Heliyon 10 (2024) e36832

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Extreme halophilic *Archaea*: *Halobacterium salinarum* carotenoids characterization and antioxidant properties

Sana Ben Hamad Bouhamed [a], Marwa Chaari [a,b], Houda Baati [b], Sami Zouari [c,d], Emna Ammar [b,d,*]

[a] *Research Group of Agri-Food Processing Engineering, Laboratory of Applied Fluids Mechanics, Process Engineering and Environment, National Engineering School of Sfax, University of Sfax, Tunisia*
[b] *National Engineering School of Sfax, University of Sfax, Laboratory of Environment Sciences and Sustainable Development, B.P. 1173 - 3038, Sfax, Tunisia*
[c] *High Institute of Biotechnology of Sfax, University of Sfax, Laboratory of Medicinal and Environmental Chemistry, Sfax, Tunisia*
[d] *National Engineering School of Sfax, University of Sfax, Tunisia*

ARTICLE INFO

Keywords:
Carotenoids
Bacterioruberin
Antioxidant activity
Halophilic *Archaea*
Halobacterium salinarum

ABSTRACT

Important marine microorganisms are resources of renewable energy that may face global population growth and needs. The application of biomass metabolites, such as carotenoids and their derivatives, may solve some agro-food health problems. Herein, a new halophilic *Archaea Halobacterium salinarum* producing carotenoid was screened from a Tunisian solar Saltworks (Sfax). The identification of the carotenoid pigments was carried out using HPLC-MS/MS. The predominant pigments produced by this *Halobacterium* were bacterioruberin and its derivatives and the carotenoids production was found to be of 21.51 mg/mL. Moreover, the data revealed that the carotenoids extract exhibited a high antioxidant activity across four oxidizing assays. The present results suggested that carotenoids extracted from halophilic *Archaea* are interesting sources of natural antioxidants for future innovative applications in agro-food, cosmetic and health fields.

1. Introduction

The halophilic *Archaea* are typically living in hypersaline environments, including solar saltern, salt lake, saline soils as well as coastal lagoons. These extremophiles are a promising source of stable biomolecules of significant biotechnological interest. Among these valuable compounds, they produce enzymes, exopolysaccharides, biosurfactants, biodegradable polyesters, and carotenoids [2]. The extreme conditions culture where the *Archaea* grow (high salt concentrations) enhance non-sterile development conditions and limit the microbial contamination risk. Consequently, halophilic *Archaea* may be suitable for high pigments recovery at low cost compared with other microorganisms [3].

Therefore, carotenoids represent the second profuse natural pigments in the environment [4,5]. These pigments are widely distributed in nature and may be colored in red, orange, or yellow, and they are belonging to isoprenoid subfamily [6]. Actually, the carotenoids have gained significant scientific interest due to their roles in photosynthesis, their antioxidant properties and health benefits [7,8]. Consequently, more than 1100 carotenoids have been found and were characterized, each one exhibiting a variety of colors and a wide range of biological and functional properties. These carotenoids are produced by diverse organisms, including plants,

* Corresponding author. National Engineering School of Sfax, University of Sfax, Tunisia.
E-mail addresses: sana.benhamad@gmail.com (S. Ben Hamad Bouhamed), ammarenis@yahoo.fr (E. Ammar).

https://doi.org/10.1016/j.heliyon.2024.e36832
Received 6 November 2023; Received in revised form 21 August 2024; Accepted 22 August 2024
Available online 23 August 2024

algae, yeast, fungi, and some bacteria [5]. The carotenoids play key roles as light protection pigments by absorbing the visible light energy spectrum (400–500 nm) quenching and protecting from photodamage, cell membrane stabilizers and antioxidant agents. Moreover, their antioxidant properties vary according to their molecular structure, which includes specific chemical characteristics such as the number of conjugated double bonds, the types of functional groups and their molecular mass [5,9]. Carotenoids with a higher number of conjugated double bonds are particularly effective in acting as radical scavengers, providing protection against oxidative damages caused by harmful molecules like reactive oxygen and nitrogen species [10].

Many research have highlighted the diverse biological activities of the carotenoids, including anti-inflammatory, anticancer, anti age-related macular degeneration, and anti-obesity [5,11–13]. Because of their health-promoting properties and color, the carotenoids have gained in popularity with its wide applications range in food, nutraceuticals, pharmaceutical and cosmetic industries [5,14]. The carotenoids chemical synthesis production (80–90 %) can yield highly pure molecules. However, the chemical synthesis process for some carotenoids requires specific and complex conditions, making it challenging and often costly.

During the last decade, considering the natural carotenoids growing demand, to substitute the chemically synthetized of some of them, the research of novel natural resources was improved, and the microbial carotenoids production was enhanced offering a great opportunity to overcome this needed in different domains. Indeed, microbial carotenoids are emerging as promising substitutes for chemically synthesized carotenoids. Such microbial production offers several advantages, including the ability to optimize yield through controlled cultivation and reduces production time. Actually, environmental conditions, such as temperature, salinity, pH, dissolved oxygen, and light, can significantly influence the regulation of the carotenoid biosynthesis pathway.

It is noteworthy that at stressing conditions, microorganisms producing pigments tend to accumulate carotenoids as a defense mechanism or as an adaptation strategy to overcome these environmental challenges. Furthermore, microorganisms' ability to use some solid waste as a substrate for the carotenoids synthesis, improve overall the process sustainability [15].

The C_{50} carotenoids are characteristic pigments generated by the halophilic *Archaea*, with bacterioruberin and its derivatives, especially the bisanhydro-bacterioruberin and the monoanhydro-bacterioruberin being the most prominent [16]. The various pigments differ in the number and stereochemistry of conjugated double bonds within the chromophore. As the number of conjugated double bonds increases, the color intensity of the pigment shifts gradually from yellow to red. The presence of oxygen in the carotenoids is inherent to their classification, this includes the xanthophyll and the carotenes represented mainly by lycopene and β-carotene; these pigments are characterized by long-chain of conjugated double bonds [5].

Other carotenoids including β-carotene, lycopene and phytoene are also biosynthesized by the halophilic *Archaea*, but at low amounts [17]. Furthermore, the bacterioruberin is characterized by a much better radical scavenging activity than the β-carotene, with its 13 pairs of conjugated double carbon bonds [6]. Nevertheless, it is widely known that bacterioruberin protect the halophilic *Archaea* from UV-radiation, H_2O_2 exposure and DNA radiography damages [5,18]. Moreover, this pigment enhances cell adaptation to hypersaline environments, affecting the cell membrane fluidity, by acting as water barrier and promoting permeability to oxygen and other molecules [19].

Few studies dealt with the isolation of *Archaea* from Mediterranean See and its saltworks, and investigated their produced carotenoids considering their identification and their potential application as antioxidants in food and pharmaceutical fields. Abbes et al. [20] isolated *Halobacterium halobium* from Tunisian solar saltern and investigated its carotenoids biological properties. In 2016, Biswas et al. [21] isolated from Indian salt works a halophilic Archaeon: *Haloferax* and investigated its carotenoid genesis, while de la Vega et al. [22] characterized a Haloarchaea producing bacterioruberin isolated from Spanish marshland. Two years later, Chaari et al. (2018) [7] isolated an Archaea in Tunisian solar saltern, and studied the encapsulation of its carotenoids in micro- and macro-emulsions. Then, two years late (2020), Ram and his collaborators [5] reviewed the carotenoids for its robust antioxidant capabilities. More recently (2023), Joshi et al. [15] investigated carotenoid production by microorganisms and their prospective applications as antioxidants in different fields. Actually, it could be noticed that no previous work considered both of halophilic *Archaea* from Tunisian saltworks and the thorough characterization of its carotenoids.

