International Journal of Food Science +Technology

Check for updates

Original article

Enhancement of *Spirulina platensis* bioactivity by probiotic fermentation and encapsulation by spray-drying

Esmeray Kuley,[1]* Leyla Uslu,[2] Mustafa Durmus,[1] Yetkin Sakarya[1] & Gulsun Özyurt[1]

1 Department of Seafood Processing Technology, Faculty of Fisheries, Cukurova University, Adana 01330, Turkey
2 Department of Basic Science, Faculty of Fisheries, Cukurova University, Adana 01330, Turkey

(Received 4 July 2023; Accepted in revised form 6 September 2023)

Summary The enhancing impact of fermentation with *Lactiplantibacillus plantarum* and drying/coating on bioactivity properties of *Spirulina platensis* was investigated. The fermentation with *Lp. plantarum* took place anaerobically for 72 h at 37 C. Encapsulation of fresh and fermented samples was made by spray-drying with a 170 °C air inlet temperature, a 20 mL/min feed flow rate and different ratios of coating material (1:0, 1:1 and 1:2 maltodextrin). Fresh *Spirulina* capsules exhibited different shapes, mostly rod form whilst fermented *Spirulina* exhibited a generally spherical shape. 1,1-diethoxy-ethane (34.02%) and tert-butyldimethylsilyl 2,2,3,3,3-pentafluoropropanoate (26.70%) were identified as the major compounds in fermented *Spirulina*. The total phenol content of *Spirulina* was in range from 14.22 mg gallic acid equivalent (GAE)/g for fermented wet spirulina to 77.27 mg GAE/g for fermented spray-dried *Spirulina* coated with maltodextrin at a ratio of 1:1. The drying and fermentation processes significantly increased the total phenol content, total antioxidant activity and diphenyl picrylhydrazyl (DPPH) radical scavenging capacity of *Spirulina* ($P < 0.05$). Bacteriostatic concentration of fermented spray-dried *Spirulina* was 25 mg/mL against *Salmonella* Paratyphi A, *Staphylococcus aureus* and *Enterococcus faecalis*, whereas bactericidal effect was at a concentration of 25 mg/mL vs. *Pseudomonas aeruginosa* and *E. faecalis*. The combination of fermentation with *Lp. plantarum* and drying after coating with maltodextrin of *Spirulina* enhanced its antimicrobial and antioxidant properties by 0.7–20-fold compared to fresh, wet *Spirulina*.

Keywords antimicrobials, bioactive compounds, encapsulation, fermentation, *Spirulina*.

Introduction

Bioactive metabolites from microalgae exhibit numerous activities like antioxidant (Kumar *et al*., 2022), anti-inflammatory (Saini & Keum, 2018), antimicrobial (Martelli *et al*., 2020), anti-carcinogenic (Fayyad *et al*., 2019), anti-hypertensive (Suo *et al*., 2022), anti-lipidemic (El-Sayed *et al*., 2018) and anti-diabetic (Guldas *et al*., 2020) activities, and therefore the pharmaceutical, nutraceuticals and food industries are rapidly increasing their demand as an interesting option to improve new products (Kaur *et al*., 2023). Among microalgae, *Spirulina* draws attention as a potential protein source due to its bioactive metabolite content with health-improving properties (Soni *et al*., 2021). *Spirulina* has a very high protein content of around 60% by dry weight and up to 70% depending on growing situations (Lafarga *et al*., 2020). The most important proteins in *Spirulina* are phycobiliproteins, water-soluble pigments that can be categorised based on their ingredient and chromophore content, namely phycocyanins, phycoerythrins and allophycocyanins (Verni *et al*., 2021). Thus, *Spirulina* emerges as a very attractive, natural and sustainable source for the manufacture of functional foods and components (Sahin *et al*., 2022).

Currently, *Spirulina* is presented in several structures in the market, primarily as powders (raw), tablets, capsules, candies, drops and bars (Vardaka *et al*., 2016). Nevertheless, since the majority of these products suffer from a fishy odour, the strong odour of *Spirulina* limits its use as a functional food as it does not suit the dietary habits of some individuals (Kurt *et al*., 2023). Recent research has indicated that fermentation can be considered a good solution to develop microalgae-based products with enhanced flavour and bioactive properties (Kurt *et al*., 2023). Fermentation, a key processing method in the food industry (Dimidi *et al*., 2019), not only efficiently removes fishy odour but also converts and break down microalgae-based

*Correspondent: E-mail: ekuley@cu.edu.tr

doi:10.1111/ijfs.16709

© 2023 Institute of Food, Science and Technology (IFSTTF).

components into an easily absorbable shape for the human body, such as phenolic compounds or proteins, complements the unique components of probiotics and prolongs shelf life (Jamnik *et al*., 2022;Yu *et al*., 2020).

Lactic acid bacteria (LAB) fermentation of *Arthrospira platensis* increased the total amount of phenol 1.73-fold, indicating that the cell walls of algae undergo biodegradation during fermentation, resulting in the discharge of smaller molecules with greater antioxidant activity (Liu *et al*., 2011). The combined use of fermentation with *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) and Alcalase R treatment enhanced the antioxidant activity (up to 60%) of *Spirulina* detected by the ABTS radical scavenging assay and its antimicrobial activity against *Escherichia coli* (Verni *et al*., 2021). LABs have long been gaining attention for their probiotic features. *Lp. plantarum* is one of the favourable LAB species, and is widely used in the food industry for its utilisation as a probiotic and starter culture. Thanks to its extensive history of safe use in human foods, most strains of LAB, especially *Lp. plantarum*, are included in the QPS approvals of the European Food Safety Authority (Leuschner *et al*., 2010) and it is a generally acknowledged as safe (GRAS) status bacterium according to the US Food and Drug Administration (2015). *Lp. plantarum* is a Gram-positive non-spore-forming LAB that yields organic acids, such as acetic acid, succinic acid and lactic acid as main metabolites (Arasu *et al*., 2016). *Lp. plantarum* has ecological and metabolic adaptations and is capable of living in a range of ecological environments, including fermented foods, meats, plants and the mammalian gastrointestinal tract (Fidanza *et al*., 2021). With probiotics, food consumption has increased enormously; *Lp. plantarum* has attracted the attention of several researchers owing to its antioxidant, anticancer, anti-inflammatory, antiproliferative, anti-obesity and anti-diabetic features and thus wide applications in medicine (Arasu *et al*., 2016).

Bioactive ingredients are generally unstable in light, humidity, oxygen and temperature. Besides, they must resist acidic stomach situations and enzymes present in all over the gastrointestinal tract (Raddatz & de Menezes, 2021). One of the other problems encountered during the processing and storage of these natural bioactive compounds is their degradation (Mehta *et al*., 2022). In this respect, microencapsulation of bioactive ingredients is a process that could help to address some of these problems (Domínguez *et al*., 2021).

