *Dunaliella* sp. at 0.5, 1.5 and 2.5%, respectively, revealed a conservation of physico-chemical and preliminary stability properties of formulations. To analyze physico-chemical and textural parameters, accelerated stability study was evaluated under two thermal conditions (25 and 40 °C) during 90 days. Results showed that pH values of all formulations were within the limits of normal skin pH range under storage time at 25 and 40 °C. During this period, the colored creams showed a significant changes of a* and b* indices, reflecting the instability of microalgae colors. Microalgae modified the textural characteristics of emulsions. The *Tetraselmis* sp. containing-cream had the lowest ($P < 0.05$) values of hardness, springiness, and cohesiveness. The 0.5% *Spirulina* containing-cream had the best stable consistency and adhesiveness under time and temperature variations. It exhibited the best properties to be used for skin care products. Thanks to their high content in bioactive macromolecules, microalgae considerably improved the antioxidant activity of the new formulated skin creams.

Keywords Eco-friendly · Microalgae · Emulsion · Stability · Antioxidant · Texture

Introduction

Regarding the appearance of aged skin caused by natural physiological changes and several environmental conditions, many additives have been used in cosmetic preparations as coloring, preserving, and skin care agents (Fendri et al. 2013; Amberg and Fogarassy 2019; Ben Halima et al. 2015). In the current tendency of using natural skin care, microalgae and *Cyanobacteria* have been studied as potential alternatives to synthetic components but also to those from seaweeds (Pereira 2018;

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Jesumani et al. 2019; Ragusa et al. 2021). Indeed, their biosourcing can be easily secured according to recent development in raceways and photobioreactors technologies and do not compete with food cultures for arable lands. Moreover, the use of cosmetic formulas with microalgae as cosmeceuticals has recently received an important acceptability and demand by biomedical industries and consumers (Mutanda et al. 2020; Morocho-Jácome et al. 2022). Among various natural ingredients, bioactive compounds derived from microalgae and cyanobacteria, including lipids, carbohydrates, proteins, pigments, and vitamins have been recently recognized for their potential in therapeutic and biological activities for the skin care as antioxidant, anti-aging, depigmentation, hydrating, antibiotics, and anti-inflammatory (Dammak et al. 2018; Pereira 2018; Barkallah et al. 2020; Hentati et al. 2020; Ragusa et al. 2021). The main microalgae and cyanobacteria genera attracting attention for cosmetic applications are *Tetraselmis*, *Dunaliella* (Chlorophyta), and *Arthrospira*/*Spirulina* (*Cyanobacteria*) (Mourelle et al. 2017). Regarding their richness on proteins (20–70% DW) (Barkallah et al. 2019), sulfated polysaccharides (De Jesus et al. 2015; Dammak et al. 2017; Ben Hlima et al. 2021), polyunsaturated fatty acids (Dammak et al. 2016; De Luca et al. 2021), pigments, and vitamins, microalgae have become a potential for medical and therapeutic applications (Mourelle et al. 2017). Extracts of *Arthrospira platensis* can be used in anti-aging creams (Ragusa et al. 2021). In addition, some proteins from *Arthrospira*/*Spirulina* are used in cosmetics to provide gloss and moisture retention on the skin cells (Ragusa et al. 2021). Cosmetics products containing essential fatty acids such as *omega*-3 and -6 were characterized by their antioxidant and anti-inflammatory properties (De Luca et al. 2021). It has been proved that natural pigments derived from green microalgae including chlorophylls and carotenoids have important antioxidant activities (Ben Amor et al. 2017; Sathasivam and Ki 2018).

The quality assessment of dermatological products is of fundamental importance, mainly, in terms of stability during storage period. To the best of our knowledge, the use of additives from natural sources, especially seaweeds, involved in the cosmetic creams stability is lacking. The aim of this work was to obtain homogeneous and stable cosmetic creams with antioxidant capacity based on microalgae and cyanobacteria supplementation. The samples were characterized by physico-chemical, textural, and colorimetric analysis. Antimicrobial and antioxidant activities were also evaluated.

Methods

Organism and culture conditions

The three species of microalgae (*Tetraselmis* sp. (V_2), *Dunaliella* sp. (Chlorophyta), and *Arthrospira platensis* (*A. platensis*) (cyanobacrteria)) used in this study were provided by biotechnology unit of algae (UBA), National Engineering School of Sfax, University of Sfax.

Microalgae were photo-autotrophically cultivated in 250 mL flasks containing sterilized culture medium. The cultivation was carried out at 25 ± 2 °C under 24 h/0 h (light/dark) photoperiod using fluorescent white lamps (100 µmol photons·m^{-2}·s^{-1}). The pre-cultures were incubated for 7 days and then transferred (10%) into a 2 L flask containing 1.2 L sterilized culture medium.

At the start of the experiment, *Tetraselmis* sp. and *Dunaliella* sp. were cultivated in autoclaved artificial seawater enriched with F/2 medium (Guillard 1975) for 15 days. In the second stage, *Tetraselmis* sp. cells were cultivated as described by Dammak et al. (2016) to induce lipid production. For *Dunaliella* sp., the cultures were maintained in nitrogen deficient artificial seawater (KNO_3, 0 mM) supplemented with modified F/2 medium ($NaNO_3$, 1 mM) and high salinity (NaCl, 2 M) to lead to more pronounced carotenoids induction (Elleuch et al. 2019). The cultures were carried out at 25 ± 2 °C under 540 µmol photons m^{-2} s^{-1} of light intensity for 20 days. *A. platensis* (*Spirulina*) was cultivated in 2 L culture Erlenmeyers containing Zarrouk's medium at pH 9 ± 0.2 for 30 days (Zarrouk 1966).

Chemical and physical compositions of microalgae and cyanobacteria

For each culture, cells were dried at 105 °C until constant weight. Biomass powders were used to evaluate the physicochemical composition of the three microalgae. The ash contents were measured by combusting biomass at 550 °C for 4 h using a muffle furnace according to the Association of Official Analytical Chemists method (AOAC) (AOAC International 2000). Total lipid amounts were determined, gravimetrically, using the modified method of Bligh and Dyer (1959), as described by Fendri et al. (2010).

The classical Kjeldahl procedure was used to determine the protein content (AOAC International, 2000). The determination of chlorophylls and carotenoids concentrations in powdered algae was, spectrophotometrically, done according to the method of Kumar et al. (2010). C-phycocyanin content was estimated using Bennet and Bogorad (1973) method. All analyses were performed in duplicates.

Formulations preparation

Cosmetic cream was obtained from VERDANT Algae Company (Sfax, Tunisia). It consists on emulsion of oil-in-water (O/W) (30/70, W/W). The materials used in the formulations were the following: olive oil, caprylic/capric triglycerides, glyceryl stearate, benzyl alcohol, and dehydroacetic acid. To determine the suitable amounts of powder

addition, different levels were added to 100 mL of cream samples and the general acceptability was determined. It was found that volunteers negatively scored creams with microalgae concentrations higher than 2.5%. Different formulations were prepared by adding microalgae powders to the control emulsion. *Tetraselmis* sp. (V_2) and *A. platensis* (Sp) powders were added to the cosmetic cream at three concentrations (0.5, 1.5 and 2.5%), while two concentrations (0.5 and 2.5%) of *Dunaliella*-cream (Ds) were used. A combined-algae formulation was prepared using 1% of *Tetraselmis* sp. and *A. platensis*. Algae-free emulsions were used as control. One hundred milliliters of cream based different microalgae powder was packed in high-density polyethylene (HDPE). Every sample of cosmetic cream, at 110 g, was packed in a separate bag.

After 48 h, formulations were separated into two groups, the first set was maintained at 25 ± 2 °C and the second was placed in an incubator at 40 ± 0.1 °C with 75% relative humidity (RH). Three replicates were carried out for every treatment.

Stability tests and physical characteristics (color, texture, and pH) of formulations were performed every 30 days for 90 days. All formulations tests were performed in duplicates.

Formulations analysis

Preliminary stability evaluation

The centrifugal test is an indicator of incoming phase separation, sedimentation, and/or precipitation. For each formulation, 5 g were centrifuged at 3000 rpm at 25 °C for 30 min (Rodrigues et al. 2016).