Therefore, the present study aimed isolating and characterizing the pigmented halophilic *Archaea* from Tunisian solar saltern with a focus on exploring their potential for carotenoids production, which were identified using High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS). The antioxidant activity of the carotenoids extracted from the halophilic *Archaea* was investigated, providing an outstanding alternative for the exploration of these pigmented compounds as natural source of antioxidants, with their potential applications in food and pharmaceutical fields.

2. Materials and methods

2.1. Samples collection

Samples of brine were taken from a local crystallizer pond (TS7) of the solar saltern of Sfax city, Tunisia (Central-Eastern coast, 34°39′N and 10°42′E). These brine samples were collected in sterile bottles from ten locations spanning randomly the whole pond, and were conveyed to the laboratory in a cool box.

2.2. Strains growth conditions

The strains were isolated in modified growth medium: the DSC-97 containing (per liter) yeast extract, 5 g; peptone, 10 g; NaCl, 250 g; $MgSO_4.7H_2O$, 20 g; KCl, 2 g; trisodium citrate, 3 g, and the pH was adjusted to 7.4 [20]. The tryptone salt dilutions were spread on DSC-97 agar plates then incubated at 37 °C in a salt saturated atmosphere. After 20 days of incubation, the grown red colonies were

selected and purified. Different colonies were purified by four streaking rounds on fresh DSC-97 modified agar medium, in plates until a pure colony isolation. The isolates were preserved in 15 % glycerol (w/v) at −80 °C until needed.

2.3. Morphological properties

The colony appearance and pigmentation were determined on DSC-97 solid media after 7 days incubation at 37 °C. The cell morphology and motility were evidenced by light microscopy on fresh growing liquid culture (72 h). The Gram staining was performed using acetic acid-fixed samples according to Dussault method [23], and the slide examined under oil immersion objective (100 ×).

2.4. Molecular identification

The genomic DNA was extracted from cells using the Quiagen DNA kit. The extracted DNA was visualized by electrophoresis on 1 % agarose gel stained with ethidium bromide. The 16 rRNA gene was amplified by polymerase chain reaction (PCR) using archaeal-specific primers 21 F (5′-TTCCGGTTGATCCTGCCGGA-3′) and the universal reverse primer 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR program used was as follows: initial denaturation step at 94 °C for 5 min, followed by 30 cycles for 30 s denaturation at 94 °C, 30 s annealing at 55 °C, 2 min elongation at 72 °C with a final extension step at 72 °C for 10 min.

The resulting sequences were compared with reference 16S rRNA gene sequences available at the GenBank and EMBL databases using advance BLAST searches at NCBI (National Center for Biotechnology Information).

2.5. Carotenoids extraction and quantification

The isolated strains were inoculated in 100 mL of DSC-97 medium in erlenmeyer flaks, and incubated on a rotary shaker at 240 rpm for 7 days at 37 °C. The carotenoids extraction was performed as described by Abbes et al. (2013) [20]. Briefly, 50 mL of the culture broth were centrifuged at 6000 rpm for 25 min at 4 °C. The harvested cells were extracted with acetone (100 mL) added with butylhydroxytoluene (BHT) (50 mg), an antioxidant minimizing the carotenoids degradation during the extraction [24]. Then, the solution was centrifuged for 10 min at 6000 rpm and 4 °C. The acetone cell suspension was re suspended in 5 mL of hexane and 5 mL of NaCl solution (25 %) until the separation achievement. The aqueous layer was re extracted in a second step with hexane. The supernatants were finally evaporated to dryness in a vacuum and weighted. The carotenoids were dissolved in ethanol and stored at −20 °C in dark. The extraction protocol practiced to assess the antioxidant activities was the same as the previous described but without BHT addition.

The extracted carotenoids were scanned in the wavelength region of 400–800 nm using a UV/Visible spectrophotometer (optizen POP, UV/VIS spectrophotometer, Mecasys CO., Ltd., Korea). The carotenoids content was determined by measuring the sample optical density at the maximum absorption wavelength (kmax = 495 nm) and calculated according to the following formula:

$$X\ (\mu g) = \frac{A \times y \times 10^{6}}{A^{1\%}_{\cdot 1cm} \times 100}$$

where X refers to the carotenoids weight (μg), A refers to the absorbance at 495 nm, y denotes the solution volume added when measuring optical density (ml) and $A^{1\%}_{\cdot 1cm}$ refers to the absorption coefficient value (2,660) proposed by Britton et al. [25].

2.6. Carotenoids analysis by High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)

An Agilent HPLC-MS/MS model 1200 HPLC system (Agilent, USA) was used, coupled to a triple quadruple mass spectrometer (3200 QTRAP (Sciex, USA)), to analyze the carotenoids. These were separated using C18 column (4.6 × 150 mm, 3.5 μm) and eluted with a mixture of dichloromethane, acetonitrile and methanol (20:70:10, v/v/v). The flow rate was 1 mL/min, the column temperature was set at 25 °C and the injection volume was 20 μL. The ultraviolet (UV) spectra were recorded at 450 nm. The mass spectra of the different compounds were determined by the MS/MS system, equipped with an atmosphere pressure chemical ionization source, operating in positive scan mode (*m/z* range 300–900) and controlled based on MassHunter Workstation Software (Agilent, USA). The ion source temperature was set at 350 °C. The working conditions were as follows: capillary voltage 3000 V, gas flow rate 11 L min^{-1}, gas temperature 290 °C, sheath gas flow rate 12 L min^{-1}, sheath gas temperature 300 °C, and nebulizer pressure 35 psi.

The ratio of each characterized carotenoid was calculated by dividing the sum of its eluted areas by the sum of all the identified carotenoids areas.

2.7. Antioxidant activities

2.7.1. DPPH radical scavenging assay

DPPH radical scavenging assay was performed by the method of Bersuder et al. [26]. The carotenoid solution was prepared at different concentrations (50, 100 200, 300 and 400 μg/mL). A 500 μL volume of each concentration was mixed with 375 μL of absolute ethanol and 125 μL of 0.02 % DPPH (dissolved in ethanol). The control was prepared with 875 μL of ethanol in 125 μL of 0.02 % DPPH. For each concentration, a blank solution containing 500 μL of the sample and 500 μL of absolute ethanol was also prepared. After keeping the mix for 60 min in the dark, the DPPH discoloration was measured at 517 nm against ethanol as blank; the butylated

hydroxytoluene (BHT) was the positive control. All the determinations were conducted in triplicate. The DPPH scavenging percentage was calculated based on absorbance (A) as follows:

$$\text{Radical scavenging activity } (\%) = \frac{(A_{control} + A_{blank}) - A_{sample}}{A_{control}} \times 100$$

2.7.2. Reducing power assay

The assay of reducing power was based on Yildirim's method [27] slightly modified. Briefly, 1 mL sample of each extract at different concentrations (50, 100 200, 300 and 400 μg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide solution at 1 % (w/v). After 30 min incubation at 50 °C, 2.5 mL of a trichloroacetic acid at 10 % (w/v) were added and the reaction mixture was centrifuged at 10 000 rpm for 10 min. From each sample, 2.5 mL of supernatant were mixed together with 2.5 mL of distilled water and 0.5 mL of 0.1 % (w/v) ferric chloride. After 10 min incubation, the solution absorbance was measured at 700 nm. In the control, distillated water replaced the sample and the BHT was the positive control. The values were the means of analyzes in triplicate.

2.7.3. Total antioxidant capacity

The carotenoids total antioxidant activity was assayed as described by Prieto et al. [28]. A sample of 100 μL at different concentrations (50–400 μg/mL) was mixed with 1 mL of the reagent solution including 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. After 90 min of incubation at 90 °C, the mixture was cooled at room temperature and its absorbance was measured at 695 nm using a control including 100 μL of distilled water and 1 mL of the reagent solution. The BHT was the positive control. The test was carried out in triplicate. The total antioxidant capacity was expressed in α-tocopherol using the following formula:

$$A = 0.001\ C + 0.0049\ (R^2 = 0.987)$$

where A was the absorbance at 695 nm, and C was the α-tocopherol concentration expressed in μmol/L.