There have been many studies regarding the biological activity of *Spirulina* and *Lp. plantarum*. However, limited studies deal with the biological activities of their microencapsulation in combination with the fermentation process by *Lp. plantarum*. Therefore, the aim of the study was to investigate the enhancing effect of fermentation and drying/coating on the bioactivity properties of microencapsulated *Spirulina* fermented with *Lp. plantarum*.

Materials and methods

Cultivation of *Spirulina platensis*

Spirulina platensis grown in Schlösser medium (Schlösser, 1982) was cultivated in in laboratory conditions at the Algal Biotechnology Laboratory of Cukurova University. *S. platensis* stock cultures were maintained in different-sized flaks at 24 ± 2 °C, under constant white fluorescence light (Philips white, 36 watts) at an intensity of 40 μmolm^2/s. The cultures in the flasks were enhanced in volume and started to be produced in 10 L jars. Then, the cultures were adapted to the outside environment to inoculate the ellipsoid pools of 5 * 1 * 0.30 m in the pilot facility (Fig. S1). At the end of the adaptation period, the culture study was started by inoculating the ponds with a capacity of 1.5 tonnes in the greenhouse of the Algal Biotechnology Pilot Plant, Fisheries Faculty, Cukurova University, Turkey. There is a pedal system at a speed of 20 cm/s that provides the culture mixture in the pools. Optical density measurements were made daily at a wavelength of 680 nm in a UV-visible spectrophotometer (Shimadzu, UV mini 1240 model; Kyoto, Japan), and the growth phases were determined. The cultures were collected by filtration when the growth phase reached the stationary phase. For the filtration process, a 3-stage filter assembly with 40, 100 and 200 micron mesh apertures was employed. The wet *Spirulina* biomass gathered at 100 and 40 μm was condensed by washing with sterile distilled water. The harvested wet biomass was kept in +4 °C until use.

Lactic acid bacteria strain

Lp. plantarum FI 8595 provided from the collection of the Department of Food Engineering (University of Sutcu Imam Kahramanmaras, Turkey) was used for the fermentation of *Spirulina*. Prior to use, the *Lp. plantarum* strain was propagated in de Man, Rogosa and Sharpe broth suggested by De Man *et al*. (1960) (MRS, Merck 1.10661, Darmstadt, Germany) at 37 °C for 24 h.

Fermentations of *Spirulina platensis*

The fermentation process of *Spirulina* with *Lp. plantarum* was made according to de Marco Castro *et al*. (2019) with minor changes. *Lp. plantarum* (10^8 cfu/mL) was inoculated in *Spirulina* medium (10% dry weight) at a rate of 5% (v/v). Then, glucose (2%, w/v) and potassium sorbate (0.2% w/v) were

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

added, followed by incubation at 37 °C for 72 h under anaerobic condition. UV treatment (20 min) was applied to stop the fermentation. This is due to the fact that 72 hours was thought to be long enough to observe and assess the main fermentation parameters (Niccolai *et al*., 2020). The fermentation effects were also investigated by the pH changes; thus, a digital pH meter (WTW 315i, Germany) was employed for assessing the pH value of fermented *Spirulina* at least three times per day. Fermentation groups were carried out in triplicate.

Microencapsulation of unfermented and fermented Spirulina

Microencapsulation of fresh and fermented *Spirulina* was made using maltodextrin (DE: 18–20, Kimbiotek Chemical Company Ltd, Istanbul, Turkey). Before emulsification, maltodextrin (MD) was dissolved in water and kept at 4 °C for overnight. Then, the coating solution is held in water bath at 50 °C for 1 h. After cooling, *Spirulina* was supplemented and homogenised at 14 000 r.p.m. for 10 min in ultra-turrax (T25, Ika-Werke, Staufen, Germany). *Spirulina* and coating material ratios (*Spirulina*:MD) were 1:0, 1:1 and 1:2. The total solid fraction of the emulsion was set at 30%. A laboratory-scale spray dryer (Buchi Mini Spray Dryer, B-290, Flawil, Switzerland) was used to dry samples. The feed flow rate of the emulsion to the device was set to 20 mL/min and the aspiration rate was ~30 m^3/h. The inlet temperature was adjusted to 170 °C, and the outlet temperature was detected as 95 ± 5 °C. The spray-dried *Spirulina* accumulated in the cyclone separator was collected and stored in dark glass bottles with a lid at 2 °C for further analysis. The groups were as follows: FWS: fermented wet *Spirulina*, NDS: unfermented (fresh) spray-dried *Spirulina* without encapsulation; NDS1: unfermented spray-dried *Spirulina* microencapsulated with MD (1:1); NDS2: unfermented spray-dried *Spirulina* microencapsulated with MD (1:2); FDS: fermented spray-dried *Spirulina* microencapsulated without encapsulation; FDS1: fermented spray-dried *Spirulina* microencapsulated with MD (1:1); FDS2: fermented spray-dried *Spirulina* microencapsulated with MD (1:2). All analyses were done immediately after spray-dried *Spirulina* was obtained, and each analysis was performed at least in triplicate.

Microencapsulation morphology

Morphological features of microencapsulated *Spirulina* were characterised *via* a scanning electron microscopy (SEM) at Cukurova University Central Research Laboratory (CUMERLAB, Adana, Turkey). The microencapsulated samples were imaged using SEM (Quanta FEG 650, USA) at 10 kV voltage after gold coating with a Q150R ES Coater (Quorum Technologies, Hillsboro, Oregon, USA).

GC–MS analysis of *Spirulina*

The chemical composition of wet unfermented and fermented *Spirulina* was determined using an Agilent 7890A gas chromatograph (GC) incorporating a mass spectrometer (MS). A non-polar (5% -phenyl)-methylpolysiloxane (30 m × 250 μm × 0.25 μm, Agilent 1901S-433HP-5MS) was used as column. The flow rate of helium benefited as carrier gas was 1.5 mL/min. GC–MS conditions were set as 50 °C initial and 240 °C final oven temperature, 1 μL injection volume and 105 min run time. Detection of peaks found in GC–MS was obtained by comparison with those in the commercial library of NIST/EPA/NIH version 2.0.

Antimicrobial properties of *Spirulina*

Food-borne pathogens

The inhibition tests were made against *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), *Enterococcus faecalis* ATCC29212 and *Candida albicans* ATCC 10231, which were purchased from the American Type Culture Collection (Rockville, MD, USA), and also against *Salmonella* Paratyphi A (NCTC13) which were obtained from the National Collection of Type Cultures (London, UK).

Agar well diffusion assay

Inhibition diameter zones of *Spirulina* samples against bacterial strains were found using the agar well diffusion method of Hwanhlem *et al*. (2017) with minor modifications. Wet *Spirulina* extracts were analysed directly. To dissolve dried or microencapsulated samples, sterile distilled water (1:3 w/v) was employed. All bacterial cultures were grown on nutrient broth (Merck 1.05443, Darmstadt, Germany) at 37 °C for 24 h. After that, cultures were adjusted to the 0.5 McFarland standard containing ~10^8 cfu/mL and inoculated into Mueller-Hinton Agar (MHA, Merck 1.05437, Darmstadt, Germany). Then, wells (diameter = 6 mm) in MHA plates were filled with 50 microliters of pure or microencapsulated samples, apart from control wells including distilled water. After incubation of plates at 37 °C for 24 h, the inhibition zone was measured in mm from the edge of the agar well. All analysis was conducted in triplicate.