The thermal stress test was performed to assess the stability of cosmetic cream ingredients at high temperatures. Five grams of each formulation was placed into a thermostatic bathwater at 40, 50, and 60 °C. After 1.5 h and after room temperature had been reached, formulation stability was evaluated regarding visual aspect, surface drying, color, and scent.

Samples were classified as follows: (*M*), modified; (*SM*), slight modified; (*NM*), no modification visualized. All stability tests were performed according to ANVISA Stability Guidelines (2004).

Accelerated stability evaluation

The accelerated stability test was assessed to examine the stability of all cosmetic formulations for 90 days at two temperatures 25 °C and 40 °C at a RH equal to75 ± 5%. After 90 days, cosmetic creams were evaluated for their organoleptic, physicochemical and textural properties, antibacterial and antioxidant activities, and their microbiological stabilities.

Sensory evaluation

The sample application procedure followed the same protocol described for the sensory analysis and was approved by the Research Ethics Committee of the Faculty of Medicine, University of Sfax (Tunisia). The sensory evaluation procedures were executed on healthy female ($n = 14$) and male ($n = 16$) volunteers from 25 to 55 years of age using a score test on the basis of hedonic ratings for color, smell, apparent viscosity, back color, hydration, penetration, and general acceptability. The organoleptic parameters were evaluated on a scale from 1 (very bad) to 9 (excellent), as described by Roland et al. (1999).

FTIR spectra analysis

Selected formulations were analyzed using FTIR spectrophotometer (Agilent Technologies Spectrophotometer, Cory 630 FT-IR) to evaluate the chemical stability. FTIR spectra were collected in the range 600 − 4000 cm^{-1} using 10 scans with spectral resolution of 4 cm^{-1}. The FTIR spectral data were processed using Essential FTIR software.

Physico-chemical properties of cosmetic cream

The pH of formulations throughout the conservation period was measured using a calibrated pH-meter (ISTEK- pH 220L, Korea). The results are mean duplicate values. The centrifugal and mechanical vibration tests were carried out in order to evaluate any separation of phases according to the method of Rodrigues et al. (2016).

Color analysis

The color of cosmetic formulations throughout the storage period was measured using a colorimeter (Konica Minolta / Chroma-meter / CR-5, Japan). The color space values (L*, lightness (0–100); a*, red (+) or green (−); and b*, yellow (+) or blue (−)) were determined from different points of the same sample.

Textural analysis

The texture analyses of cosmetic formulations throughout the storage period (90 days) were carried out using a texture analyzer (Texture Analyzer, TA Plus, LLOYD instruments, England) with a penetration distance of 30 mm. The texture parameters (firmness, hardness (N), stiffness ($N \cdot s^{-1}$), springiness (cm), and cohesiveness) were calculated as described by Bourne (1978). All analyses were done in duplicate.

Microbiological tests

The microbiological stability of cosmetic formulations throughout the storage period (90 days) was determined using colony counting in Petri dishes. The cosmetic creams were tested for the presence of coliforms, *Escherichia coli*, molds, and yeasts basing on the method of APHA (2001).

Bacterial strains, media, and culture conditions

The target bacterial strains were obtained from international culture collections (ATCC). They included Gram-positive (*Staphylococcus aureus* ATCC 6538) and Gram-negative (*Escherichia coli* ATCC 8739) bacteria. These strains were used as indicator microorganisms for the antibacterial activity assays. Indicator microorganisms were grown overnight in *Luruia-Bertani* broth (LB, Oxoid Ltd, UK) at 30 °C. For the antagonist tests, the final inoculum concentration used for each indicator bacterium was 10^6 colony-forming units of bacteria per milliliter ($CFU{\cdot}mL^{-1}$).

Agar diffusion method

The antimicrobial activity was evaluated by means of agar-well diffusion assays, as described by Valgas et al. (2007). Fifteen milliliters of the molten agar (45 °C) was poured into sterile Petri dishes (Ø 90 mm). Working cell suspensions were prepared at 10^6 $CFU{\cdot}mL^{-1}$, and 100 µL was evenly spread onto the surface of the agar plates of *Luria–Bertani* (LB) agar (Oxoid Ltd, UK). Once the plates had been aseptically dried, 06 mm wells were punched into the agar with a sterile Pasteur pipette. Each cosmetic formulation was dissolved in distilled water to a final concentration of 100 $mg{\cdot}mL^{-1}$. After filtration, 50 µL were placed into the wells and the plates were incubated at 37 °C for 24 h. Antibacterial activities were evaluated measuring the diameter of circular inhibition zones around the well. The un-inoculated media were also tested for inhibitory zones as a control. Tests were performed in triplicate.

Antioxidant activity (DPPH assay)

The DPPH scavenging activity of formulations was evaluated as described by Bersuder et al. (1998). Briefly, 500 µL of cream at different concentrations was mixed with 125 µL of DPPH solution (0.02%, w/v) and 375 µL of ethanol (99.5%). The mixtures were incubated in dark for 30 min at room temperature. The antiradical activity was determined measuring absorbance at 517 nm using the T70 UV–visible spectrophotometer (PG Instruments Ltd., Beijing, China).

DPPH scavenging activity was determined as follows:

$$\text{DPPH scavenging activity}(\%) = \left(\left(OD_{control} - OD_{sample}\right)/OD_{sample}\right) \times 100$$

where: $OD_{control}$ (using distilled water instead of sample) is the absorbance of the control reaction, OD_{sample} is the absorbance of cosmetic cream solution with the DPPH solution. The experiment was done in duplicate.

Statistical analysis

The analysis of variance (ANOVA) followed by one-way test with $P\text{-}value \leq 0.05$ was used to determine the statistical significance of data using SPSS 19 statistical software package (SPSS Ltd.Woking, UK). The obtained values were expressed as mean ± standard deviation (SD).

Results and discussion

Chemical composition of microalgae and cyanobacteria

Chemical compositions of microalgae and cyanobacteria are given in Table 1. *A. platensis* had higher ($P < 0.05$) protein content (65% DW) than *Tetraselmis* sp. (20.4% DW) and *Dunaliella* sp. (24% DW). Barkallah et al. (2019) and Yilmaz (2012) revealed high values of protein in *Arthrospira platensis*, which are in agreement with results from this study. In addition, significantly ($P < 0.05$) higher lipid content (54% DW) was obtained in *Tetraselmis* sp. It was stated by Dammak et al. (2016) that *Tetraselmis* sp. is known to produce high lipid level (49% DW). Ash content was high (11% DW) in *Dunaliella* sp., while *Tetraselmis* sp. had the lowest ash content (6.5% DW). A high phycocyanin content (1.1% DW) was obtained only in *Spirulina* powder. In addition, it was shown a high carotenoids content in *Dunaliella* sp. ($P < 0.05$). Table 1 proved also that *A. platensis* had higher chlorophylls content than *Dunaliella* sp. and *Tetraselmis* sp.