2.7.4. Antioxidant assay using the β-carotene bleaching method

The carotenoids ability to prevent bleaching of β-carotene was assessed as described by Koleva et al. [29]. An emulsion of β-carotene-linoleic acid was prepared by dissolving 0.5 mg of β-carotene, 25 μL of linoleic acid and 200 μL of Tween 40 in 1 mL of chloroform. Then, the chloroform was evaporated at 40 °C under vacuum in a rotatory vapor and 100 mL of bi-distilled water were added. The established emulsion was freshly prepared before each experiment. A volume of 2.5 mL of the β-carotene-linoleic acid emulsion was added to 0.5 mL of carotenoids extract, at different concentrations. After incubation for 2 h at 50 °C, the absorbance was measured at 470 nm against a blank made of β-carotene-linoleic acid emulsion without β-carotene reagent. A control was used including 0.5 mL distilled water instead of the sample, and the BHT was the positive standard. The antioxidant activity was calculated as follows:

$$\text{Antioxidant activity } (\%) = \left[1 - \frac{(A_0 - A_t)}{(A_{00} - A_{t0})}\right] \times 100$$

where A_0 and A_t were the absorbance's of the samples initially at time zero, and after 2 h of incubation, respectively, and A_{00} and A_{t0} were the absorbance's of the control at time zero and after 2 h of incubation, respectively.

2.8. Hemolytic activity

The carotenoid hemolytic activity was determined according to the Singh & Kaur method [30]. One mL of fresh human blood sample was centrifuged for 10 min at 13 000 rpm to isolate erythrocytes. Then, these were washed with physiological NaCl solution and re centrifuged. The supernatant was discarded and the erythrocytes portion was washed with phosphate buffered saline (PBS, pH 7.5). Then, 0.1 mL of erythrocytes suspension was mixed with various concentrations of the extracted carotenoids (50 μg/mL and 2 mg/mL), dissolved in a buffer to give a final volume of 1 mL. Then, the reaction mixture was incubated at 37 °C for 2 h and finally centrifuged at 5000 rpm for 5 min. The solution absorbance was measured at 450 nm. The positive control (100 % hemolysis) and negative control (0 % hemolysis) were obtained by incubating erythrocytes with distilled water and PBS solution, respectively.

2.9. Statistical analysis

All the determinations were carried out in triplicate. The data were presented as mean ± standard deviation.

3. Results and discussion

3.1. Colony and cell morphologies

On solid media (DSC-97), the isolate produced regular circular cells that were approximately of 1 mm in diameter, convex, and domed, displaying a distinctive red pigmentation. The cells microscopic observation showed motile bacilli, stained Gram negative.

3.2. Bacterial strain identification

The fragmented DNA migration was affected by molecular mass and therefore DNA size and conformation. The electrophoresis exhibited a single band of 16S rDNA, which migrated in the 1500 base pairs region (exactly 1844 base pair) (Fig. 1).

The BLAST analysis showed that the rRNA gene sequencing of the isolated bacteria shared a significant degree of identity with the *Halobacteriales* order, including up to 99 % homology with *Halobacterium salinarum* genus. The sequence of this fragment has been deposited in the European Bioinformatic Institute (EBI) database, under accession number MH350847.

3.3. Total carotenoid content and their UV–Visible spectrum

The total carotenoids concentration produced by the isolated *Halobacterium salinarum* was 21.51 ± 0.39 mg/L, exceeding the previous published results in the literature [1,20,31,32]. However, this concentration matches with results from other *Archaea* [22]. Indeed, while comparing the results reported by de la Vega et al. [22], we might notice that the maximum carotenoids production by *Halorobrum* sp. SH1 reached 20 mg/L at the optimum NaCl concentration of 250 g/L, the same salt concentration used in our experiment. Nevertheless, this value was found in different culture conditions regarding the growth medium composition used lacking peptone and trisodium citrate, but including glucose (1 %), $MgCl_2$, $6H_2O$ (13 g), NaBr (0.5 g), agar (1 %), and at a pH of 7.0 instead of 7.4. Moreover, while in our experiment the oxygenation rate was 250 rpm, this rate was doubled (550 rpm) in the case of *Halorobrum*. This need of oxygen would be related to the added salt in the medium where the carotenoids were produced [22], since the salinity increase of 10 % reduced by 50 % the oxygen solubility.

Besides, total carotenoid produced by the *Halobacterium salinarum* was higher than those produced by the ten *Halobacterium halobium* strains (5.66–7.63 mg/L), the halophilic *Archaea* selected from the same location (local crystallizer pond: TS7) of the solar saltern in Sfax city, Tunisia. These concentrations were found in an identical culture medium (DSC-97) and at the same growth conditions (pH, oxygenation rate and temperature incubation) [20].

Three peaks exhibiting maximum absorption at 470, 495 and 530 nm (Fig. 2 (A)) characterized extract UV–Visible spectrum analysis displaying the halophilic *Archaea* carotenoids content. The first wavelength of a maximum absorption at 470 nm corresponded to lycopene, the most unsaturated acyclic carotenoid characterized by its 11 conjugated double bonds absorbing at 444, 470 and 502 nm [33]. The maxima wavelength absorptions at 495 and 530 nm exhibited the bacterioruberin, the specific halophilic *Archaea* carotenoid. Britton et al. [25] reported that the three distinct peaks at 467, 494 and 527 nm along with two maxima absorption at lower wavelengths were attributed to bacterioruberin and its derivatives, the characteristic red carotenoids. In the present case, *Halobacterium salinarum* carotenoids absorbance's were different from those previously characterized.

3.4. Identification of the Halobacterium salinarum' carotenoids

The *Halobacterium salinarum* carotenoids extracted from a Tunisian solar saltern were identified for the first time based on their chromatographic and spectroscopic characteristics. Fig. 2 (B) showed their HPLC elution profile, and their chromatographic peaks identification was listed in Table 1. The HPLC analysis revealed seven distinct peaks all of which displayed identical absorption spectrum, characteristic of bacterioruberin and its derivatives (peaks 1 to 6) and lycopene isomers (peak 7).

Mass spectrometric analysis exhibited that peaks 1 to 4 presented the same pseudo molecular ion $[M+H]^+$ at *m/z* 741.5 corresponding to bacterioruberin isomers and were revealed at different retention times along the elution process, ranging from 2.37 to

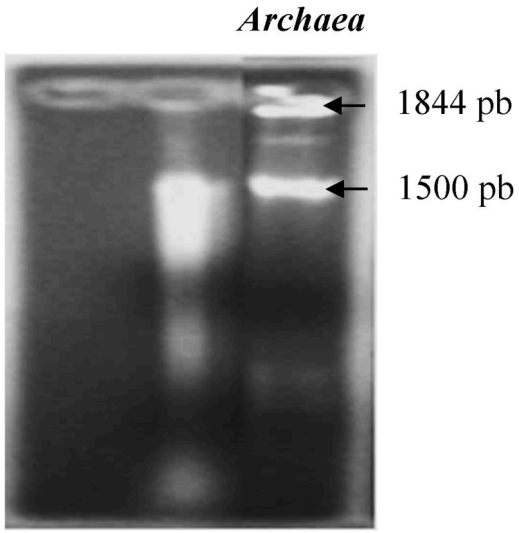


Fig. 1. Gel visualizing DNA extracted from halophilic *Archaea* with phenol-chloroform.

