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Chemical and physical characterization of microencapsulated *Spirulina* fermented with *Lactobacillus plantarum*

Gülsün Özyurt [a,*], Leyla Uslu [b], Mustafa Durmuş [a], Yetkin Sakarya [a], Türkan Uzlaşir [c], Esmeray Küley [a]

[a] Department of Seafood Processing Technology, Faculty of Fisheries, University of Cukurova, 01330 Adana, Turkey
[b] Department of Basic Science, Faculty of Fisheries, University of Cukurova, 01330 Adana, Turkey
[c] Department of Food Engineering, Faculty of Engineering, Adana Alparslan Turkes Science and Technology University, 01250 Adana, Turkey

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ABSTRACT

Spirulina fermented by lactic acid bacteria (LAB) stands out among innovative fermented products with bioactive properties. In addition, the fermentation of algae with LAB has a significant effect on protein, essential amino acid content, essential fatty acid, etc. Changes in nutritional components, colour and morphological appearances of unfermented and fermented *Spirulina* as a result of microencapsulation with different ratios of coating material were investigated. The fermentation of wet *Spirulina* was carried out with *Lactobacillus plantarum* FI 8595 at 37 °C for 72 h under anaerobic condition. Microencapsulation of unfermented (USp) and fermented *Spirulina* (FSp) was performed by spray drying using 170 °C air inlet temperature, 20 mL/min feed flow rate and different ratios of maltodextrin. After fermentation, there was an increase in dry matter and crude ash contents unlike the protein content. Aspartic acid, histidine and lysine concentrations were found to be significantly higher in FSp compared to USp. The main fatty acids in USp and FSp were palmitic (27.39–31.11 %), gamma linolenic (17.41–18.93 %), linoleic (8.11–10.45 %) and palmitoleic acids (4.36–5.05 %). A significant decrease was observed in the phycocyanin content of *Spirulina* after fermentation. However, the highest total organic acid content was observed in spray dried fermented *Spirulina* groups (9–22 g/100 g). Increasing maltodextrin (MD) concentrations caused a significant reduction in moisture content of USp and FSp and provided more effective drying. In addition, with the increase of MD addition, the essential/nonessential amino acid ratio raised from 0.83 to 1.26 and from 0.72 to 0.76 in USp and FSp, respectively. According to the SEM results, it was observed that the fermented *Spirulina* were coated more effectively with the coating material. In addition, fermentation and microencapsulation revealed positive effects on colour properties of *Spirulina*. Therefore, it can be concluded that microencapsulation of fermented *Spirulina* has significant potential for the production of new functional foods.

1. Introduction

Algae, which make up 90–95 % of the aquatic ecosystem, are used for many different purposes nowadays. Although it is known that microalgae species such as *Aphanizomenon*, *Spirulina platensis*, and *Nostoc* are consumed as human food for centuries, their cultivation dates back to the last few decades. *Spirulina platensis* is one of the most important cultivated microalgae species. The fact that *S. platensis* is a significant protein source among non-traditional food sources is due to its high protein content and various bioactive components. In addition to its high protein and bioactive components, *Spirulina* stands out with its gamma-linolenic acid, iron, B_{12} content and especially its pharmacological effects. Many researchers have stated that *Spirulina* had beneficial properties such as anticancer, antidiabetic, antibiotic, antioxidant, and immune system booster [1–3]. Therefore, it has gained more attention of consumers who want to have a healthy and balanced diet.

Fermented foods are another food group known to be beneficial to human health today. It is known that the nutraceutical profiles of food products are improved by fermentation with lactic acid bacteria. Therefore, attention for fermented foods has increased in recent years. Bioactive peptides can be released through hydrolysis by proteolytic starter cultures from parent proteins. When released, these peptides, which are inactive in the precursor protein sequences, can interact with

* Corresponding author at: Cukurova University, Fisheries Faculty, Department of Seafood Processing Technology, 01330, Balcalı, Adana, Turkey.
E-mail address: beklevik@cu.edu.tr (G. Özyurt).

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selected receptors and adjust the physiological functions of the body. Many researchers reported that these peptides are involved in varied biological functions such as immunomodulatory, antithrombotic, antihypertension, anticancer, antimicrobial, and antioxidant activities [4–7]. Fermented foods are available worldwide and are prepared from a wide variety of food sources. It is stated that 5000 kinds of fermented beverages and foods produced and consumed worldwide which provide 5–40 % of our daily food consumption [8]. Of these products, beverages, dairy products, fruit and vegetable products, cereal products and meat products can be counted among the main product groups, but the production of fermented products from algae is not common yet [9]. However, algae have great potential for producing fermented products as they are inexpensive sources of protein and rich sources of biologically active peptides [9,10]. Some researchers stated that fermentation of algae with yeast and lactic acid bacteria enriches protein, mineral, vitamin, essential fatty acid and essential amino acid content, increases nutritional values and improves digestibility of algae-based foods [10–12]. Ovando et al. [13] reported that the bioactive peptides obtained from *Spirulina* have antiallergic, antibacterial, antitumor, antihypertensive and immunomodulatory properties. Although recent studies have focused on the production and bioavailability of fermented *Spirulina* [14–18], they have not focused on changes that may occur during drying.