Table 1 Chemical composition of algae (% DW)

Composition	*A. platensis*	*Tetraselmis* sp.	*Dunaliella* sp.
Protein (g)	65 ± 1.7^{b}	20.4 ± 1.3^{a}	28 ± 1.1^{a}
Lipid (g)	6.3 ± 0.4^{a}	54 ± 0.9^{b}	26 ± 0.34^{a}
Carbohydrates (g)	18.32 ± 0.32^{a}	16.8 ± 1.2^{a}	27 ± 0.1^{b}
Ash (g)	8.09 ± 0.08^{ab}	6.5 ± 1.7^{a}	11 ± 1.4^{b}
Phycocyanin (g)	1.1 ± 0.1	-	-
Chlorophylls (mg)	790.43 ± 2.8^{b}	200.78 ± 1.4^{b}	320 ± 0.1^{b}
Carotenoids (mg)	140.5 ± 1.6^{b}	159.5 ± 0.4^{b}	$5\ g \pm 1.6^{a}$

A *t*-Student test was applied to compare each parameter

Preliminary formulation studies

Physicochemical properties

The pH and color properties were evaluated after 24 h of cosmetic cream preparation (Table 2). The pH of formulations was slightly acid in the range of 5.97–6.24, which is suitable for human skin health (Lukić et al., 2021). In addition, it was shown a slight decrease of mean values of pH depending on microalgae concentration, compared with that of the control cream. The decline of pH may be due to the acid nature of microalgae, which are very rich in various acid compounds such as fatty and amino acids, as reported by Dammak et al. (2016) and Hentati et al. (2019). Similarly, instrumental color indices (a*, b*, L*) were measured. Lightness values (L*), which represent the brightness of cosmetic formulations, were ranged between 0 (black) and 100 (white). Compared to the control samples, a significant ($P < 0.05$) decrease of L* was measured depending on *Arthrospira* sp. and *Tetraselmis* sp. concentrations increasing. The darkness observed in formulations containing these two microalgae might be due to their green color (Barkallah et al. 2019). In addition, a* values, which represent the redness, showed a significant decrease ($P < 0.05$) in formulations enriched with *Tetraselmis* sp., *Arthrospira* sp., and the combination of *Tetraselmis* sp. and *Arthrospira* sp., compared to the control samples. These results might be explained by the fact that *Tetraselmis* sp. is rich in chlorophylls and *Arthrospira* sp. is rich in chlorophylls and phycocyanins. Whereas, the redness of formula enriched *Dunaliella* sp. was intensified, compared to others formulations, which is related to the high carotenoids content of this strain. Concerning b* data, the significant ($P < 0.05$) increased of yellowness in formulations enriched with *Tetraselmis* sp. and *Dunaliella* sp. was explained by the richness of these two algae in lipids and carotenoids, respectively. Nevertheless, the highest *Arthrospira* sp. concentrations, the more b* values reduces, and in their turn, formulations tend to be bluer. Therefore, it is clear that color values were significantly affected by microalgae species.

Preliminary stability analysis

After 24 h of formulations preparation, preliminary physical-stability tests (centrifuging and thermal stress) were assessed and the suitable prepared formulations were selected. It was clear that all emulsions remained stable without any separation phase throughout the centrifuging. In addition, thermal stress tests revealed good physical properties (visual aspect and smell, *NM*; phase separation, *NM*; color, *NM*; surface drying, *NM*), except formulations containing, separately, 2.5% *Spirulina* and 2.5% *Tetraselmis* sp. which showing a slight modification (*SM*) after thermal stress in visual aspect, smell, and color.

Table 2 Effect of adding microalgae on pH and color of cosmetic creams

	Formulations									
	Control cream	0.5% *Spirulina* cream	1.5% *Spirulina* cream	2.5% *Spirulina* cream	0.5% *Tetraselmis sp.* cream	1.5% *Tetraselmis sp.* cream	2.5% *Tetraselmis sp.* cream	0.5% *Dunaliella sp.* cream	2.5% *Dunaliella sp.* cream	1% *Spirulina* and *Tetraselmis sp.* cream
L*	88.5 ± 0.07^{b}	64.08 ± 0.02^{b}	47.6 ± 0.02^{a}	42.74 ± 0.09^{a}	76.21 ± 0.04^{b}	62.86 ± 0.02^{b}	46.16 ± 0.02^{a}	81.7 ± 0.04^{b}	76.05 ± 0.02^{b}	62.88 ± 0.04^{b}
a*	-0.61 ± 0.007^{c}	-18.68 ± 0.04^{a}	-23.74 ± 0.01^{a}	-24.69 ± 0.07^{a}	-6.05 ± 0.02^{b}	-8.01 ± 0.007^{b}	-9.19 ± 0.007^{b}	1.52 ± 0.04^{c}	2.67 ± 0.04^{c}	-14.92 ± 0.01^{a}
b*	0.5 ± 0.02^{a}	6.38 ± 0.01^{b}	5.72 ± 0.02^{b}	5.02 ± 0.03^{b}	21.99 ± 0.04^{c}	31.75 ± 0.01^{d}	32.48 ± 0.007^{d}	29.4 ± 0.2^{d}	32.96 ± 0.01^{d}	24.99 ± 0.04^{c}
pH	6.24 ± 0.03	6.19 ± 0.02	6.14 ± 0.05	6.1 ± 0.01	6.12 ± 0.03	6.02 ± 0.05	5.78 ± 0.02	6.18 ± 0.06	6.04 ± 0.02	6.06 ± 0.04

Tukey's post hoc test was used to determine the significant differences between treatments for each parameter

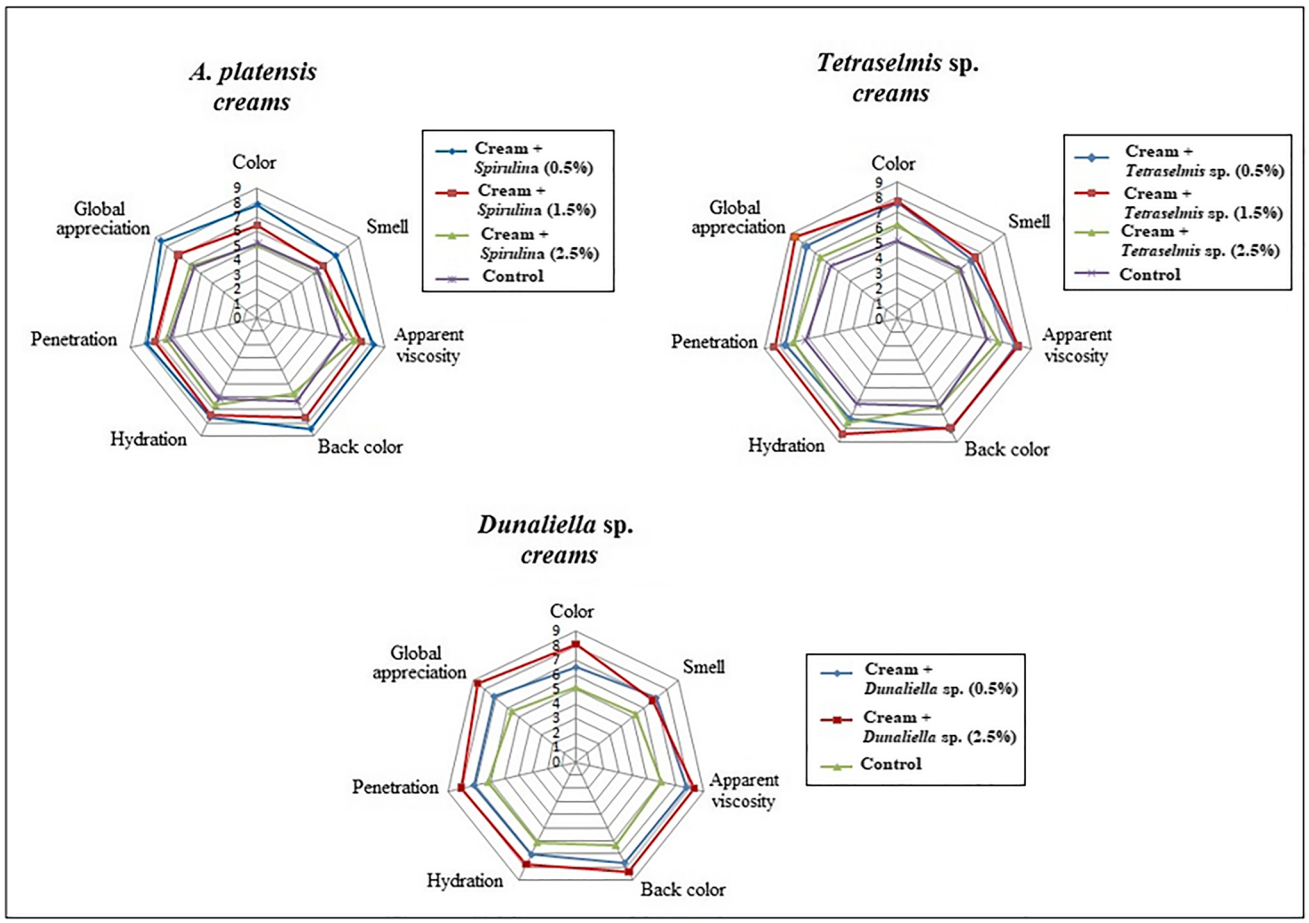

Fig. 1 Mean scores of tasting panelists ($n = 30$) for sensory properties of control and fortified cosmetic creams with different percentage of algae