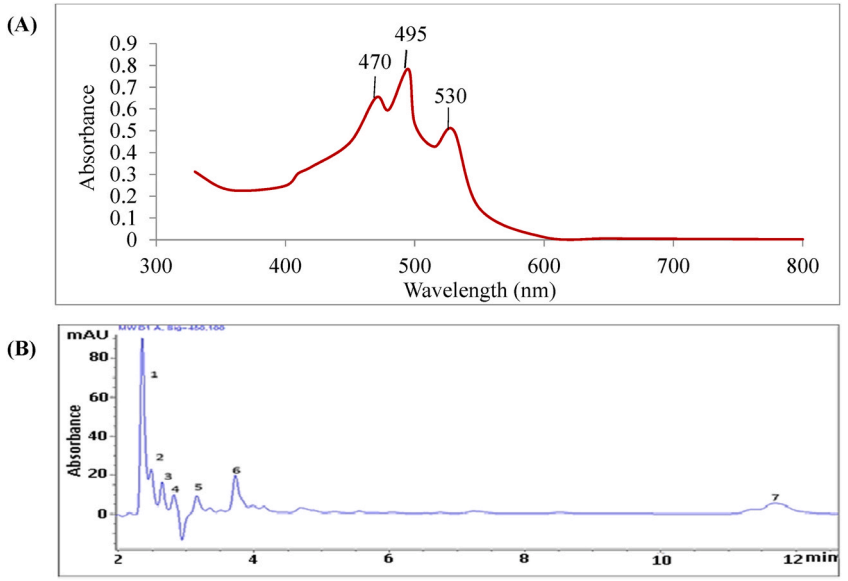


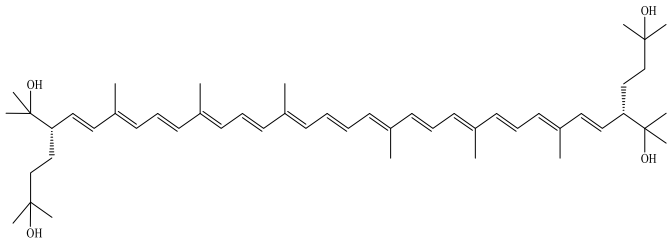
Fig. 2. UV/Visible spectrum (A) and HPLC elution profiles (B) of carotenoids extracted from *Halobacterium salinarum*. Peak identification is revealed in Table 1.

Table 1
Identification of the carotenoids produced by *Halobacterium salinarum* based on their HPLC-MS/MS technique.

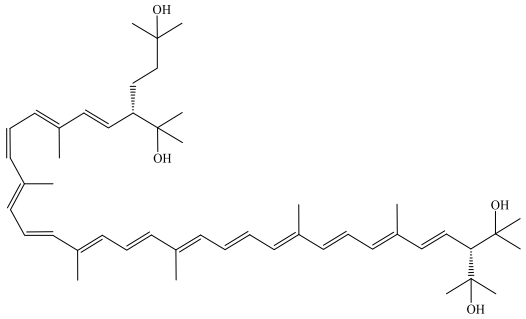
Peak[a]	Carotenoid (tentative identification)	Molecular formula	Retention time (min)	$[M+H]^+$ (*m/z*)	MS/MS fragments ion (*m/z*)
1	All-*trans*-Bacterioruberin	$C_{50}H_{76}O_4$	2.37	741.5	723.5 $[M + H-18]^+$, 705.5 $[M + H-18-18]^+$, 687.5 $[M + H-18-18-18]^+$, 683.5 $[M + H-58]^+$, 665.5 $[M + H-18-58]^+$, 647.5 $[M + H-18-18-58]^+$, 635.5 $[M + H-106]^+$, 631.5 $[M + H-18-92]^+$, 629.5 $[M + H-18-18-18-58]^+$, 599.5 $[M + H-18-18-106]^+$, 591.5 $[M + H-92-58]^+$, 581.5 $[M + H-18-18-18-106]^+$, 563.5 $[M + H-18-18-18-18-106]^+$, 549.5 $[M + H-18-18-18-69-69]^+$, 545.5 $[M + H-58-69-69]^+$, 537.5 $[M + H-18-18-18-58-92]^+$, 507.5 $[M + H-18-18-106-92]^+$, 369.5 $[M + H-106-92-69-69-18-18]^+$
2	5-*cis*, 9′-*cis*-Bacterioruberin	$C_{50}H_{76}O_4$	2.55	741.5	723.5 $[M + H-18]^+$, 705.5 $[M + H-18-18]^+$, 687.5 $[M + H-18-18-18]^+$, 683.5 $[M + H-58]^+$, 665.5 $[M + H-18-58]^+$, 647.5 $[M + H-18-18-58]^+$, 635.5 $[M + H-106]^+$, 629.5 $[M + H-18-18-18-58]^+$, 599.5 $[M + H-18-18-106]^+$, 549.5 $[M + H-18-18-18-69-69]^+$, 529.4 $[M + H-106-106]^+$, 505.5 $[M + H-106-58-18-18-18-18]^+$
3	Bacterioruberin isomer	$C_{50}H_{76}O_4$	2 0.68	741.5	723.5 $[M + H-18]^+$, 705.5 $[M + H-18-18]^+$, 687.5 $[M + H-18-18-18]^+$, 665.5 $[M + H-18-58]^+$, 599.5 $[M + H-18-18-106]^+$, 631.5 $[M + H-18-92]^+$, 537.5 $[M + H-18-18-18-58-92]^+$, 507.5 $[M + H-18-18-92-106]^+$, 397.5 $[M + H-18-18-18-92-92-106]^+$
4	15-*cis*-Bacterioruberin	$C_{50}H_{76}O_4$	2.93	741.5	723.5 $[M + H-18]^+$, 705.5 $[M + H-18-18]^+$, 687.5 $[M + H-18-18-18]^+$, 665.5 $[M + H-18-58]^+$, 647.5 $[M + H-18-18-58]^+$, 631.5 $[M + H-18-92]^+$, 599.5 $[M + H-18-18-106]^+$, 591.5 $[M + H—92-58]^+$, 537.5 $[M + H-18-18-18-58-92]^+$, 507.5 $[M + H-18-18-106-92]^+$, 447.5 $[M + H-18-92-92-92]^+$
5	Monoanhydro-Bacterioruberin isomer	$C_{50}H_{74}O_3$	3.13	723.5	705.5 $[M + H-18]^+$, 687.5 $[M + H-18-18]^+$, 647.5 $[M + H-18-58]^+$, 629.5 $[M + H-18-18-58]^+$, 617.5 $[M + H-106]^+$, 563.5 $[M + H-18-18-18-106]^+$, 549.5 $[M + H-18-18-69-69]^+$, 537.5 $[M + H-18-18-58-92]^+$, 523.5 $[M + H-18-18-58-106]^+$, 507.5 $[M + H-18-106-92]^+$, 479.5 $[M + H-18-18-58-58-92]^+$
6	Monoanhydro-Bacterioruberin	$C_{50}H_{74}O_3$	3.64	723.5	705.5 $[M + H-18]^+$, 687.5 $[M + H-18-18]^+$, 647.5 $[M + H-18-58]^+$, 629.5 $[M + H-18-18-58]^+$, 617.5 $[M + H-106]^+$, 563.5 $[M + H-18-18-18-106]^+$, 549.5 $[M + H-18-18-69-69]^+$, 537.5 $[M + H-18-18-58-92]^+$, 523.5 $[M + H-18-18-58-106]^+$, 507.5 $[M + H-18-106-92]^+$, 479.5 $[M + H-18-18-58-58-92]^+$
7	All-*trans*-lycopene	$C_{40}H_{56}$	11.65	537	467, 455, 413, 347, 321, 269, 177

[a] The peak numbers are those presented on Fig. 2B.