Broth microdilution method

The methods of the Clinical and Laboratory Standards Institute (2008) were applied to determine the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) of wet, dried and microencapsulated *Spirulina*. One

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

milliliter of stock solution (50 mg/mL) was transferred into each tube containing Mueller Hinton Broth (MHB, Merck1.10293) to achieve a dilution series ranging from 50 to 0.19 mg/mL. Subsequently, each tube was inoculated with 1 mL of an individual bacterial strain (10^8 cfu/mL). The tube having the bacterial suspension with no MHB and sample was a positive control, while the tubes lacking MHB were considered negative control. The MIC was determined to be the lowest concentration at which no growth could be seen by naked eye detection in tubes after incubation at 37 °C for 18–24 h. MBC/MFC was considered by inoculation of tubes of MIC displaying no invisible proliferation on MHA. The lowest concentration at which no growth could be seen by naked eye detection was determined to be the MIC.

Total phenol contents and antioxidant activities

Total phenol content of *Spirulina* was determined by a Folin–Ciocalteu method (Gamez-Meza *et al*., 1999). Results are expressed in mg gallic acid equivalent (GAE)/g of sample. Briefly, gallic acid standards come in concentrations between 20 and 1000 µL. The first standard contained 7980 mL of distilled water, 1.5 mL of Na2CO3, 0.5 mL of Folin Ciocalteu's reagent (Merck 109 001), 20 L of gallic acid (Sigma G7384) and 0.5 mL of Folin Ciocalteu's reagent. In later standards, gallic acid concentration increased while water concentration declined, despite Na_2CO_3 and Folin Ciocalteu's reagent remaining constant. A spectrophotometer (Shimadzu UV 1800) was used to measure the samples at 760 nm. *In vitro* antioxidant activities were performed using total antioxidant activity (TAA) and diphenyl picrylhydrazyl (DPPH) radical scavenging capacity assays suggested by Prieto *et al*. (1999) and Wu *et al*. (2003), respectively. Briefly, the samples was mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) for the total antioxidant capacity assay. After that they were kept in a water bath at 95 °C for 90 min and then, the absorbance measurements of samples were made at 695 nm in the spectrophotometer. DPPH radical scavenging capacity analysis was conducted by respective addition of 100 µL of 2 mL of distilled water and 2 mL of 0.16 M DPPH (in methanol) on *Spirulina* samples. The mixture obtained was homogenised and hold in the dark place at room temperature for 30 min. The scavenging activity was measured by sample absorbance at 517 nm.

Statistical analysis

The obtained data were exposed to analysis of variance (one-way ANOVA), at a 5% confidence level using the Duncan multiple range test.

Results and discussions

Morphology of encapsules

Microparticles are mostly in the 1–1000 µm size range and frequently the spheroid form is desired as it offers the further processing advantages (Lengyel *et al*., 2019). In the current study, encapsules showed size varying between 1.48 and 19.96 µm. Unfermented *Spirulina* capsules exhibited irregularly wrinkled structure with different shapes mostly rod form (Fig. 1), whilst fermented *Spirulina* exhibited irregularly wrinkled structure with mostly spherical shape (Fig. 2). Compared to unfermented form, fermented *Spirulina* was better coated resulting in a more spherical shape since the spiral form is broken down by lactic acid bacteria during fermentation. When *Spirulina* are dried without maltodextrin, the rod form appears, but when coated with maltodextrin in a 1:1 ratio, it is seen that they are not encapsulated very well and still have a rod-like form. *Spirulina* coated with 1:2 maltodextrin looked better but did not have a sufficiently rounded form. Similarly, da Silva *et al*. (2019) found *S. platensis* covered with maltodextrin showed a spiral structure and a fragmented pattern (resulting from drying). Concentration of coating material affected the encapsulation efficiency of core particles (Arepally & Goswami, 2019). Different spray-drying conditions also effects spherical shape of particles (Piñón-Balderrama *et al*., 2020). As a result, in this study, the group of fermented samples that coated with 1:2 maltodextrin had the best encapsulation results.

Chemical content of *Spirulina platensis*

Table 1 shows chemical compounds of unfermented and fermented wet *Spirulina* determined by GC/MS. Unfermented wet *Spirulina* contained 7 compounds, of which heptadecane (51.51%), propanamide (20.27%) and 10,18-bisnorabieta-5,7,9(10),11,13-pentaene (11.32%) were the main compounds. Other compounds found in significant levels were 1,1-diethoxy- ethane, (8.07%) and 10-methoxybenz[a]azulen-1,4-dione (4.27%). Fermented wet *Spirulina* included 6 compounds, the ones with the highest levels were 1,1-diethoxy-ethane (34.02%), tert-butyldimethylsilyl 2,2,3,3,3-pentafluoropropanoate (26.70%), butyl ester acetic acid (15.35%) and 2-methyl-5-(1-adamantyl) pentan-2-ol (11.33%). 2-Ethoxy-1-propanol (8.58%) and 1-(2-adamantylidene) semicarbazide (4.02%) are among the other compounds found in the fermented *Spirulina*. 1,1-Diethoxyethane (acetaldehyde, diethyl acetal or acetal) is a major flavouring component of distilled beverages, especially malt whisky and sherry (Haaz *et al*., 2021). Main components of *S. platensis* extract were reported

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

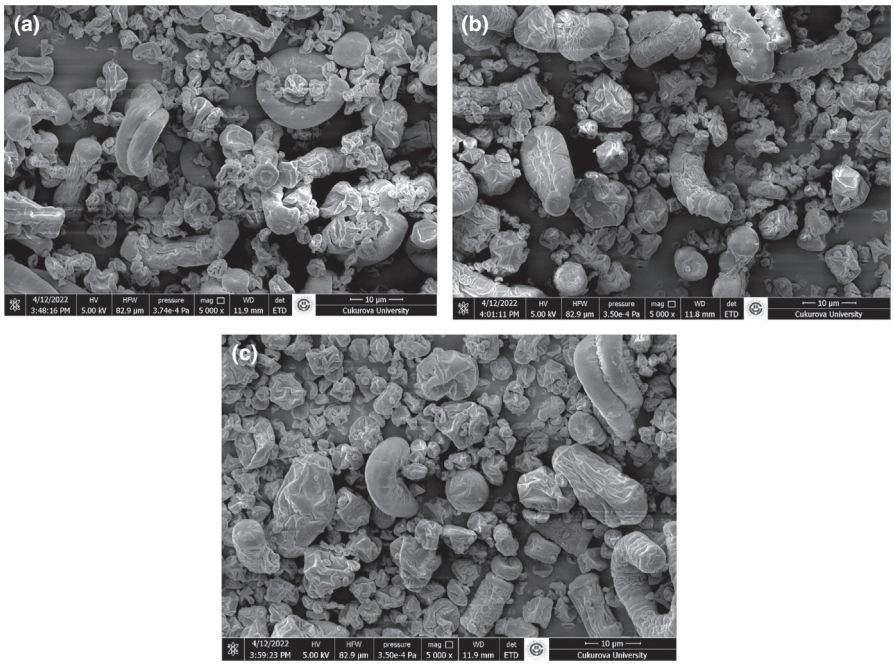

Figure 1 SEM analysis of *Spirulina* encapsules (a) NDS: unfermented spray-dried *Spirulina* without encapsulation (b) NDS1: unfermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:1), (c) NDS2: unfermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:2).