Sensory evaluation

As shown in Fig. 1, fortifying cosmetic cream with algae had a significant ($P < 0.05$) effect on all sensory attributes. The addition of microalga modified the color of cosmetic creams from white to green (cream with *Tetraselmis* sp.), blue-green (cream with *A. platensis*), or yellow-orange (cream with *Dunaliella* sp.) depending on type and concentration of microalga (Fig. 2). Color scores varied between 5.12 and 8. In this line, the lowest color scores (5.12 and 6.21) were determined for cosmetic creams containing 2.5% of *A. platensis* and *Tetraselmis* sp., respectively. This fact can be explained by the high phycocyanin and chlorophylls levels (Table 1). According to these results, it shows also that 0.5% *Tetraselmis* sp. and *Dunaliella* sp. concentrations have no significant ($P > 0.05$) effects on sensory quality of the cosmetic creams. Formulation with 2.5% of *Tetraselmis* sp. has the lowest ($P < 0.05$) odor score compared to other formulations. Statistically, the cosmetic creams enriched with 0.5% of *A. platensis*, 1.5% of *Tetraselmis* sp., and 2.5% of *Dunaliella* sp. received higher ($P < 0.05$) scores for the major sensory traits (Fig. 1). Thus, these concentrations were chosen for further analyses, to evaluate their textural assessments, microbiological, physicochemical, antioxidant, and stabilities' properties, which were compared to a base cream (without microalgae) used as a reference.

FTIR spectroscopy was usually employed to detect any interaction or variation of polymorphic form to another by intensity of absorption band or wavelength change corresponding to chemical groups or new peaks appearance. As shown in the Fig. 3, FTIR spectra of studied samples revealed similar profiles that indicated the chemical stability. As expected, all formulations showed the same characteristic bands, except some changes in control-cream spectrum. The different biomolecules such as lipids, proteins, and carbohydrates can be identified by their distinct absorption bands in different regions over the wavenumber range 4000–500 cm^{-1} on the basis of reference standards (Duygu et al. 2012). Hydroxyl (O–H) stretching vibration band in the products' spectra appeared at 3300 cm^{-1} corresponding to the proteins, polysaccharides, and water. The FTIR spectra showed two strong absorption bands in the 3000–2800 cm^{-1} spectral region at 2915 and 2848 cm^{-1} indicating the presence of asymmetric and symmetric stretching vibrations of

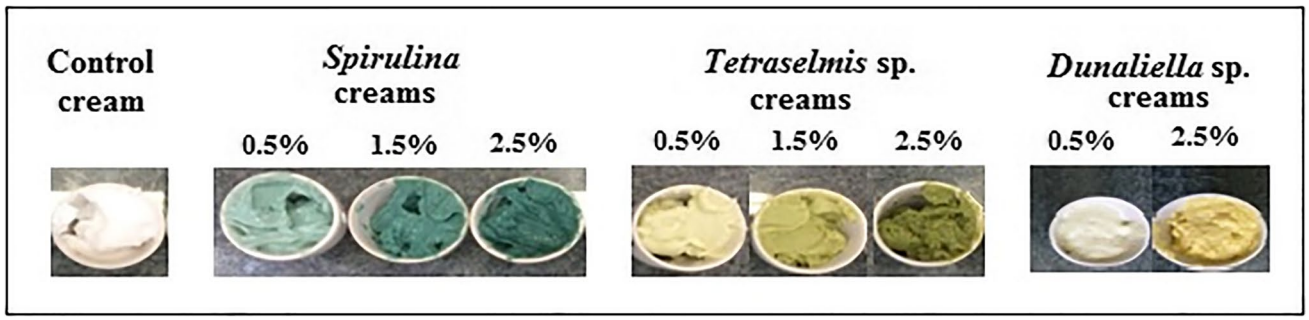

Fig. 2 Photos of cosmetic creams at different level of microalgae

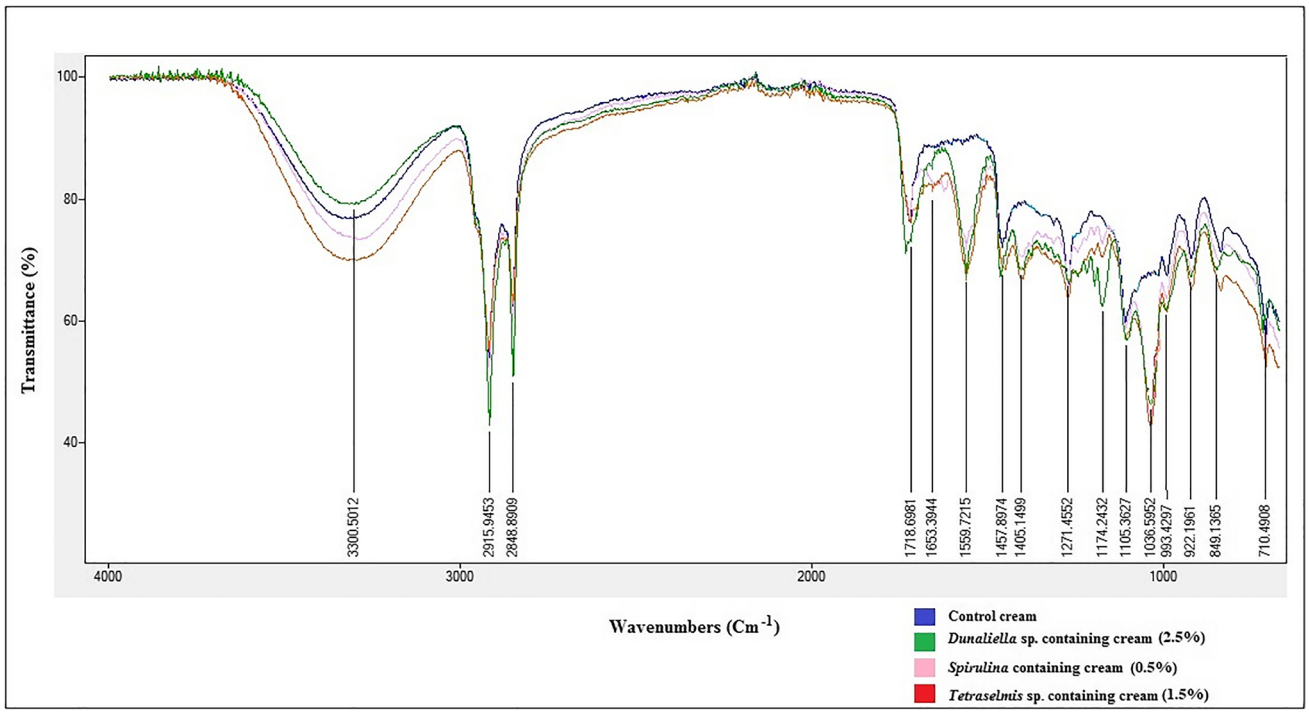

Fig. 3 FT-IR spectra of cosmetic creams containing *Dunaliella* sp. (2.5%), *Spirulina* (0.5%), and *Tetraselmis* sp. (1.5%)

$\nu(CH_3)$ and $\nu(CH_2)$ bending from lipids and carbohydrates, which reflects the high lipid and polysaccharides contents in the tested cosmetic cream. The latter may be confirmed by the presence of two bands in the 1460–1379 cm^{-1} region at 1457 and 1405 cm^{-1} from protein $\delta as(CH_2)$ and $\delta as(CH_3)$ bending of methyl and lipid $\delta as(CH_2)$ bending of methyl. In microalgae containing-creams, protein spectra were characterized by two peaks at 1655 and 1559 cm^{-1} corresponding to v(C = O) stretching of amide I and δ(N–H) bending and v(C-N) stretching vibrations of amide II, which indicated the high protein content in the microalgae-creams. As expected, fatty acid spectra were characterized by a vibration at 1718 cm^{-1} due to v(C = O) stretching of esters. Except control cream, microalgae containing-creams were characterized by three sets of vibrations v(C–O–C) attributed to polysaccharides at 1174, 1036, and 993 cm^{-1}. The FTIR spectra showed clearly a signal at ~ 1271 cm^{-1} corresponded to the v_{as}(> P = O) stretching of phosphodiesters and nucleic acids. Compared to control, microalgae containing creams spectra showed a clear variation due to microalgae richness in lipids, proteins, and carbohydrates.