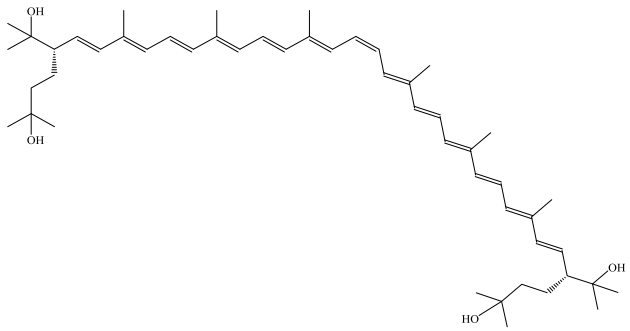
Peaks 1 and 3: All-*trans*-bacterioruberin (28.04% and 8.92%)



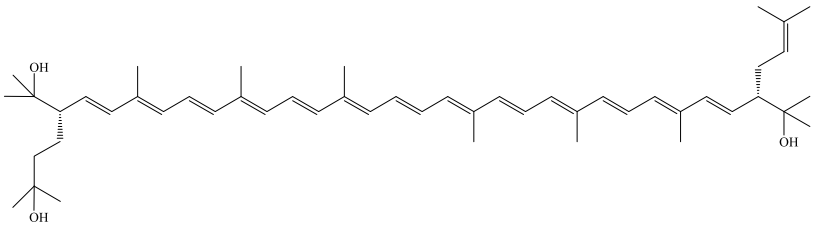
Peak 2: 5-*cis*, 9'-*cis*-Bacterioruberin (9.77%)



Peak 4 : 15-*cis*-bacterioruberin (6.47%)



Peaks 5 and 6 : Monoanhydrobacterioruberin (14.28% and 17.83%)



Peak 7 : All-*trans*-lycopene (14.66%)

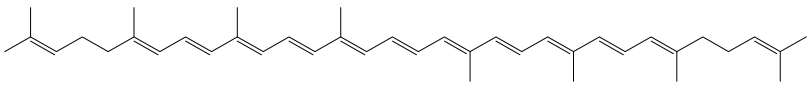


Fig. 3. Rate and structure of the produced carotenoids by *Halobacterium salinarum*.

2.93 min; this is in agreement with previous reports [22,32]. Indeed, de la Vega et al. [22] reported different bacterioruberin isomers detected in *Halorubrum* sp. SH1, with retention time ranged between 2.15 and 2.55 min. More recently, Flores et al. reported the bacterioruberin isomers detection in *Haloterrigena* sp. strain SGH1, with retention times varying from 10.4 to 12.1 min [31]. Because of the difference in techniques applied and data expression, it will be difficult to compare specifically both of the results. The numerous peaks at the different retention times indicated the presence of bacterioruberin isomers, as revealed by the increase in cis-peak intensity as the cis-double bond moves closer to the center of the molecule [34]. The bacterioruberin represented 53.2 % of the detected carotenoids. This finding aligns with previous studies that have shown bacterioruberin to be the most abundant pigment in halophilic *Archaea* [19,31]. More precisely, peak 1 was detected as all-*trans*-bacterioruberin, amounting to 28.04 % of the total carotenoid content (Fig. 3), similar to Giani and collaborators [32] results (28–44 %) described recently; peak 3 was identified as bacterioruberin isomer (8.92 %). Meaningfully, both of the peaks 2 and 4, were geometric *cis* isomers accounting for 9.77 and 6.47 % of the total area, respectively, and were characterized by lower fine structure and higher *cis* peak intensity compared to the *trans* isomer [34]. As expected, all these bacterioruberin isomers exhibited similar protonated ion at *m/z* 741.5 and mass fragments resulting from the losses of water molecules (−18), as well as fragments of C_4H_{10} (−58), C_5H_9 (−69) from the end groups, and toluene (−92) and xylene (−106) from the polyenic chain (Table 1).

The peaks 5 and 6 had a $[M+H]^+$ ion at *m/z* 723.5 which would be attributed to mono-anhydro-bacterioruberin isomers, at the retention time 3.13 and 3.64 min, respectively. Recently, Lizama et al. [35] reported the detection of anhydro-bacterioruberin isomers in *Haloarcula* sp. at 9.52 min. The anhydro-bacterioruberin isomers represented 32.11 % (peak 5: 14.28 % and peak 6: 17.83 %) of the total carotenoids content (Fig. 3). These rates were higher than those previously reported [6,19,32,35]. Mandelli and his collaborators [34] analyzed the carotenoids extract composition obtained from *Halococcus morrhuae* and *Halobacterium salinarium*. In addition, they identified 12 % of different anhydro-bacterioruberin isomers at retention time between 12- and 35-min. The peaks 5 and 6 had similar mass fragments resulting from the loss of water molecules (−18) included several characteristic fragments, such as C_4H_{10} (−58), C_5H_9 (−69), toluene (−92) and xylene (−106).

Peak 7 was identified as all-*trans*-lycopene (14.66 %) at a retention time of 11.65 min, with a protonated molecule at *m/z* 537, in accordance with Squillaci et al. results' [17].

According to the literature, the bacterioruberin and the other archaeal C_{50} carotenoids found in the halophile microorganisms may increase membrane rigidity by their incorporation in their bacterial structure, the relatively long chain, and their two polar end-groups facilitates the adjustment and stabilization of the cell membrane [34]. Indeed, recently it was reported that they act as water barrier allowing the ions and oxygen molecules cross through the cell membrane [36,37]. In addition, the carotenoids produced by these halophiles would be implicated in membrane stabilization, UV light protection as well as in protection against oxidizing agents. Actually, the high number of carbon atoms and the presence of hydroxyl groups in these carotenoids would be responsible for their unique properties that may present industrial interests [6,37].

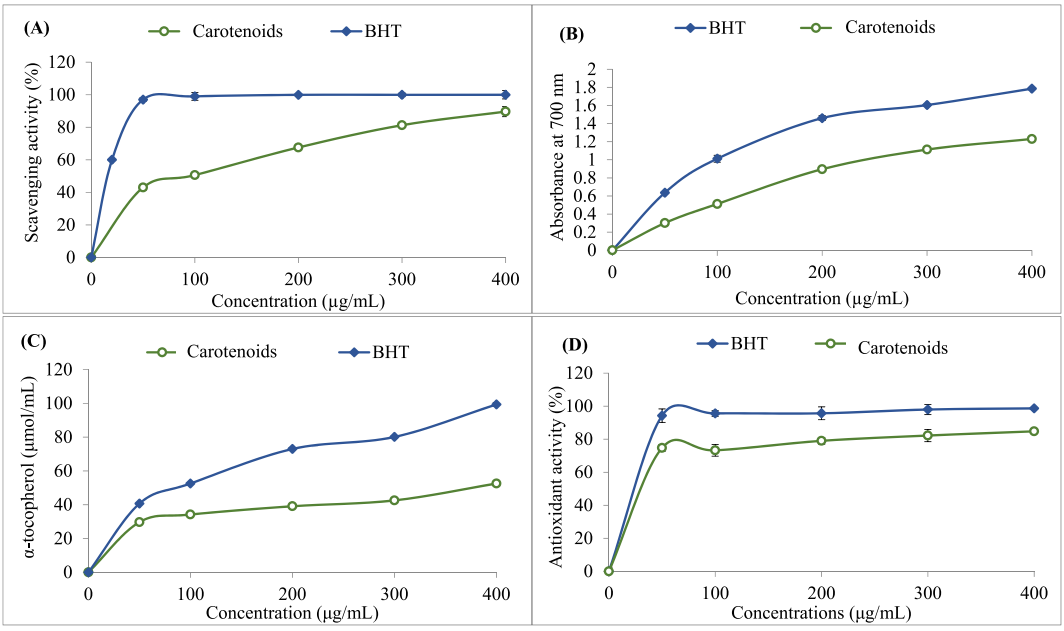


Fig. 4. Antioxidant activities of carotenoids at different concentrations. **(A)** DPPH-radical scavenging activity, **(B)** reducing power, **(C)** Total antioxidant activity and **(D)** Inhibition of β-carotene bleaching. Values are means of three replications ± SD.