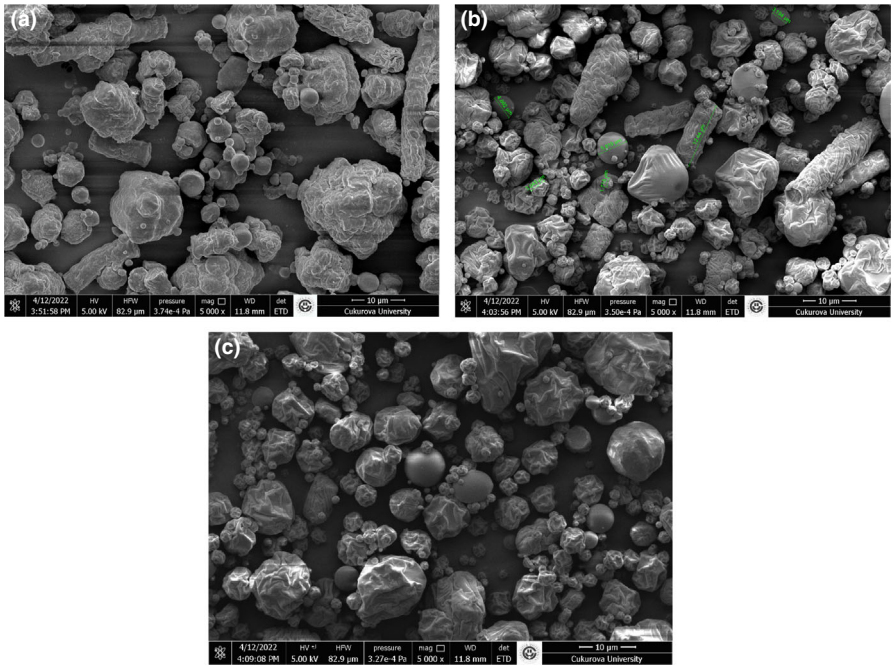

Figure 2 SEM analysis of *Spirulina* encapsules (a) FDS: fermented spray-dried *Spirulina* microencapsulated without encapsulation, (b) FDS1: fermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:1), c) FDS2: fermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:2).

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1 Chemical content of unfermented and fermented wet *Spirulina*

Compounds	RT	Unfermented wet spirulina %	Fermented wet spirulina %
Acetic acid, butyl ester	2.125	–	15.35
2-ethoxy-1-Propanol	2.153	–	8.58
Propanamide	2.176	20.27	
tert-Butyldimethylsilyl 2,2,3,3,3-pentafluoropropanoate	2.187	–	26.70
2-Methyl-5-(1-adamantyl) pentan-2-ol	2.262	–	11.33
1-(2-Adamantylidene) semicarbazide	2.508	–	4.02
1,3,3,3-Tetrachloro-2,2-difluoropropane	2.525	2.38	
Ethane, 1,1-diethoxy-	3.209	8.07	34.02
Heptadecane	39.266	51.51	–
10,18-Bisnorabieta-5,7,9 (10),11,13 -pentaene	39.895	11.32	–
10-Methoxybenz[a]azulen-1,4-dione	40.004	4.27	–
4-Heptanone, 2-methyl-	46.121	2.18	–

RT, retention time.

Table 2 Total phenol content and antioxidant activity of *Spirulina* samples

	Total phenol (mg GA/g)	DPPH (BHT equivalent mcg/g)	Total Antioxidant (mg AA/g)
NWS	$16.87 \pm 0.09^{*gD}$	1.61 ± 0.13^{fC}	55.6 ± 1.20^{fD}
NDS	68.62 ± 0.20^{bA}	16.46 ± 1.36^{bA}	342.02 ± 12.50^{aA}
NDS1	51.08 ± 0.04^{cB}	11.77 ± 1.54^{cdB}	215.77 ± 1.19^{cB}
NDS2	37.01 ± 0.06^{eC}	10.52 ± 0.43^{dB}	135.66 ± 2.96^{dC}
FWS	14.22 ± 0.08^{hD}	3.16 ± 0.19^{fD}	39.61 ± 2.64^{fD}
FDS	77.27 ± 0.27^{aA}	31.43 ± 2.10^{aA}	311.92 ± 3.42^{bA}
FDS1	42.94 ± 0.12^{dB}	13.58 ± 0.39^{cB}	143.89 ± 1.48^{dB}
FDS2	35.63 ± 0.04^{fC}	7.69 ± 0.48^{eC}	83.07 ± 1.19^{eC}

*Mean value ($n = 3$) ± Standard deviation. Different lowercase letters in a column indicate significant differences ($P < 0.05$) among fresh or fermented groups (A–D) and, among all groups (a–h). FDS, fermented spray-dried *Spirulina* microencapsulated without encapsulation; FDS1, fermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:1); FDS2, fermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:2); FWS, fermented wet *Spirulina*; NDS, unfermented spray-dried *Spirulina* without encapsulation; NDS1, unfermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:1); NDS2, unfermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:2).

as hydrocarbons (toluene, heptadecane and tetradecane, dodecane and pentadecane), dioctylamine, nitrogenous components (hexadecaneamide and 3-methyl-1H-indole), oxygenated components (phenol, o-cresol and nonadecanol), n-hexadecanoic acid, 2- ethylhexyl ester octanoic acid, 8-hexadecyn-1-o, bis (2-ethylhexyl) phthalate, 1,2-benzendicarboxilic acid, bis (2-ethylhexyl)-ester, jasmolone, isopulegone and cymarin (Özçakır & Karaduman, 2020; Özogul *et al.*, 2021; Hidhayati *et al.*, 2022). The chemical components of *Spirulina* may be influenced by cultivation, environmental conditions and the growth season of algae, as well as algae extraction methods (AlFadhly *et al.*, 2022).

Total phenol content and antioxidant activities of spirulina samples

Significant differences were found in total phenol content among groups ($P < 0.05$). The total phenol content of *Spirulina* samples ranged from 14.22 mg GAE/g for FWS to 77.27 mg GAE/g for FDS (Table 2). Dried unfermented and fermented *Spirulina* had the highest total phenol content, whilst the lowest value was observed for unfermented (16.87 mg GAE/g) and fermented wet *Spirulina* (14.22 mg GAE/g). Total phenolics rose by more than 35% after *Arthrospira platensis* was fermented by *Lp. plantarum* ATCC 8014 in water or a vegetal soybean beverage, which suggests that during fermentation, the cell walls of algae degrade, releasing smaller compounds with enhanced antioxidant action (Niccolai *et al.*, 2020).