The biggest challenge of incorporating *Spirulina* into foods is its high moisture content and specific sensory properties. It is typically characterized by an unpleasant taste and aroma in addition to an undesirable green colour appearance [3,19]. One of the most suitable methods for the industrial use of *Spirulina* is spray drying, but due to the process used, losses in nutritional and bioactive components can be observed. Coating the core material with a carrier agent, in other words encapsulation, can mask the sensory properties of the product while preserving the bioactive properties of the product. Maltodextrin (MD) is the mostly preferred wall material in the industry due to its low cost, neutral flavour and aroma [20]. In addition, maltodextrin provides a strong barrier against oxidation of core materials and protects against external factors. It is clear that microencapsulation of fermented *Spirulina* with a carrier agent such as MD will provide industrial advantages and allow the production of new functional foods. Therefore, in this study, changes in nutritional components (proximate composition, amino acid composition, fatty acid composition, organic acid and phycocyanin content), colour and morphological appearances of unfermented and fermented *Spirulina* as a result of microencapsulation with different ratios of coating material were investigated.

2. Materials and methods

2.1. Production of S. platensis

Spirulina platensis cultivated in *Spirulina* medium [21] was grown in laboratory conditions at Algal Biotechnology Laboratory of Çukurova University. *Spirulina platensis* stock cultures were maintained in different sized flaks at 24 ± 2 °C, under continuous illumination with white fluorescence (Philips white, 36 watts). The light intensity was measured with the Radiation Sensor LI-COR (LI250, Nebraska, USA) and determined as 80 $\mu molm^{-2}\ s^{-1}$. The cultures in the flasks were increased in volume and started to be produced in 10 L jars. Then, the cultures were adapted to the external environment to inoculate the ellipsoid pools of 5 * 1 * 0.30 m in the pilot facility. At the end of the adaptation period, the culture study was started by inoculating the ponds with a capacity of 1.5 tons. Optical density was recorded daily to determine growth in cultures. Wet biomass was harvested when the cultures reached the stationary phase.

The culture at the harvest stage was filtered with 200, 100 and 40 μm porous cloths, respectively. The wet *Spirulina* biomass accumulated at 100 and 40 μm was condensed by washing with distilled water. The harvested wet biomass was stored at a temperature of +4 °C for the experiment.

2.2. Lactic acid bacteria strain (LAB)

Lactobacillus plantarum FI 8595 belonging to 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 *Lactobacillus* strain was activated in de Man, Rogosa and Sharpe broth (MRS, Merck 1.10661, Darmstadt, Germany) at 37 °C. Culture in concentration 2 McFarland (6.0×10^8 cfu/mL) was inoculated into *Spirulina* media.

2.3. Fermentations of S. platensis

Fermented *Spirulina* with *L. plantarum* was produced according to Choi et al. [15] and De Marco Castro et al. [16] with minor modifications. Cultivated *L. plantarum* (log 8 CFU/mL) was inoculated in harvested wet *Spirulina* with a dry weight of 10 % at a rate of 5 % (v/v). Then glucose (2 %, w/v) and potassium sorbate (0.2 % w/v) were added, followed by incubation at 37 °C for 72 h under anaerobic condition. UV treatment (20 min) was applied to stop the fermentation. Because a 72-h period was considered appropriate for the observation and evaluation of the main fermentation parameters [21,22]. The fermentation effects were also investigated by the pH changes; therefore, a digital pH meter (WTW 315i, Germany) was used for measuring of pH value of fermented *Spirulina*. Fermentations were carried out in triplicate.

2.4. Microencapsulation of unfermented and fermented Spirulina

Maltodextrin (DE: 18–20, Kimbiotek Chemical Company Ltd., Istanbul) was used for the microencapsulation of unfermented and fermented *Spirulina*. The total solids content of the emulsion was set at 30 % [23]. Before emulsification, maltodextrin (MD) was dissolved in water and kept at 4 °C for overnight. Then, the wall solution kept in benmari (50 °C) for 1 h. After cooling, wet unfermented or fermented *Spirulina* was added according to the dry matter ratios and homogenized at 14000 rpm for 10 min in ultra-turrax. Spirulina and coating material ratio (*Spirulina*:MD, w/w) were 1:0, 1:1 and 1:2. A laboratory-scale spray dryer (Buchi Mini Spray Dryer, B-290, Switzerland) was used to dry the emulsions. The feed flow rate of the emulsion was set to 20 mL/min and the aspiration rate was approximately 30 m^3/h. The inlet temperature was adjusted to 170 °C, and the outlet temperature was 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. All analyses were performed immediately after spray-dried *Spirulina* was produced, and each analysis was performed at least in triplicate. All chemicals were of analytical grade and provided from Merck (Darmstadt, Germany).