3.5. Carotenoids antioxidant potentiality

3.5.1. DPPH scavenging activity

The DPPH is well known as a stable free radical compound often used to assay the antioxidants free radical scavenging ability. The DPPH radical scavenging capacities of both of the carotenoids extract and the BHT (the positive control) are presented in Fig. 4 (A). The result disclosed that the carotenoid extracted from the *Halobacterium salinarum,* a halophilic *Archaea*, exhibited a high inhibiting effect on free radical production, demonstrating a clear dose-effect relationship. At a concentration of 400 μg/mL, DPPH activity displayed by carotenoid and BHT were 89.65 % and 99.96 %, respectively. Consequently, the carotenoid extracted from halophilic *Archaea* was a strong radical scavenger with an inhibition concentration (IC_{50}) of 86.67 μg/mL. Similar results were reported for natural carotenoid [37–39]. However, while extracting carotenoid from *Haloferax* sp. strain BKW301, Biswas et al. [21] proved that a concentration of 400 μg/mL was able to scavenge 70 % of DPPH radicals (0.25 mM); this represents a lower activity than that evidenced by the isolated *Halobacterium salinarum* in the present work. Indeed, concentrations exceeding 120 μg/mL would be needed to inhibit 50 % of the DPPH radicals with carotenoids extracted from *Haloferax. mediterranei* isolated in control cell culture [32]; this concentration was higher than that obtained for *Halobacterium salinarum*, reflecting the isolate high performance. Considering other extracts, *Dunaliella salina*, a renowned producer of carotenoids, has reported to inhibit 50 % at a concentration of 250 μg/mL [40]. In comparison, *Halobacterium salinarum* demonstrated significantly higher effectiveness, being 3.64 times more effective in DPPH scavenging activity than *Dunaliella salina* extracts.

Consequently, the bacterioruberin, the major carotenoid produced by this halophilic strain, exhibited a strong antioxidant ability and exhibited significant radical scavenging capacity. This potent antioxidant effect is attributed to the presence of 13 conjugated double bonds and four hydroxyl groups in the carotenoids extract [6]. This fact may be explained by the synergistic action of many compounds confirming the advantage of using total carotenoids extract instead of the single isolated and/or purified bacterioruberin isomers [17].

3.5.2. Reducing power

The reducing power is widely used to assess the antioxidant capability to donate hydrogen or electron [41]. In the extract, the antioxidant effect can be observed through the reduction of a Fe^{3+}/ferric cyanide complex to its ferrous form (Fe^{2+}). It has been widely accepted that the higher absorbance at 700 nm, the greater is the reduction degree. Fig. 4 (B) showed the carotenoids ability to reduce the Fe^{3+} to Fe^{2+}. It could be noticed that the carotenoid pigment reducing power was a function of concentration while compared to BHT used as a positive control, and it increased linearly with the concentration. At 100 and 200 μg/mL, the carotenoids extract reducing power were 0.51 and 0.89, respectively. Ours finding are in line with other published studies [17,40,42]. Regarding the others extracts, astaxinthin reducing power was 1.71 at a concentration of 100 μg/mL [42], which is higher than that obtained for the *Halobacterium salinarum* extracts. *Dunaliella salina* extracts presented a reducing power of 0.77 at a concentration of 200 μg/mL.

Free radical reducing reaction of carotenoids was conversed to be related to three main mechanisms namely, electron transfer reaction (donate or accepted unpaired electrons), hydrogen atom transfer from the carotenoid and radical addition to the carotenoid [43].

3.5.3. Total antioxidant activity

While assessing quantitative phosphomolybdenum antioxidant activity, the carotenoid extract exhibited an important effect as presented in Fig. 4 (C). Indeed, an increasing antioxidant activity was revealed with carotenoid concentration. The carotenoid extract demonstrated a remarkable antioxidant activity (52.55 μmol/mL α-tocopherol equivalents) at a concentration of 400 μg/mL. However, butylated hydroxytoluene (BHT), used as a positive control in the experiment, demonstrated higher efficiency, with an antioxidant activity of 99.37 μmol/mL α-tocopherol equivalents at the same concentration.

However, the BHT, used as the positive control in the experiment, exhibited higher efficiency, with an antioxidant activity of 99.37 μmol/mL α-tocopherol equivalents at the same concentration. Table 2 summaries the antioxidant activities model of carotenoids and BHT. For the total antioxidant activity, the equation model for carotenoids and BHT were very closely. The estimated regression coefficient ($R^2 = 0.96$ for carotenoids and 0.99 for BHT) reflects the strong correlation between data and the equation.

It should be noticed that no previous data regarding total antioxidant activity carried out on carotenoid extracted from halophilic *Archaea* have been reported in the literature. Therefore, this study investigated for the first time the interesting total antioxidant capacity of halophilic carotenoid extract and their potent use in food without proceeding to the separation of the different pigment' molecules.

3.5.4. β-carotene bleaching inhibition

In the current study, carotenoid hindered the extent of β-carotene bleaching. This effect is due to the carotenoid's ability to neutralize linoleic hydroperoxyl radicals generated in the system. The carotenoid pigments from halophilic *Archaea* were able to decrease the β-carotene concentration, and consequently a discoloration was noticed (Fig. 4 (D)). The carotenoid antioxidant activity was dose-dependent, increasing with carotenoids concentration and reaching 73.27 % and 84.77 % at a concentration of 100 and 400 μg/mL, respectively. Moreover, BHT displayed the highest antioxidant capacity at all the experimented concentrations. However, β-carotene bleaching inhibition of the carotenoid extracts was lower than that found with astaxanthin obtained from shrimp shell (98.20 % at 100 μg/mL concentration) [42]. The carotenoid extracted from *Halobacterium salinarum* presented an IC_{50} value of 24.11 μg/mL, which is higher than those reported for other Haloarchaeal species. Giani et al. [32] found that the IC_{50} of the β-carotene bleaching assay amounted to 28.10 μg/mL with carotenoids extracted from *Haloferax. mediterranei* isolated in controlled cell culture

Table 2
Antioxidant activities models of carotenoids and BHT.

Activities	Model	
	Carotenoids	BHT
Scavenging activity	$Y = 3E\text{-}06x^3\text{-}0.0027x^2+0.7338x+3.8542$ $R^2 = 0.974$	$Y = -8E\text{-}08x^4+7E\text{-}05x^3\ \text{-}0.0223x^2+2.6228x+7.1775$ $R^2 = 0.958$
Reducing power	$Y = -7E06x^2+0.006x+0.008$ $R^2 = 0.999$	$Y = 5E08x^3\text{-}5E05x^2+0.014x+0.008$ $R^2 = 0.999$
Total antioxidant activity	$Y = 4E06x^3\text{-}0.002x^2+0.556x+2.384$ $R^2 = 0.967$	$Y = 4E06x^3\text{-}0.003x^2+0.815x+1.976$ $R^2 = 0.992$
β-carotene bleaching inhibition	$Y = -6E08x^4+5E\text{-}05x^3\ \text{-}0.016x^2+1.952x+2.412$ $R^2 = 0.961$	$Y = -7E08x^4+7E\text{-}05x^3\ \text{-}0.021x^2+2.550x+2.548$ $R^2 = 0.970$

(without any additional carbon source).