DPPH values were well correlated with the total phenol content of *Spirulina* (Table 2). Significant differences in DPPH values were observed among groups ($P < 0.05$). NDS, FDS and FDS1 showed the highest DPPH activity. The drying and fermentation processes significantly increased the antioxidant activity of *Spirulina*. Since fermented *Spirulina* coated with maltodextrin did not exhibit a sufficient spherical form in order to be considered adequately coated, DPPH ratios in the coated group were comparable to those in the uncoated dried group. Moreover, the encapsulation process of *Spirulina* with maltodextrin at a ratio of 1:1 showed better results in DPPH than that applied at a ratio of 1:2. Similar results were also observed for total antioxidant values. The highest total antioxidant activity was found for the NDS and FDS groups, with respective values of 342.02 and 311.92 mg AA/g (Table 2). The free radical scavenging activity of microalgae *Pavlova lutheri* fermented with *Candida rugopelliculosa* was dose-dependent (0.01–1000 µg/mL of fermented microalgae) (Ryu *et al.*, 2012). Most studies regarded the higher total phenolic content and DPPH radical scavenging activity of lactic acid fermentation of samples compared with unfermented samples (Jamnik *et al.*, 2022; Pérez-Alva *et al.*, 2022). Moreover, a slight or no difference in antioxidant capacity between fermented and unfermented seaweed has been recorded (Takei *et al.*, 2017). de Marco Castro *et al.* (2019) found that after 36 h fermentation of

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 3 Inhibition zones (mm) of *Spirulina* samples against food-borne pathogens

	Salmonella Paratyphi A	*Staphylococcus aureus*	*Candida albicans*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*
NWS	–	–	14.00 ± 0.58aA	–	8.25 ± 0.29^{dC}
NDS	4.00 ± 0.00*^{dC}	12.00 ± 0.58^{abA}	–	9.38 ± 0.75^{dC}	11.33 ± 0.85^{abA}
NDS1	5.17 ± 0.24^{cB}	5.50 ± 0.41^{eC}	–	13.88 ± 1.03^{bB}	12.13 ± 1.03^{aA}
NDS2	5.83 ± 0.47^{bA}	8.17 ± 0.62^{dB}	4.50 ± 0.00^{fB}	15.00 ± 0.58^{aA}	9.50 ± 0.71^{cB}
FWS	6.50 ± 0.41^{aA}	12.50 ± 0.82^{abA}	12.67 ± 0.24^{bA}	12.50 ± 0.41^{cA}	10.17 ± 0.24^{bcB}
FDS	5.00 ± 0.41^{cB}	12.83 ± 0.94^{aA}	9.50 ± 0.82^{dB}	7.33 ± 0.47^{eC}	11.13 ± 0.25^{abcAB}
FDS1	6.17 ± 0.24^{abA}	11.50 ± 0.82^{bcAB}	8.67 ± 0.47^{eC}	9.00 ± 0.82^{dB}	10.75 ± 0.87^{bAB}
FDS2	6.50 ± 0.00^{aA}	10.50 ± 0.82^{cB}	12.00 ± 0.41^{cA}	13.13 ± 1.03^{bcA}	11.50 ± 0.91^{abA}

*Mean value (n = 3) ± Standard deviation. Different lowercase letters in a column indicate significant differences ($P < 0.05$) among fresh or fermented groups (A–C) and, among all groups (a–e). FDS, fermented spray-dried Spirulina microencapsulated without encapsulation; FDS1, fermented spray-dried Spirulina microencapsulated with maltodextrin (1:1); FDS2, fermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:2); FWS, fermented wet Spirulina; NDS, unfermented (fresh) spray-dried Spirulina without encapsulation; NDS1, unfermented spray-dried Spirulina microencapsulated with maltodextrin (1:1); NDS2, unfermented spray-dried Spirulina microencapsulated with maltodextrin (1:2).

Arthrospira platensis by *Lp. plantarum*, total phenolic content was enhanced by 112%, whilst after 24 h, the DPPH radical scavenging capacity increased by 60%. During fermentation, structural disruption of cell walls happens because of bacterial enzyme activity. This induces the release and/or induction of the synthesis of various bioactive components responsible for enhanced total phenols and antioxidant activity (Masten Rutar *et al*., 2021).

Antimicrobial activity of samples

Table 3 shows the minimum inhibition zones of *Spirulina* samples against food-borne pathogens. Significant differences in inhibition zones were found among groups ($P < 0.05$). Unfermented wet *Spirulina* (NWS) did not show any inhibition zones against *S. Paratyphi* A, *S. aureus* and *P. aeruginosa*, although it exerted high activity (Fig. S2) towards the food-borne fungus *C. albicans* (14.00 mm). Coating of unfermented *Spirulina* with maltodextrin, especially at a ratio of 1:2 (NDS2 group) resulted in a higher inhibition zone than the group with dried without coating material (NDS). The highest inhibition zones of NDS1 and NDS2 were observed against *P. aeruginosa* (13.88 vs. 15.00 mm) and *E. faecalis* (12.13 vs. 9.5 mm). The essential oil of *S. platensis* showed antibacterial effects against both Gram-negative and Gram-positive bacteria, and a higher inhibition zone (28 mm) was recorded for *E. coli* (Ould Bellahcen *et al*., 2019). Ethyl acetate extract of *S. platensis* exhibited inhibition activity against various bacteria with varying diameter zones of 11.7–16.9 mm at concentrations 20.000 ppm and 30.000 ppm (Hidhayati *et al*., 2022). Moreover, fermentation of *Spirulina* led to the strongest antimicrobial activity against all food-borne pathogens tested ($P < 0.05$). Fermented *Spirulina* showed the lowest antimicrobial activity against *S. Paratyphi* A with lower than 7 mm inhibition zones. FDS1 showed the highest inhibition zones towards *S. aureus* (11.50 mm), whilst FDS2 exerted strongest activity against *C. albicans* (12.00 mm) and *P. aeuginosa* (13.13 mm). Jamnik *et al*. (2022) reported that lactic acid fermentation of *Arthrospira platensis* by *Lactobacillus plantarum* inhibited pathogen growth in broth. (Yu *et al*., 2020) found that the diameters zone of *Spirulina* supernatants of the combinations of lactic acid bacteria or the mixture of LAB and *Bacillus* strains were 28.0 and 26.8 mm individually against *S. typhimurium*, but their antibacterial impact on *S. aureus* was poor.