Accelerated stability test

Physico-chemical parameters stabilities

The accelerated stability centrifugation test is an important parameter to test the formulation stability through storage period. After centrifugation, any phase separation was detected in all cosmetic formulations kept at 25 and 40 °C

Table 3 Organoleptic characteristics of cosmetics creams after 30, 60, and 90 days of preparation

T (°C)	Time(day)	Formulations														
		Control cream			*Spirulina* cream			*Tetraselmis sp.* cream			*Dunaliella sp.* cream			*Spirulina* and *Tetraselmis sp.* cream		
		Aspect	Odor	Color	Aspect	Odor	Color	Aspect	Odor	Color	Aspect	Odor	Color	Aspect	Odor	Color
25	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	60	N	N	N	N	N	N	N	N	N	N	N	SM	N	N	N
	90	N	N	N	N	N	SM	N	N	SM	N	N	SM	N	N	SM
40	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	30	N	N	N	SM	N	SM	SM	N	SM	SM	N	SM	SM	N	SM
	60	SM	N	N	M	SM	M	M	SM	M	SM	N	M	M	SM	M
	90	SM	N	N	M	SM	M	M	SM	M	M	N	M	M	SM	M

N, non modified; SM, slow modified; M, modified

during 90 days of storage, which means the stability of all formulations (data not shown).

The appeared aspect (homogeneity, phase separation, texture, viscosity), odor, and color of the formulations were evaluated. Results are described in Table 3. Overall, stored at 25 °C, creams were odorless, have good homogeneity and without color variation through storage period (90 days). Although, except base cream, changes were detected in cosmetic creams with microalgae exposed to high temperature (40 °C) and after 60 days, such as altered odor and viscosity, disappeared color, dried surface, and little phase separation.

Testing pH change in emulsions is very important. Table 4 showed the results of pH of different formulations stored at 25 °C and 40 °C for 90 days. In this study, it was found that the pH value changes of all formulations were within the range of 5.75 and 6.24. Sousa and Leal (2018) suggested that the changes of pH values of cosmetics creams between 5.5 and 6.5 are considered acceptable and suitable for skin health application. At the same period storage, pH changes between 25 and 40 °C, control cream and cream containing *Tetraselmis* sp. have the same ($P > 0.05$) behavior. Although, the pH of creams with *Spirulina*, *Dunaliella* sp., and the combination of *Spirulina* and *Tetraselmis* sp. show a significant ($P < 0.05$) decrease through storage period at 40 °C. The greater decrease of pH may be due to the accumulation of acidic products caused by esters bonds hydrolysis (Mahmood and Akhtar 2013). It was also reported by An et al. (2019) that the reason for the decline of pH values is the generating of oxidative products caused by oxidation of phenolic hydroxyl groups of microalgae.

Color analyses stabilities

Color parameters values (L*, a*, b*) of cosmetic creams from the stability study are shown in Table 5. It was found a significant ($P < 0.05$) differences between the control cream and samples with microalgae at all storage conditions and this could be attributed to the darker colors of pigments from microalgae. With respect to the storage time, color differences were found in all cosmetic creams (Table 5). The formulations with *Spirulina* and *Tetraselmis* sp. had not significantly lower lightness ($P > 0.05$) at 25 and 40 °C

Table 4 pH values of formulations kept at 25 and 40 °C

	Storage period (days)							
	0	30	60	90	0	30	60	90
Formulation	25 °C				40 °C			
Control cream	6.24 ± 0.03^{cA}	6.20 ± 0.00^{cA}	6.19 ± 0.01^{cA}	6.19 ± 0.00^{cA}	6.24 ± 0.03^{cA}	6.2 ± 0.03^{bA}	6.18 ± 0.00^{bA}	6.15 ± 0.00^{bA}
Spirulina cream	6.19 ± 0.02^{bA}	6.22 ± 0.02^{cA}	6.18 ± 0.01^{cB}	6.17 ± 0.02^{cB}	6.19 ± 0.02^{bA}	6.03 ± 0.06^{aB}	5.97 ± 0.1^{aA}	5.86 ± 0.01^{aA}
Tetraselmis sp. cream	6.02 ± 0.05^{aA}	5.97 ± 0.01^{aA}	5.92 ± 0.02^{aA}	5.92 ± 0.01^{aA}	6.02 ± 0.05^{aA}	5.95 ± 0.02^{aA}	5.83 ± 0.0^{aA}	5.75 ± 0.02^{aA}
Dunaliella sp. cream	6.04 ± 0.02^{aA}	6.08 ± 0.04^{abA}	6.01 ± 0.00^{aB}	6.02 ± 0.00^{aB}	6.04 ± 0.02^{aA}	5.93 ± 0.01^{aA}	5.91 ± 0.01^{aA}	5.89 ± 0.01^{aA}
Spirulina and Tetraselmis sp. cream	6.06 ± 0.01^{aA}	6.13 ± 0.01^{bB}	6.12 ± 0.00^{bB}	6.10 ± 0.00^{bB}	6.06 ± 0.01^{aA}	5.96 ± 0.02^{aA}	5.94 ± 0.0^{aA}	5.91 ± 0.00^{aA}

Tukey's post hoc test was used to determine the significant differences between treatments at a fixed time (capital letters). A *t*-Student test was applied to compare each formulations kept at 25 and 40 °C at the same time (0, 30, 60, and 90 days) (lowercase letters)