3.6. Hemolytic activity

The carotenoid hemolytic capacity was performed on human erythrocytes (data not shown). Different concentrations of samples were tested (50 μg/mL and 2 mg/mL) and no hemolysis was detected for the experimented concentrations. These findings exhibited that carotenoids extracted from halophilic *Archaea* has no toxicity, even when tested at relatively high concentrations.

Thus, this result would confirm the potential practice of the halophilic carotenoids extracts as food additives, without any negative effect on human blood. It should be mentioned that no previous work reported such activity; the goal of this tested activity was to assess the carotenoids safety effect on hemolysis.

4. Conclusion

Carotenoids represent a class of diverse pigments, naturally available issued from plants, algae, and several microorganisms. The current research focused on carotenoids production by halophilic *Archaea*, which exhibited an identity of 99 % with *Halobacterium salinarum* genus. The characterization of the carotenoids profile using HPLC-MS/MS presented a complex of 7 compounds. This study equally highlighted the potential antioxidant activity of carotenoids extract that could be considered as natural source of antioxidant, with prospective uses in food as well as in pharmaceutical field.

Accordingly, in a future work, the improvement of the carotenoids yield production should be studied to better explore such natural pigments at relatively low cost, especially for food industry applications.

Funding

The authors declare no specific funding for this work.

Data availability statement

The data that support the findings of this study will be made available on request from the corresponding author.
Data associated with this study had not been deposited into a publicly available repository.

CRediT authorship contribution statement

Sana Ben Hamad Bouhamed: Writing – original draft, Data curation. **Marwa Chaari:** Visualization, Validation, Investigation, Formal analysis, Conceptualization. **Houda Baati:** Conceptualization, Data curation, Methodology, Visualization. **Sami Zouari:** Writing – original draft, Visualization, Validation, Data curation. **Emna Ammar:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, 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.

Acknowledgments

This research was supported by the Ministry of Higher Education and Scientific Research, Tunisia. We greatly appreciate COTUSAL (General Company of the Saltworks of Tunisia, Sfax) for their help with samples collection and Aristotelis Xenakis from National Hellenic Research Foundation (Athens, Greece) for their help of data analysis.

References

[1] K. Sahli, M.A. Gomri, J. Esclapez, P. Gómez-Villegas, O. Ghennai, M.-J. Bonete, R. León, K. Kharroub, Bioprospecting and characterization of pigmented halophilic archaeal strains from Algerian hypersaline environments with analysis of carotenoids produced by Halorubrum sp. BS2, J. Basic Microbiol. 60 (2020) 624–638, https://doi.org/10.1002/jobm.202000083.

[2] V. Kumar, S.K. Tiwari, Halocin diversity among halophilic Archaea and their applications, in: T. Satyanarayana, B.N. Johri, S.K. Das (Eds.), Microb. Divers. Ecosyst. Sustain. Biotechnol. Appl. Vol. 1 Microb. Divers. Norm. Extreme Environ., Springer, Singapore, 2019, pp. 497–532, https://doi.org/10.1007/978-981-13-8315-1_16.

[3] A. Grivard, I. Goubet, L.M. de S. Duarte Filho, V. Thiéry, S. Chevalier, R.G. de Oliveira-Junior, N. El Aouad, J.R. Guedes da Silva Almeida, P. Sitarek, L. J. Quintans-Junior, R. Grougnet, H. Agogué, L. Picot, Archaea carotenoids: natural pigments with unexplored innovative potential, Mar. Drugs 20 (2022) 524, https://doi.org/10.3390/md20080524.

[4] N. Nisar, L. Li, S. Lu, N.C. Khin, B.J. Pogson, Carotenoid metabolism in plants, Mol. Plant 8 (2015) 68–82, https://doi.org/10.1016/j.molp.2014.12.007.

[5] S. Ram, M. Mitra, F. Shah, S.R. Tirkey, S. Mishra, Bacteria as an alternate biofactory for carotenoid production: a review of its applications, opportunities and challenges, J. Funct.Foods 67 (2020) 103867, https://doi.org/10.1016/j.jff.2020.103867.

[6] R. Yatsunami, A. Ando, Y. Yang, S. Takaichi, M. Kohno, Y. Matsumura, H. Ikeda, T. Fukui, K. Nakasone, N. Fujita, M. Sekine, T. Takashina, S. Nakamura, Identification of carotenoids from the extremely halophilic archaeon Haloarcula japonica, Front. Microbiol. 5 (2014) 100, https://doi.org/10.3389/fmicb.2014.00100.

[7] M. Chaari, I. Theochari, V. Papadimitriou, A. Xenakis, E. Ammar, Encapsulation of carotenoids extracted from halophilic Archaea in oil-in-water (O/W) micro- and nano-emulsions, Colloids Surf. B Biointerfaces 161 (2018) 219–227, https://doi.org/10.1016/j.colsurfb.2017.10.042.

[8] H. Maeda, S. Kanno, M. Kodate, M. Hosokawa, K. Miyashita, Fucoxanthinol, metabolite of fucoxanthin, improves obesity-induced inflammation in adipocyte cells, Mar. Drugs 13 (2015) 4799–4813, https://doi.org/10.3390/md13084799.

[9] M. Furubayashi, D. Umeno, Directed evolution of carotenoid synthases for the production of unnatural carotenoids, Methods Mol. Biol. Clifton NJ 892 (2012) 245–253, https://doi.org/10.1007/978-1-61779-879-5_14.

[10] N.J. Miller, J. Sampson, L.P. Candeias, P.M. Bramley, C.A. Rice-Evans, Antioxidant activities of carotenes and xanthophylls, FEBS Lett. 384 (1996) 240–242, https://doi.org/10.1016/0014-5793(96)00323-7.

[11] J. Ávila-Román, S. García-Gil, A. Rodríguez-Luna, V. Motilva, E. Talero, Anti-inflammatory and anticancer effects of microalgal carotenoids, Mar. Drugs 19 (2021) 531, https://doi.org/10.3390/md19100531.

[12] T.-Y. Lau, H.-Y. Kwan, Fucoxanthin is a potential therapeutic agent for the treatment of breast cancer, Mar. Drugs 20 (2022) 370, https://doi.org/10.3390/md20060370.

[13] C. Thevamirtha, A. Balasubramaniyam, S. Srithayalan, P.M. Selvakumar, An Insight into the antioxidant activity of the facial cream, solid soap and liquid soap made using the carotenoid extract of palmyrah (Borassus flabellifer) fruit pulp, Ind. Crops Prod. 195 (2023) 116413, https://doi.org/10.1016/j.indcrop.2023.116413.

[14] G. Sandmann, Carotenoids of biotechnological importance, Adv. Biochem. Eng. Biotechnol. 148 (2015) 449–467, https://doi.org/10.1007/10_2014_277.

[15] K. Joshi, P. Kumar, R. Kataria, Microbial carotenoid production and their potential applications as antioxidants: a current update, Process Biochem. 128 (2023) 190–205, https://doi.org/10.1016/j.procbio.2023.02.020.

[16] C.-J. Fang, K.-L. Ku, M.-H. Lee, N.-W. Su, Influence of nutritive factors on C50 carotenoids production by Haloferax mediterranei ATCC 33500 with two-stage cultivation, Bioresour. Technol. 101 (2010) 6487–6493, https://doi.org/10.1016/j.biortech.2010.03.044.

[17] G. Squillaci, R. Parrella, V. Carbone, P. Minasi, F. La Cara, A. Morana, Carotenoids from the extreme halophilic archaeon Haloterrigena turkmenica: identification and antioxidant activity, Extrem. Life Extreme Cond. 21 (2017) 933–945, https://doi.org/10.1007/s00792-017-0954-y.

[18] H.R. Shahmohammadi, E. Asgarani, H. Terato, T. Saito, Y. Ohyama, K. Gekko, O. Yamamoto, H. Ide, Protective roles of bacterioruberin and intracellular KCl in the resistance of Halobacterium salinarium against DNA-damaging agents, J. Radiat. Res. 39 (1998) 251–262, https://doi.org/10.1269/jrr.39.251.