The MIC and MBC value of NWS were 50 and >50 mg/mL against most bacteria, respectively (Table 4). Unfermented, coated and/or dried *Spirulina* had generally 12.5 mg/mL of MIC, whilst their bactericidal and fungicidal concentrations against *S. Paratyphi* A and *C. albicans* were above 50 mg/mL. The higher MIC for essential oil of *Spirulina* were reported against *Bacillus antharcis*, *Staphylococcus epidermidis* and *E. coli*, with value of 16.37, 19.13 and 17.81 μL/mL, respectively (Ould Bellahcen *et al*., 2019). *Arthrospira platensis* extract also exerted bacteriostatic and bactericidal effects at doses of 0.45% and 0.90% towards *Listeria monocytogenes* (Martelli *et al*., 2020). Fermented wet *Spirulina* (FWS) showed similar MIC and MBC/MFC values against all pathogens, with respective values of 12.5 and >50 mg/mL. The bacteriostatic concentration of FDS was 25 mg/mL against all bacteria apart from *P. aeruginosa*, whereas the bactericidal effect was found at a concentration of 25 mg/mL vs. *P. aeruginosa* and *E. faecalis*. FDS2 had the lowest MIC and MFC against *C. albicans*, with values of 6.25 and 50 mg/mL, respectively. FDS1 and FDS showed 12.5 mg/mL MICs towards food-borne bacteria. Many studies have shown *in vitro* antimicrobial activity of different algae extracts against *Salmonella* spp., *L. monocytogenes*, *E. coli* and *S. aureus*, indicating that the antimicrobial activity is

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 4 Minimum inhibitory (MIC) and bactericidal/fungicidal concentration (MBC/MFC) of *Spirulina* samples against food-borne pathogens

Groups		*S. Paratyphi* A	*S. aureus*	*C. albicans*	*P. aeruginosa*	*E. faecalis*
NWS	MIC	50	50	12.5	50	25
	MBC/MFC	>50	>50	>50	>50	>50
NDS	MIC	25	12.5	25	12.5	12.5
	MBC/MFC	>50	50	>50	25	25
NDS1	MIC	12.5	12.5	25	12.5	12.5
	MBC/MFC	>50	50	>50	50	50
NDS2	MIC	12.5	12.5	12.5	12.5	12.5
	MBC/MFC	>50	50	>50	50	50
FWS	MIC	12.5	12.5	12.5	12.5	12.5
	MBC/MFC	>50	>50	>50	25	>50
FDS	MIC	25	25	50	50	25
	MBC/MFC	50	50	50	25	25
FDS1	MIC	12.5	12.5	12.5	12.5	12.5
	MBC/MFC	>50	>50	>50	50	>50
FDS2	MIC	12.5	12.5	6.25	12.5	12.5
	MBC/MFC	>50	50	50	>50	50

FDS, fermented spray-dried *Spirulina* microencapsulated without encapsulation; FDS1, fermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:1); FDS2, fermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:2); FWS, fermented wet *Spirulina*; NDS, unfermented (fresh) spray-dried *Spirulina* without encapsulation; NDS1, unfermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:1); NDS2, unfermented spray-dried *Spirulina* microencapsulated with maltodextrin (1:2).

variable according to the algae species, used concentrations and extraction method (Kim *et al.*, 2018; Martelli *et al.*, 2020). The existence of fatty acid compounds, flavonoids and polyphenolic compounds could be linked to the antimicrobial activity of *Spirulina* (Martelli *et al.*, 2020; Sowmya *et al.*, 2021). Antimicrobial compounds like caffeic and coumaric acids, bis (2-ethylhexyl) phthalate, 1,2-benzendicarboxilic acid, bis (2-ethylhexyl) ester, benzophenone, dinitrobenzoate, propanediamine, isoquinoline, piperidin, oxazolidin, pyrrolidine and dioctylamine were also reported for *Spirulina* (Ama Moor *et al.*, 2016; Alshuniaber *et al.*, 2021; Özogul *et al.*, 2021; Hidhayati *et al.*, 2022). Similar to these studies, unfermented and fermented *Spirulina* contained significant levels of heptadecane, propenamide, 1,1-diethoxyethane, tert-butyldimethylsilyl 2,2,3,3,3-pentafluoropropanoate and other compounds that may be responsible for the antimicrobial properties of *Spirulina*.

Conclusion

In conclusion, spherical form was achieved in *Spirulina* samples thanks to the fermentation process and the drying process in a spray-dryer after coating with maltodextrin. As well, the best encapsulation outcomes were achieved by the group with fermented *Spirulina* coated with 1:2 maltodextrin (FDS2). *Spirulina*'s antibacterial and antioxidant capabilities were improved by 0.7–20 fold when combined with fermentation with *L. plantarum* and drying after coating with maltodextrin. *Salmonella* Paratyphi A seemed to be the most resistant bacteria to all *Spirulina* forms, whilst bacteriostatic concentration of *Spirulina* was ≥12.5 mg/mL against all food-borne pathogens tested. *Salmonella* Paratyphi A appeared to be the most resistant bacteria against all *Spirulina* forms, while the bacteriostatic concentration of *Spirulina* samples was 12.5 mg/mL against all food-borne pathogens tested. This result revealed the potential use of microencapsulated fermented *Spirulina* as an antimicrobial and antioxidant in various food matrixes. However, further studies are needed to determine the sensory, physical and biochemical effects of improved *Spirulina* forms as well as the antimicrobial properties of foods.

Funding

The authors would like to thank the Scientific Research Projects Unit of Cukurova University for their financial support.

Author contributions

Esmeray Kuley: Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Leyla Uslu:** Formal analysis (equal); investigation (equal); methodology (equal). **Mustafa Durmus:** Formal analysis (equal); methodology (equal). **Yetkin Sakarya:** Formal analysis (equal); methodology (equal). **Gulsun Özyurt:** Conceptualization (equal); funding acquisition (equal); project

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

administration (equal); validation (equal); writing – review and editing (equal).

Data availability statement

All related data and methods are available within the article

References

AlFadhly, N.K., Alhelfi, N., Altemimi, A.B., Verma, D.K., Cacciola, F. & Narayanankutty, A. (2022). Trends and technological advancements in the possible food applications of *Spirulina* and their health benefits: a review. *Molecules*, **27**, 5584.

Alshuniaber, M.A., Krishnamoorthy, R. & AlQhtani, W.H. (2021). Antimicrobial activity of polyphenolic compounds from spirulina against food-borne bacterial pathogens. *Saudi Journal of Biological Sciences*, **28**, 459–464.

Ama Moor, V., Pieme, C.A. & Biapa, P.C.N. (2016). Chemical composition of Spirulina platensis of nomayos-yaounde. *Annals Food Science and Technology*, **17**, 524–528.

Arasu, M.V., Al-Dhabi, N.A., Ilavenil, S., Choi, K.C. & Srigopalram, S. (2016). *In vitro* importance of probiotic *Lactobacillus plantarum* related to medical field. *Saudi Journal of Biological Sciences*, **23**, S6–S10.

Arepally, D. & Goswami, T.K. (2019). Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. *LWT*, **99**, 583–593.

da Silva, S.C., Fernandes, I.P., Barros, L. *et al*. (2019). Spray-dried *Spirulina platensis* as an effective ingredient to improve yogurt formulations: testing different encapsulating solutions. *Journal of Functional Foods*, **60**, 103427.