Table 5 Color analysis of control cream and formulations with microalgae

		Storage period (days)							
		0		30		60		90	
		25 °C	40 °C	25 °C	40 °C	25 °C	40 °C	25 °C	40 °C
L*	Control cream	88.70 ± 0.05^{eA}	88.55 ± 0.02^{eA}	92.02 ± 0.005^{dA}	90.06 ± 0.02^{dA}	92.94 ± 0.02^{dA}	90.88 ± 0.01^{cA}	92.75 ± 0.11^{eA}	90.33 ± 0.00^{dA}
	Spirulina cream	67.43 ± 0.03^{cA}	67.04 ± 0.04^{cA}	67.85 ± 0.01^{aA}	67.58 ± 0.005^{bA}	65.77 ± 0.04^{bA}	67.01 ± 0.02^{bA}	71.24 ± 0.05^{cA}	67.66 ± 0.03^{bA}
	Tetraselmis sp. cream	59.07 ± 0.01^{aA}	59.48 ± 0.07^{aA}	58.26 ± 0.02^{aA}	50.57 ± 0.07^{aA}	56.87 ± 0.02^{abA}	40.35 ± 0.03^{aA}	54.95 ± 0.02^{aA}	31.43 ± 0.01^{aA}
	Dunaliella sp. cream	76.05 ± 0.01^{dA}	76.24 ± 0.04^{dA}	79.15 ± 0.04^{cA}	78.7 ± 0.03^{cA}	80.98 ± 0.00^{cB}	83.54 ± 0.15^{bA}	81.63 ± 0.03^{dB}	84.00 ± 0.005^{cA}
	Spirulina and Tetraselmis sp. cream	62.88 ± 0.03^{bA}	62.73 ± 0.01^{bA}	61.65 ± 0.05^{bB}	47.75 ± 0.01^{aA}	49.61 ± 0.13^{aA}	41.76 ± 0.05^{aA}	48.17 ± 0.07^{bA}	37.29 ± 0.09^{aA}
a*	Control cream	-0.62 ± 0.01^{cA}	-0.69 ± 0.03^{cA}	-0.73 ± 0.01^{cA}	-0.68 ± 0.00^{cA}	-0.7 ± 0.02^{aA}	-0.68 ± 0.002^{aA}	-0.66 ± 0.00^{bA}	-0.69 ± 0.005^{bA}
	Spirulina cream	-16.19 ± 0.00^{aA}	-16.27 ± 0.05^{aA}	-4.92 ± 0.02^{bA}	-4.54 ± 0.01^{aB}	-4.87 ± 0.02^{bA}	-3.54 ± 0.03^{cB}	-4.03 ± 0.01^{aA}	-2.13 ± 0.005^{aB}
	Tetraselmis sp. cream	-10.07 ± 0.01^{bA}	-10.09 ± 0.09^{bA}	-3.51 ± 0.01^{bA}	-1.27 ± 0.03^{bA}	1.65 ± 0.02^{aA}	1.43 ± 0.01^{bA}	1.91 ± 0.00^{bA}	1.41 ± 0.13^{bA}
	Dunaliella sp. cream	2.67 ± 0.01^{dA}	2.7 ± 0.06^{dA}	2.37 ± 0.01^{dB}	0.52 ± 0.01^{cA}	1.79 ± 0.03^{aB}	-1.87 ± 0.04^{bA}	0.18 ± 0.02^{bB}	-2.98 ± 0.005^{aA}
	Spirulina and *Tetraselmis sp.* cream	-14.92 ± 0.01^{aA}	-14.57 ± 0.03^{aA}	-7.03 ± 0.01^{aA}	-2.42 ± 0.01^{abB}	-5.65 ± 0.01^{bA}	1.44 ± 0.01^{bB}	-4.18 ± 0.01^{aA}	1.48 ± 0.00^{bB}
b*	Control cream	0.13 ± 0.015^{aA}	0.14 ± 0.03^{aA}	1.60 ± 0.005^{aA}	1.45 ± 0.01^{aA}	1.34 ± 0.03^{aA}	1.56 ± 0.03^{aA}	1.13 ± 0.03^{aA}	1.64 ± 0.01^{aA}
	Spirulina cream	10.62 ± 0.01^{bA}	10.8 ± 0.04^{bA}	10.92 ± 0.01^{dB}	14.67 ± 0.01^{bA}	10.96 ± 0.04^{dB}	15.97 ± 0.03^{bA}	10.69 ± 0.05^{bA}	17.76 ± 0.05^{cB}
	Tetraselmis sp. cream	32.86 ± 0.005^{dA}	32.71 ± 0.05^{dA}	26.25 ± 0.03^{bA}	23.3 ± 0.02^{cA}	22.65 ± 0.01^{bB}	15.75 ± 0.02^{bA}	19.72 ± 0.05^{cB}	11.61 ± 0.03^{bA}
	Dunaliella sp. cream	32.96 ± 0.08^{dA}	32.87 ± 0.01^{dA}	45.76 ± 0.02^{cB}	39.2 ± 0.15^{dA}	38.56 ± 0.00^{cB}	22.98 ± 0.04^{cA}	25.6 ± 0.16^{dB}	16.22 ± 0.01^{cA}
	Spirulina and *Tetraselmis sp.* cream	24.99 ± 0.03^{cA}	24.78 ± 0.01^{cA}	22.39 ± 0.14^{bA}	20.92 ± 0.015^{cA}	21.88 ± 0.02^{bB}	18.65 ± 0.04^{bcA}	20.08 ± 0.03^{cB}	16.13 ± 0.02^{cA}

Tukey's post hoc test was used to determine the significant differences between each formulation time (capital letters) kept 25 and 40 °C during the storage. Tukey's post hoc test was used to determine the significant differences between treatments at a fixed time (lowercase letter)

after 90 days of storage. Although, lightness of the formulated creams with *Dunaliella* sp. and with the combination of *Spirulina* and *Tetraselmis* sp. was stable up to 60 and 30 days, respectively. Afterwards, significant ($P < 0.05$) increases were revealed under both storage temperatures.

Moreover, cream with *Spirulina* showed an important increase of the green color (a*) up to 1 month under both temperatures. Afterwards, a slight increase was detected with time at 25 and 40 °C. At high temperature (40 °C), the same formulation showed increasing of yellow color (b*). This result was explained by the presence of oxidation products (Del Castillo et al. 2016). After 3 months of storage, formulated cream with *Tetraselmis* sp. showed increased ($P < 0.05$) green tone values (a*) and a decrease of yellow color ones (b*) with a significant difference between treatments at 25 and 40 °C. The same results were obtained in the cream with the combined microalgae (*Tetraselmis* sp. and *Spirulina*), probably due to the decreasing of water content when stored under high temperature condition.

Furthermore, Table 5 shows a reduction of the green color (a*) and the yellow color (b*) after 90 days at both temperatures in *Dunaliella* sp. formulated cream. It is well known that the decreased of pH in the *Dunaliella* sp. formulated cream can induce acid-initiated carotenoids degradation (Boon et al. 2010). Additionally, a significant ($P < 0.05$) differences in a* and b* values were found between treatments at 25 and 40 °C, except control cream and a* values of *Tetraselmis* formulated cream. The results presented above were in accordance with color changes in visual aspect analyses.

Textural analyses stabilities

The textural parameter values (hardness, springiness, cohesiveness, and adhesiveness) of cosmetic formulations are

Table 6 Effect of microalgae addition on the textural properties of cosmetic creams