[19] J. Hou, H.-L. Cui, In vitro antioxidant, antihemolytic, and anticancer activity of the carotenoids from halophilic Archaea, Curr. Microbiol. 75 (2018) 266–271, https://doi.org/10.1007/s00284-017-1374-z.

[20] M. Abbes, H. Baati, S. Guermazi, C. Messina, A. Santulli, N. Gharsallah, E. Ammar, Biological properties of carotenoids extracted from Halobacterium halobium isolated from a Tunisian solar saltern, BMC Compl. Alternative Med. 13 (2013) 255, https://doi.org/10.1186/1472-6882-13-255.

[21] J. Biswas, F.N. Haque, A.K. Paul, Carotenogenesis in Haloferax sp. strain BKW301, a halophilic archaeon from Indian solar saltarns, J. Adv. Microbiol. (2016) 1–11, https://doi.org/10.9734/JAMB/2016/31559.

[22] M. de la Vega, A. Sayago, J. Ariza, A.G. Barneto, R. León, Characterization of a bacterioruberin-producing Haloarchaea isolated from the marshlands of the Odiel river in the southwest of Spain, Biotechnol. Prog. 32 (2016) 592–600, https://doi.org/10.1002/btpr.2248.

[23] H.P. Dussault, An improved technique for staining red halophilic bacteria, J. Bacteriol. 70 (1955) 484–485, https://doi.org/10.1128/jb.70.4.484-485.1955.

[24] R. Cui, F. Zhu, Physicochemical properties and bioactive compounds of different varieties of sweetpotato flour treated with high hydrostatic pressure, Food Chem. 299 (2019) 125129, https://doi.org/10.1016/j.foodchem.2019.125129.

[25] G. Britton, S. Liaaen-Jensen, H. Pfander, Carotenoids. Vol. 1B, Spectroscopy, Birkhäuser Verlag, Basel, 1995.

[26] P. Bersuder, M. Hole, G. Smith, Antioxidants from a heated histidine-glucose model system. I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography, J. Am. Oil Chem. Soc. 75 (1998) 181–187, https://doi.org/10.1007/s11746-998-0030-y.

[27] A. Yildirim, A. Mavi, A.A. Kara, Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts, J. Agric. Food Chem. 49 (2001) 4083–4089, https://doi.org/10.1021/jf0103572.

[28] P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Anal. Biochem. 269 (1999) 337–341, https://doi.org/10.1006/abio.1999.4019.

[29] I.I. Koleva, T.A. van Beek, J.P.H. Linssen, A. de Groot, L.N. Evstatieva, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, Phytochem. Anal. PCA 13 (2002) 8–17, https://doi.org/10.1002/pca.611.

[30] R.P. Singh, G. Kaur, Hemolytic activity of aqueous extract of Livistona chinensis fruits, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 46 (2008) 553–556, https://doi.org/10.1016/j.fct.2007.08.037.

[31] N. Flores, S. Hoyos, M. Venegas, A. Galetović, L.M. Zúñiga, F. Fábrega, B. Paredes, C. Salazar-Ardiles, C. Vilo, C. Ascaso, J. Wierzchos, V. Souza-Egipsy, J. E. Araya, R.A. Batista-García, B. Gómez-Silva, Haloterrigena sp. strain SGH1, a bacterioruberin-rich, perchlorate-tolerant halophilic archaeon isolated from halite microbial communities, atacama desert, Chile, Front. Microbiol. 11 (2020) 324, https://doi.org/10.3389/fmicb.2020.00324.

[32] M. Giani, L. Gervasi, M.R. Loizzo, R.M. Martínez-Espinosa, Carbon source influences antioxidant, antiglycemic, and antilipidemic activities of Haloferax mediterranei carotenoid extracts, Mar. Drugs 20 (2022) 659, https://doi.org/10.3390/md20110659.

[33] D. Rodriguez-Amaya, A guide to carotenoid analysis in foods. https://www.semanticscholar.org/paper/A-Guide-to-Carotenoid-Analysis-in-Foods-Rodriguez-Amaya/a70fa5a74e79a526d8ea898d193da3588a09a9b6, 1999 accessed January 18, 2023.

[34] F. Mandelli, V.S. Miranda, E. Rodrigues, A.Z. Mercadante, Identification of carotenoids with high antioxidant capacity produced by extremophile microorganisms, World J. Microbiol. Biotechnol. 28 (2012) 1781–1790, https://doi.org/10.1007/s11274-011-0993-y.

[35] C. Lizama, J. Romero-Parra, D. Andrade, F. Riveros, J. Bórquez, S. Ahmed, L. Venegas-Salas, C. Cabalín, M.J. Simirgiotis, Analysis of carotenoids in Haloarchaea species from atacama saline lakes by high resolution UHPLC-Q-orbitrap-mass Spectrometry: antioxidant potential and biological effect on cell viability, Antioxidants 10 (2021) 1230, https://doi.org/10.3390/antiox10081230.

[36] P. Mapelli-Brahm, P. Gómez-Villegas, M.L. Gonda, A. León-Vaz, R. León, J. Mildenberger, C. Rebours, V. Saravia, S. Vero, E. Vila, A.J. Meléndez-Martínez, Microalgae, seaweeds and aquatic bacteria, Archaea, and yeasts: sources of carotenoids with potential antioxidant and anti-inflammatory health-promoting actions in the sustainability era, Mar. Drugs 21 (2023) 340, https://doi.org/10.3390/md21060340.

[37] D. Rathakrishnan, A.K. Gopalan, Screening for anti-neoplastic enzymes producing halophilic bacterial extract and their antioxidant activity due to carotenoid synthesis, Bioresour. Technol. Rep. 19 (2022) 101138, https://doi.org/10.1016/j.biteb.2022.101138.
[38] Y. Li, K. Hu, C. Huang, Y. Hu, H. Ji, S. Liu, J. Gao, Improvement of solubility, stability and antioxidant activity of carotenoids using deep eutectic solvent-based microemulsions, Colloids Surf. B Biointerfaces 217 (2022) 112591, https://doi.org/10.1016/j.colsurfb.2022.112591.
[39] M. Sharma, S. Hussain, T. Shalima, R. Aav, R. Bhat, Valorization of seabuckthorn pomace to obtain bioactive carotenoids: an innovative approach of using green extraction techniques (ultrasonic and microwave-assisted extractions) synergized with green solvents (edible oils), Ind. Crops Prod. 175 (2022) 114257, https://doi.org/10.1016/j.indcrop.2021.114257.
[40] P. Singh, M. Baranwal, S.M. Reddy, Antioxidant and cytotoxic activity of carotenes produced by Dunaliella salina under stress, Pharm. Biol. 54 (2016) 2269–2275, https://doi.org/10.3109/13880209.2016.1153660.
[41] Kazuko Shimada, Kuniko Fujikawa, Keiko Yahara, Takashi Nakamura, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, J. Agric. Food Chem. 40 (1992) 945–948, https://doi.org/10.1021/jf00018a005.
[42] A. Sila, Y. Ayed-Ajmi, N. Sayari, M. Nasri, O. Martinez-Alvarez, A. Bougatef, Antioxidant and anti-proliferative activities of astaxanthin extracted from the shell waste of deep-water pink shrimp (parapenaeus longirostris), Nat. Prod. J. 3 (2013) 82–89.
[43] A. Martínez, M.A. Rodríguez-Gironés, A. Barbosa, M. Costas, Donator acceptor map for carotenoids, melatonin and vitamins, J. Phys. Chem. A 112 (2008) 9037–9042, https://doi.org/10.1021/jp803218e.