In this study, Spirulina platensis was added to yoghurt in three different forms, all of which had their chemical composition and bioactivity previously assessed: free, microencapsulated in maltodextrin, and microencapsulated in maltodextrin crosslinked with citric acid. With the intention of enhancing their nutritional content and providing them with the bioactive chemicals found in spirulina, these ingredients were further utilised to generate several yoghurt compositions. Throughout storage, it was able to preserve the nutritional profile and increase antioxidant activity. Additionally, and according to qualitative investigation, the application of microencapsulation forms decreased the bright green colour and unpleasant fish-like odour of Spirulina. These findings on the evaluation of colour and odour offer crucial information for the future use of microencapsulated Spirulina as an additive.

De Man, J.C., Rogosa, D. & Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Microbiology*, **23**, 130–135.

de Marco Castro, E., Shannon, E. & Abu-Ghannam, N. (2019). Effect of fermentation on enhancing the nutraceutical properties of *Arthrospira platensis* (*Spirulina*). *Fermentation*, **5**, 28.

The overall goal of this study was to determine the ideal fermentation time for maximising the nutraceutical properties of spirulina. Specific objectives included quantifying and comparing the total antioxidant activity, total phenolic, C-phycocyanin, and free methionine content of LAB fermented spirulina to untreated spirulina. The increased antioxidant capacity and protein fragmentation of the fermented samples demonstrate that cyanobacterial peptides and other beneficial substances entrapped inside the spirulina cell wall were liberated during fermentation. This study plays a significant role in defining fermented spirulina as a functional ingredient for nutraceuticals and pharmaceuticals

Dimidi, E., Cox, S.R., Rossi, M. & Whelan, K. (2019). Fermented foods: definitions and characteristics, impact on the gut microbiota and effects on gastrointestinal health and disease. *Nutrients*, **11**, 1806.

Domínguez, R., Pateiro, M., Munekata, P.E., McClements, D.J. & Lorenzo, J.M. (2021). Encapsulation of bioactive phytochemicals in plant-based matrices and application as additives in meat and meat products. *Molecules*, **26**, 3984.

El-Sayed, E.-S., Hikal, M., Abo El- Khair, B., El-Ghobashy, R. & El-Assar, A. (2018). Hypoglycemic and hypolipidemic effects of *Spirulina platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin on male diabetic rats. *Arab Universities Journal of Agricultural Sciences*, **26**, 1121–1134.

Fayyad, R.J., Ali, A.N.M., Dwaish, A.S. & Al-Abboodi, A.K.A. (2019). Anticancer activity of Spirulina platensis methanolic extracts against L20B and MCF7 human cancer cell lines. *Plant Archives*, **19**, 1419–1426.

Fidanza, M., Panigrahi, P. & Kollmann, T.R. (2021). *Lactiplantibacillus plantarum*–nomad and ideal probiotic. *Frontiers in Microbiology*, **12**, 712236.

Gamez-Meza, N., Noriega-Rodriguez, J.A., Medina-Juarez, L.A., Ortega-Garcia, J., Cazarez-Casanova, R. & Angulo-Guerrero, O. (1999). Antioxidant activity in soybean oil of extracts from *Thompson grape bagasse*. *Journal of the American Oil Chemists' Society*, **76**, 1445–1447.

Guldas, M., Ziyanok-Demirtas, S., Sahan, Y., Yildiz, E. & Gurbuz, O. (2020). Antioxidant and anti-diabetic properties of *Spirulina platensis* produced in Turkey. *Food Science and Technology*, **41**, 615–625.

Haaz, E., Fozer, D. & Toth, A.J. (2021). Development of anhydrous ethanol purification: reduction of acetal content and vapor–liquid equilibrium study of the ethanol–acetal binary system. *ACS Omega*, **6**, 1289–1298.

Hidhayati, N., Agustini, N.W.S., Apriastini, M. & Diaudin, D.P.A. (2022). Bioactive compounds from microalgae *Spirulina platensis* as antibacterial candidates against pathogen bacteria. *Jurnal Kimia Sains Dan Aplikasi*, **25**, 41–48.

Hwanhlem, N., Ivanova, T., Haertlé, T., Jaffrès, E. & Dousset, X. (2017). Inhibition of food-spoilage and foodborne pathogenic bacteria by a nisin Z-producing *Lactococcus lactis* subsp. *lactis* KT2W2L. *LWT*, **82**, 170–175.

Jamnik, P., Mahnič, N., Mrak, A. *et al*. (2022). Fermented biomass of *Arthrospira platensis* as a potential food ingredient. *Antioxidants*, **11**, 216.

Kaur, M., Bhatia, S., Gupta, U. *et al*. (2023). Microalgal bioactive metabolites as promising implements in nutraceuticals and pharmaceuticals: inspiring therapy for health benefits. *Phytochemistry Reviews*, **22**, 1–31.

Kim, H.-J., Dasagrandhi, C., Kim, S.-H., Kim, B.-G., Eom, S.-H. & Kim, Y.-M. (2018). *In vitro* antibacterial activity of phlorotannins from edible brown algae, *Eisenia bicyclis* against streptomycin-resistant *Listeria monocytogenes*. *Indian Journal of Microbiology*, **58**, 105–108.

Kumar, A., Ramamoorthy, D., Verma, D.K. *et al*. (2022). Antioxidant and phytonutrient activities of *Spirulina platensis*. *Energy Nexus*, **6**, 100070.

Kurt, H., Hosoglu, M.I., Guneser, O. & Karagul-Yuceer, Y. (2023). Influence of different bacteria species in chemical composition and sensory properties of fermented *Spirulina*. *Food Chemistry*, **400**, 133994.

Lafarga, T., Fernández-Sevilla, J.M., González-López, C. & Acién-Fernández, F.G. (2020). *Spirulina* for the food and functional food industries. *Food Research International*, **137**, 109356.

Lengyel, M., Kállai-Szabó, N., Antal, V., Laki, A.J. & Antal, I. (2019). Microparticles, microspheres, and microcapsules for advanced drug delivery. *Scientia Pharmaceutica*, **87**, 20.

Leuschner, R.G., Robinson, T.P., Hugas, M. *et al*. (2010). Qualified presumption of safety (QPS): a generic risk assessment approach for biological agents notified to the European food safety authority (EFSA). *Trends in Food Science & Technology*, **21**, 425–435.

Liu, J.-G., Hou, C.-W., Lee, S.-Y., Chuang, Y. & Lin, C.-C. (2011). Antioxidant effects and UVB protective activity of Spirulina (Arthrospira platensis) products fermented with lactic acid bacteria. *Process Biochemistry*, **46**, 1405–1410.

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Martelli, F., Cirlini, M., Lazzi, C., Neviani, E. & Bernini, V. (2020). Edible seaweeds and spirulina extracts for food application: *In vitro* and in situ evaluation of antimicrobial activity towards foodborne pathogenic bacteria. *Food*, **9**, 1442.

Masten Rutar, J., Cillero-Pastor, B., Mohren, R., Poklar Ulrih, N., Ogrinc, N. & Jamnik, P. (2021). Insight into the antioxidant effect of fermented and non-fermented spirulina water and ethanol extracts at the proteome level using a yeast cell model. *Antioxidants*, **10**, 1366.