Time (day)	Formulation	Hardness (N)		Springiness (cm)		Cohesiveness		Adhesiveness (Ns)	
		25 °C	40 °C	25 °C	40 °C	25 °C	40 °C	25 °C	40 °C
0	Control cream	0.64 ± 0.03^{bA}	0.64 ± 0.03^{bB}	0.17 ± 0.87^{aA}	0.17 ± 0.87^{aA}	0.82 ± 0.04^{aA}	0.82 ± 0.04^{aA}	0.54 ± 0.02^{bA}	0.54 ± 0.02^{bA}
	Spirulina cream	0.47 ± 0.02^{aA}	0.47 ± 0.02^{aB}	0.22 ± 1.11^{cA}	0.22 ± 1.11^{cA}	0.81 ± 0.03^{aA}	0.81 ± 0.03^{aB}	0.38 ± 0.02^{aA}	0.38 ± 0.02^{aB}
	Tetraselmis sp. cream	0.64 ± 0.03^{bB}	0.64 ± 0.03^{bB}	0.17 ± 1.25^{aA}	0.17 ± 1.25^{aA}	0.73 ± 0.02^{aB}	0.73 ± 0.02^{aB}	0.48 ± 0.02^{bB}	0.48 ± 0.02^{bB}
	Dunaliella sp. cream	0.54 ± 0.02^{abA}	0.54 ± 0.02^{abB}	0.18 ± 0.98^{aA}	0.18 ± 0.98^{aA}	0.82 ± 0.01^{aB}	0.82 ± 0.01^{aB}	0.46 ± 0.01^{bA}	0.46 ± 0.01^{bb}
	Spirulina and *Tetraselmis* sp. cream	0.47 ± 0.02^{aA}	0.47 ± 0.02^{a}	0.19 ± 0.36^{bA}	0.19 ± 0.36^{b}	0.81 ± 0.02^{a}	0.81 ± 0.02^{aB}	0.39 ± 0.01^{aA}	0.39 ± 0.01^{aC}
30	Control cream	0.94 ± 0.04^{abB}	0.46 ± 0.01^{bA}	0.26 ± 0.13^{aA}	0.28 ± 1.41^{cA}	0.68 ± 0.09^{aA}	0.82 ± 0.02^{cA}	0.68 ± 0.01^{aA}	0.39 ± 0.01^{bcA}
	Spirulina cream	0.81 ± 0.04^{aB}	0.43 ± 0.01^{bB}	0.27 ± 1.17^{aA}	0.27 ± 1.4^{cA}	0.75 ± 0.02^{aA}	0.69 ± 0.00^{bA}	0.63 ± 0.04^{aB}	0.38 ± 0.01^{bB}
	Tetraselmis sp. cream	1.23 ± 0.06^{cC}	0.25 ± 0.01^{aA}	0.28 ± 0.23^{aA}	0.19 ± 0.97^{aA}	0.79 ± 0.04^{aB}	0.45 ± 0.01^{aA}	0.98 ± 0.03^{bC}	0.11 ± 0.02^{aA}
	Dunaliella sp. cream	1.30 ± 0.06^{cB}	0.64 ± 0.03^{cAB}	0.29 ± 1.24^{aB}	0.27 ± 1.35^{cA}	0.81 ± 0.03^{aB}	0.69 ± 0.02^{bA}	1.08 ± 0.04^{cC}	0.47 ± 0.02^{cB}
	Spirulina and *Tetraselmis* sp. cream	1.00 ± 0.05^{bB}	0.32 ± 0.00^{aA}	0.27 ± 1.02^{aA}	0.24 ± 1.24^{bA}	0.88 ± 0.02^{aB}	0.68 ± 0.02^{bA}	0.89 ± 0.05^{bB}	0.22 ± 0.01^{ab}
60	Control cream	1.37 ± 0.06^{dC}	0.68 ± 0.02^{bB}	0.26 ± 1.00^{bA}	0.24 ± 1.2^{cA}	0.75 ± 0.04^{bA}	0.79 ± 0.01^{cA}	1.06 ± 0.04^{dC}	0.56 ± 0.03^{cA}
	Spirulina cream	1.01 ± 0.05^{cC}	0.53 ± 0.01^{bB}	0.22 ± 1.12^{aA}	0.19 ± 0.97^{aA}	0.66 ± 0.04^{aA}	0.71 ± 0.01^{cAB}	0.69 ± 0.05^{cB}	0.39 ± 0.02^{bB}
	Tetraselmis sp. cream	0.38 ± 0.01^{aA}	0.17 ± 0.02^{aA}	0.21 ± 1.07^{aA}	0.18 ± 0.92^{aA}	0.53 ± 0.03^{aA}	0.40 ± 0.02^{aA}	0.22 ± 0.03^{aA}	0.10 ± 0.00^{aA}
	Dunaliella sp. cream	1.32 ± 0.06^{dB}	1.26 ± 0.02^{cC}	0.21 ± 1.08^{aA}	0.23 ± 1.19^{cA}	0.78 ± 0.03^{bB}	0.67 ± 0.07^{bA}	1.03 ± 0.01^{dC}	0.86 ± 0.04^{dc}
	Spirulina and *Tetraselmis* sp. cream	0.63 ± 0.03^{bA}	0.22 ± 0.00^{aA}	0.21 ± 1.07^{aA}	0.22 ± 1.14^{bA}	0.73 ± 0.02^{bAB}	0.57 ± 0.03^{aA}	0.46 ± 0.05^{bA}	0.13 ± 0.0^{aB}
90	Control cream	1.31 ± 0.06^{dC}	0.47 ± 0.00^{cA}	0.26 ± 1.35^{bA}	0.21 ± 1.05^{bA}	0.60 ± 0.03^{aA}	0.72 ± 0.02^{bA}	0.82 ± 0.02^{bB}	0.34 ± 0.01^{cA}
	Spirulina cream	1.19 ± 0.06^{cD}	0.24 ± 0.00^{bA}	0.27 ± 1.4^{cA}	0.18 ± 0.91^{aA}	0.67 ± 0.0^{aA}	0.66 ± 0.01^{bA}	0.82 ± 0.04^{bB}	0.16 ± 0.00^{aA}
	Tetraselmis sp. cream	0.45 ± 0.02^{aA}	0.15 ± 0.02^{aA}	0.24 ± 1.22^{aA}	0.18 ± 0.9^{aA}	0.76 ± 0.03^{aB}	0.38 ± 0.05^{aA}	0.34 ± 0.01^{aA}	0.10 ± 0.00^{aA}
	Dunaliella sp. cream	1.25 ± 0.06^{dB}	0.24 ± 0.01^{bA}	0.25 ± 1.23^{aA}	0.20 ± 1.04^{bA}	0.61 ± 0.03^{aA}	1.26 ± 0.06^{cC}	0.72 ± 0.03^{bB}	0.29 ± 0.01^{bA}
	Spirulina and *Tetraselmis* sp. cream	0.69 ± 0.03^{bA}	0.20 ± 0.0^{bA}	0.25 ± 1.2^{abA}	0.21 ± 1.09^{bA}	0.64 ± 0.03^{aA}	0.48 ± 0.02^{aA}	0.47 ± 0.02^{aA}	0.10 ± 0.00^{aA}

Tukey's post hoc test was used to determine the significant differences between each formulation kept 25 and 40 °C during the storage time (capital letters). Tukey's post hoc test was used to determine the significant differences between treatments at a fixed time (lowercase letters)

summarized in Table 6. Compared to control cream, only the addition of *A. platensis* and the combination of *A. platensis* and *Tetraselmis* sp. led to a significant ($P < 0.05$) increase in springiness and a decrease in hardness from 0.64 to 0.47 N and adhesiveness from 0.54 to 0.38 and 0.39 Ns, respectively. However, the cohesiveness values were not affected ($P > 0.05$) by the addition of microalgae. This could be due to the composition differences between control and added microalgae creams, which are essential element for emulsion consistency.

Along storage time, hardness values increased for all formulations kept at 25 °C, except *Tetraselmis* sp. and combined *A. platensis* and *Tetraselmis* sp. formulated creams showed a decrease of hardness values after 30 days of storage. All formulations showed a decrease of hardness values at 40 °C, whereas the *Dunaliella* sp. formulated cream was increased.

Except *Tetraselmis* sp. formulated cream kept at 40 °C, an increase of springiness values was observed for the other formulations at 25 and 40 °C along time and the difference between both temperatures was not significative ($P > 0.05$) up to 30 days of storage. Afterwards, a slight decrease of springiness values was detected with time. Comparing the initial time storage of formulations with those after 90 days, the cohesiveness values showed an obvious decline and the decreases at 40 °C were a little more than those at 25 °C (Table 6). The adhesiveness values of the different formulations investigated in this study were found to increase up to 30 days of storage at 25 °C. After that, except control

and *Spirulina* formulated creams, creams showed a decrease of adhesiveness. Moreover, storage at 40 °C led to a decrease of adhesiviness values along storage time, except for *Dunaliella* sp. prepared cream. Additionally, the adhesiveness values decreasing of creams storage at 40 °C were a little more than those at 25 °C ($P < 0.05$) (Table 6).

In the present case, among the six studied formulations, cream prepared with *Tetraselmis* sp. presenting significantly ($P < 0.05$) the lowest hardness, springiness, and cohesiveness. Consequently, the *Tetraselmis* sp. prepared cream presented less consistency and stability than the others samples through storage period. This is probably due to the fact that *Tetraselmis* sp. cells are rich in lipids. Many reports have suggested that decreasing consistency parameters values during storage time mentioning instability of the formulation (Guaratini et al. 2006).

Microbiological quality of cosmetic creams

Along storage time, no yeast, mold, Enterobacteriaceae, or coliform bacteria were detected in any of the creams during 90 days of storage at 25 and 40 °C. These microbiological results suggest that cosmetic creams production was carried out with good hygienic and sanitary practices.

Antioxidant and antimicrobial activity analysis throughout storage period

The antioxidant activities of cosmetic creams were carried out by the DPPH scavenging activity (Table 7). The antioxidant activity of cream with microalgae was higher than base cream. Thus, microalgae exhibited important antioxidant activities due mainly to the presence of poly-unsaturated fatty acids and pigments as demonstrated by Dammak et al. (2016, 2018) and Barkallah et al. (2019). Many reports expected that bioactive components from microalgae were qualified by their important antioxidant activities which justify their addition in cosmetic creams (Pangestuti and Kim 2011; Singh et al. 2016; Ariede et al. 2017; Widowati et al. 2017).

The most potential radical scavenging effects of cosmetic creams were seen in cream with *Spirulina* followed by *Tetraselmis* sp. and then the combination of these two microalgae (98.83%, 98.33%, and 97.47%, respectively). The cream supplemented with *Dunaliella* sp. exhibited the lowest activity (80.91%). According to Park et al. (2011), some amino acids and proteins inhibit lipid oxidation by free radical scavenging in O/W emulsions.