Mehta, N., Kumar, P., Verma, A.K. *et al*. (2022). Microencapsulation as a noble technique for the application of bioactive compounds in the food industry: a comprehensive review. *Applied Sciences*, **12**, 1424.

Niccolai, A., Bažec, K., Rodolfi, L. *et al*. (2020). Lactic acid fermentation of *Arthrospira platensis* (*spirulina*) in a vegetal soybean drink for developing new functional lactose-free beverages. *Frontiers in Microbiology*, **11**, 560684.

Ould Bellahcen, T., Cherki, M., Sánchez, J.A.C., Cherif, A. & El Amrani, A. (2019). Chemical composition and antibacterial activity of the essential oil of *Spirulina platensis* from Morocco. *Journal of Essential Oil Bearing Plants*, **22**, 1265–1276.

Özçakır, G. & Karaduman, A. (2020). Detecting chemicals with high yield in pyrolytic liquid of spirulina sp. microalgae via GC-MS. *International Journal of Energy Applications and Technologies*, **7**, 107–114.

Özogul, İ., Kuley, E., Durmus, M., Özogul, Y. & Polat, A. (2021). The effects of microalgae (*Spirulina platensis* and *Chlorella vulgaris*) extracts on the quality of vacuum packaged sardine during chilled storage. *Journal of Food Measurement and Characterization*, **15**, 1327–1340.

The purpose of the study was to determine how sensory, chemical, and microbiological alterations in vacuum-packed sardine fillets stored at 4 °C and 1 °C for 15 days were affected by water extracts of *Spirulina platensis* and *Chlorella vulgaris*. The findings demonstrated the high mineral content of *S. platensis* and *C. vulgaris*. 3,5-dichloro-6-nitrocholestane and dioctylamine were the main ingredients in the extracts of *C. vulgaris* and *S. platensis*, respectively. The extracts extended the sardine's sensory shelf life by 3 days, despite the fact that the *S. platensis* group was preferred over the C. vulgaris group. With the exception of Gram-positive lactic acid bacteria in sardine meat, these microalgae extracts postponed lipid oxidation and prevented bacterial development. This research is crucial in identifying the potential use of Spirulina as antimicrobial and antioxidant food additives.

Pérez-Alva, A., MacIntosh, A.J., Baigts-Allende, D.K., García-Torres, R. & Ramírez-Rodrigues, M.M. (2022). Fermentation of algae to enhance their bioactive activity. *Algal Research*, **64**, 102684.

Piñón-Balderrama, C.I., Leyva-Porras, C., Terán-Figueroa, Y., Espinosa-Solís, V., Álvarez-Salas, C. & Saavedra-Leos, M.Z. (2020). Encapsulation of active ingredients in food industry by spray-drying and nano spray-drying technologies. *PRO*, **8**, 889.

Prieto, P., Pineda, M. & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, **269**, 337–341.

Raddatz, G.C. & de Menezes, C.R. (2021). Microencapsulation and co-encapsulation of bioactive compounds for application in food: challenges and perspectives. *Ciência Rural*, **51**, 1–8.

Ryu, B., Kang, K.-H., Ngo, D.-H., Qian, Z.-J. & Kim, S.-K. (2012). Statistical optimization of microalgae *Pavlova lutheri* cultivation conditions and its fermentation conditions by yeast, *Candida rugopelliculosa*. *Bioresource Technology*, **107**, 307–313.

Sahin, B., Hosoglu, M.I., Guneser, O. & Karagul-Yuceer, Y. (2022). Fermented spirulina products with *saccharomyces* and non-*saccharomyces* yeasts: special reference to their microbial, physico-chemical and sensory characterizations. *Food Bioscience*, **47**, 101691.

Saini, R.K. & Keum, Y.S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance. *Life Sciences*, **203**, 255–267.

Schlösser, U.G. (1982). Sammlung von algenkulturen. *Berichte der Deutschen Botanischen Gesellschaft*, **95**, 181–276.

Soni, R.A., Sudhakar, K., Rana, R.S. & Baredar, P. (2021). *Food Supplements Formulated with Spirulina*. Pp. 201–226. Algae: Multifarious Applications for a Sustainable World.

Sowmya, S., Suba Sri, M. & Dineshkumar, R. (2021). *In vitro* therapeutic effect of *spirulina* extract. *Asian Journal of Biological and Life Sciences*, **10**, 583.

Suo, Q., Yue, Y., Wang, J., Wu, N., Geng, L. & Zhang, Q. (2022). Isolation, identification and in vivo antihypertensive effect of novel angiotensin I-converting enzyme (ACE) inhibitory peptides from *spirulina* protein hydrolysate. *Food & Function*, **13**, 9108–9118.

Takei, M., Kuda, T., Eda, M., Shikano, A., Takahashi, H. & Kimura, B. (2017). Antioxidant and fermentation properties of aqueous solutions of dried algal products from the Boso peninsula, Japan. *Food Bioscience*, **19**, 85–91.

US Food and Drug Administration. (2015). Microorganisms & microbial-derived ingredients used in food (Partial list). FDA (Aug. 21, 2013).

Vardaka, E., Kormas, K.A., Katsiapi, M., Genitsaris, S. & Moustaka-Gouni, M. (2016). Molecular diversity of bacteria in commercially available "*spirulina*" food supplements. *PeerJ*, **4**, e1610.

Verni, M., Dingeo, C., Rizzello, C.G. & Pontonio, E. (2021). Lactic acid bacteria fermentation and endopeptidase treatment improve the functional and nutritional features of *Arthrospira platensis*. *Frontiers in Microbiology*, **12**, 744437.

Wu, H.-C., Chen, H.-M. & Shiau, C.-Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International*, **36**, 949–957.

Yu, J., Ma, D., Qu, S. *et al*. (2020). Effects of different probiotic combinations on the components and bioactivity of *Spirulina*. *Journal of Basic Microbiology*, **60**, 543–557.

In this study, three different probiotic combination types were utilised to ferment spirulina and examine how the nutritional, functional, and flavour components changed along with the antioxidant and antibacterial properties. The fermentation of Spirulina was studied using three probiotic combination groups: lactic acid bacteria, Bacillus strains, and their mixtures. In comparison to unfermented Spirulina, the results showed that lactic acid bacteria considerably enhanced the amount of amino acids and the proportion of essential amino acids to total amino acids in the fermented Spirulina. Additionally, fermentation with the Bacillus strain increased the amount of flavonoids and polyphenols in the Spirulina compared to unfermented spirulina, and it also dramatically improved the capacity of the Spirulina to scavenge free radicals and act as an overall antioxidant.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. *Spirulina*-growing ellipsoid pools.

Figure S2. Inhibitory zone (mm) of NWS and NDS1 against *C. albicans* and *E. faecalis*.

© 2023 Institute of Food, Science and Technology (IFSTTF).

13652621, 0, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.16709 by Cukurova University, Wiley Online Library on [03/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License