From the results, it was noticed a stable DPPH radical scavenging activity for cream with *A. platensis* and with the combination *A. platensis* and *Tetraselmis* sp. among the storage period at 25 ± 2 °C and 40 ± 2 °C (Table 7). Cream with *Dunaliella* sp. revealed significant ($P < 0.05$) decrease in DPPH activity among the storage period at 25 ± 2 °C and 40 ± 2 °C. In addition, cream fortified by *Tetraselmis* sp. showed more decrease in the antioxidant activities under both temperatures. Zheng et al. (2020) revealed that many factors can affect antioxidant activity of formulation such as particles size, emulsion stability, and interface composition. Thus, radical scavenging activity demonstrated the instability of formulations. We can conclude that creams with *Spirulina* and with the combination of *Spirulina* and *Tetraselmis* sp. are the most stable formulations with sustainable antioxidant activity.

For antimicrobial assays, as can be seen in Table 8, cream samples were found to have moderate antimicrobial activities against all tested microorganisms. The anti-*S. aureus* activity varied from 0 to 18.3 mm, was significantly ($P < 0.05$) affected by the temperature of storage (Table 8). In fact, the best inhibition zone was obtained from creams with *Dunaliella* sp. samples. After 60 days of storage at 40 °C, the *Tetraselmis* formulated cream has been reported to be weakly inhibitory against *S. aureus*.

After 60 days of storage at 25 °C, interestingly, the anti-*E. coli* activity was > 14.3 mm in all cream samples (Table 8). Anti-*E. coli* activity followed the order: $C + V_2 > C + Ds > C + (Sp + V_2) > C + Sp$ and ranged from 14.3 to 17.3 mm.

Microalgae-derived ω-3 and ω-6 fatty acids have been previously demonstrated to possess antimicrobial activity against a range of pathogens (Little et al. 2021). The presence of antimicrobial fatty acids is very likely in the

Table 7 Antioxidant activities of control and microalgae-cosmetic creams

Formulation	0 days		30 days		60 days		90 days	
	25 °C	40 °C	25 °C	40 °C	25 °C	40 °C	25 °C	40 °C
Control cream	53.22 ± 4.04	55.97 ± 0.72	50.08 ± 3.54	48.4 ± 4.02	51.11 ± 2.14	49.23 ± 1.8	49.07 ± 1.74	48.21 ± 2.13
Spirulina cream	98.33 ± 2.35	98.97 ± 3.02	97.82 ± 2.88	95.97 ± 3.73	96.89 ± 1.3	96.07 ± 2.47	96.77 ± 4.43	96.07 ± 2.03
Tetraselmis sp. cream	98.83 ± 2.12	98.03 ± 1.35	96.05 ± 3.72	79.44 ± 3.87	93.84 ± 1.81	74.21 ± 2.07	87.15 ± 3.04	69.11 ± 0.45
Dunaliella sp. cream	80.91 ± 0.11	81.73 ± 1.23	81.71 ± 2.79	80.36 ± 3.14	79.04 ± 1.09	75.07 ± 4.18	77.08 ± 3.46	70.04 ± 3.83
Spirulina and *Tetraselmis* sp. cream	97.47 ± 2.12	96.21 ± 0.54	97.31 ± 1.11	97.43 ± 1.49	95.09 ± 2.04	98.44 ± 1.57	94.11 ± 1.32	98.21 ± 0.52

Table 8 Antimicrobial activities measured as inhibition zone (cm) of control and microalgae-cosmetic creams

Time (days)	T (°C)	Formulations					
		Test organism	Control cream	*Spirulina* cream	*Tetraselmis sp.* cream	*Dunaliella sp.* cream	*Spirulina and Tetraselmis sp.* cream
0	25	*S. aureus*	1.23 ± 0.15^{aA}	1.4 ± 0.06^{bA}	1.26 ± 0.08^{aB}	1.26 ± 0.04^{aA}	1.23 ± 0.04^{aA}
		E. coli	1.46 ± 0.04^{aA}	1.43 ± 0.08^{aC}	1.46 ± 0.04^{aC}	1.46 ± 0.11^{aA}	1.43 ± 0.08^{aA}
	40	*S. aureus*	1.23 ± 0.15^{aA}	1.4 ± 0.06^{bA}	1.26 ± 0.08^{aB}	1.26 ± 0.04^{aA}	1.23 ± 0.04^{aA}
		E. coli	1.46 ± 0.04^{aA}	1.43 ± 0.08^{aC}	1.46 ± 0.04^{aC}	1.46 ± 0.11^{aA}	1.43 ± 0.08^{aA}
30	25	*S. aureus*	1.26 ± 0.04^{aA}	1.43 ± 0.04^{bA}	1.46 ± 0.04^{bC}	1.53 ± 0.04^{bcB}	1.6 ± 0.06^{cC}
		E. coli	1.4 ± 0.06^{bA}	1.33 ± 0.11^{aB}	1.46 ± 0.04^{bC}	1.46 ± 0.04^{bA}	1.43 ± 0.11^{bA}
	40	*S. aureus*	1.23 ± 0.04^{aA}	1.63 ± 0.11^{dB}	1.23 ± 0.11^{aB}	1.5 ± 0.06^{cB}	1.4 ± 0.13^{bB}
		E. coli	1.43 ± 0.04^{cA}	1.13 ± 0.08^{aA}	1.33 ± 0.08^{bB}	1.4 ± 0.04^{cA}	1.46 ± 0.04^{cA}
60	25	*S. aureus*	1.3 ± 0.06^{aA}	1.46 ± 0.04^{bA}	1.56 ± 0.04^{cD}	1.83 ± 0.04^{dC}	1.46 ± 0.04^{bB}
		E. coli	1.46 ± 0.04^{aA}	1.43 ± 0.11^{aC}	1.73 ± 0.04^{cD}	1.63 ± 0.04^{bB}	1.5 ± 0.13^{aAB}
	40	*S. aureus*	1.26 ± 0.08^{bA}	1.63 ± 0.11^{cB}	0.00 ± 0.00^{aA}	1.6 ± 0.04^{cB}	1.16 ± 0.11^{bA}
		E. coli	1.4 ± 0.06^{bA}	1.46 ± 0.11^{bC}	0.00 ± 0.00^{aA}	1.6 ± 0.06^{cB}	1.53 ± 0.11^{bcB}

Tukey's post hoc test was used to determine the significant differences between treatments at a fixed time (capital letters). A *t*-Student test was applied to compare each formulations kept at 25 and 40 °C at a same time (0, 30, 60, and 90 days) (lowercase letters)

present study as extracts tested showed activity against two pathogen bacteria. Inhibition was the greatest for Gram-positive bacteria, in particular *S. aureus*, which is in agreement with previous study reported by Mc Gee et al. (2020). It has been proposed that fatty acids can inhibit Gram-positive cell growth through a range of mechanisms including cell membrane rupture, cellular respiration inhibition, or peroxidative mechanisms (Singh et al. 2011). The length of the carbon chain and their degree of saturation influences the antibacterial potency of fatty acids (Yoon et al. 2018). The activity of cosmetic creams formulated with different kind of algae could therefore be related to the total cellular lipid content and the fatty acid composition.

Conclusion

In this study, the addition of microalgae significantly ameliorated the physicochemical properties and the sensory acceptability of cosmetic cream and preserved its microbiological quality. The physico-chemical and textural stabilities of *Spirulina*-containing skin cream were proven. This study concluded also that this formulation retained a high and stable antioxidant efficiency. Therefore, the use of microalgae as bioactive ingredients might be a potential alternative to chemical and synthetic additives, which can cause harmful effects on consumers. As a conclusion, it was proved that this innovative skin formulation has a strong capacity to reduce free radicals and the potential to be developed as cosmeceuticals with better stability.

Author contribution Mouna Dammak, Hajer Ben Hlima, and Slim Abdelkafi were responsible for the design of the present study and obtaining funding. Mouna Dammak, Slim Smaoui, Philippe Michaud, Hajer Ben Hlima, Imen Fendri, and Slim Abdelkafi were responsible for the review of the manuscript. Mouna Dammak, Hajer Ben Hlima, Slim Smaoui, Mohamed Ali Ayadi, Imen Fendri, Philippe Michaud, and Slim Abdelkafi conducted data collection, performed the statistical analyses, drafted, and revised the manuscript. All authors contributed to the data acquisition, manuscript revision, and final version approval.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

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