The prepared samples were coded as below:

USp, unfermented wet *Spirulina*; USp1:0, spray dried unfermented *Spirulina* without MD; USp1:1, spray dried unfermented *Spirulina* at a ratio of 1USp:1MD; USp1:2, spray dried unfermented *Spirulina* at a ratio of 1USp:2MD; FSp, fermented wet *Spirulina*; FSp1:0, spray dried fermented *Spirulina* without MD; FSp1:1, spray-dried fermented *Spirulina* at a ratio of 1FSp:1MD; FSp1:2, spray-dried fermented *Spirulina* at a ratio of 1FSp:2MD.

2.5. Proximate composition

To determine the moisture and crude ash contents of spray-dried *Spirulina*, they were kept at 103 °C and 550 °C, respectively, until the weight of the samples was stabilized. Lipid content was measured according to procedure of Bligh and Dyer [24] and crude protein was determined by Kjeldahl's procedure [25].

2.6. Amino acid composition

Amino acid compositions of spray dried *Spirulina* were determined by the centre laboratory of Food Institute of Marmara Research Centre, TUBITAK (Scientific and Technological Research Council of Turkey). The instrument used for the analysis was a Shimadzu 20 series ultrafast liquid chromatograph with UV detection. The method was carried out according to Dimova [26] and modified by TÜBİTAK MAM.

2.7. Fatty acid composition

The extracted oils of the powders and wet *Spirulina* were pipetted into a small tube and converted to their constituent FAME (fatty acid methyl esters). Sample (10 mg) was dissolved in n-heptane (2 mL) followed by 2 M methanolic KOH (4 mL). The mixture was centrifuged for 10 min at 4000 rpm and the separated n-heptane layer was used for GC analyses.

The analyses performed by an Agilent GC–MS/MS (7890B GC-7010B MS, Gerstel, Germany) with a flame ionization detector and a capillary Agilent J&W DB-WAX column (60 m × 0.25 μm × 0.25 μm). The oven temperature was set at 50 °C for 1 min, and raised to 200 °C at a rate of 25 °C/min. After 10 min, it was adjusted to 230 °C at a rate of 3 °C/min held this temperature was kept for 18 min. The injector and detector temperatures were set to 250 °C and 300 °C, respectively. Sample size and flow rate of carrier gas were 1 μL and 1 mL/min, respectively. The split ratio was 1:40. Fatty acids were identified using a comparison with the exact times of the FAME containing 37 standard components (Supelco 37, Bellefonte, PA, USA).

2.8. Organic acid composition

A 0.5 g sample was taken for the analysis of organic acids in powder samples, and 10 mL of phase A (0.1 % formic acid) was first kept in an ultrasonic water bath for 3.5 h at a temperature not exceeding 25 °C, and then was kept in a magnetic stirrer for 1 night and extracted. The extracts were obtained at the end of the period were centrifuged at 6500 rpm at 4 °C (Hettich Universal 320R, Germany) and the upper clear part was taken and passed through 0.45 μm filters. The obtained filtrates were injected into the Shimadzu Nexera X2 model PDA and HPLC with RID detector, and the organic acid amounts in the samples were detected at a wavelength of 210 nm. In the study, a Phenomenex Luna reversed-phase C-18 column (4.6 mm × 250 mm, 5 μm) and a 0.1 % formic acid solution were used as the carrier phase, and the flow rate of this phase was 0.5 mL/min with an isocritic system. The column furnace temperature was set at 55 °C. In order to determine the amounts of the compounds, five different concentrations of solutions were prepared for each standard substance, and calibration curves were created for each compound. The amounts of the compounds were then determined. For this purpose, calibration solutions at 5 different concentrations were prepared from oxalic, pyruvic, malic, and succinic acid standards.

2.9. C-Phycocyanin analysis

For C-phycocyanin analysis, 100 mL of NaNO3 (15 g $NaNO_3$/1 L) is added to 1 g of powder sample. It is kept in the shaker for 2 h. 5 mL of the samples are taken and centrifuged at 3500–5000 rpm for 5 min. The supernatant is analysed in the spectrophotometer at 620 nm. The amount of C-phycocyanin (μg/mL) is calculated by the following equation [27].

CPC=OD 620 nm × 137 = μg/mL (137, factor determined from absorption coefficient for *Spirulina*) [27].

2.10. Morphology of microencapsules

Morphological characterization of spray-dried unfermented/fermented *Spirulina* samples was investigated using SEM (scanning electron microscopy) at CUMERLAB (Çukurova University Central Research Laboratory, Adana, Turkey). Microencapsulated samples were gold coated to a thickness of 2 nm using a Q150R ES Coater (Quorum Technologies, UK). Coated samples were observed at 1000 and 2500 magnifications using SEM (Quanta FEG 650, USA) at 10 kV voltage.

2.11. Colour assessment of microencapsules

Colours of spray dried samples were measured using a chromameter (CR-400, Konica-Minolta, Osaka, Japan) as described by Hunter [28]. Calibration of the colorimeter prior to measurements was performed using a white standard plate. The L^* value (lightness) is measured between 0 (black) and 100 (white). The value "$+a^*$" indicates reddish and "$-a^*$" value indicates greenish colours. Positive b^* values are observed for yellowish colour and negative for bluish colours. Total colour differences (ΔE) values were calculated by following formula and used to describe the colour change after fermentation and drying [29].

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)2\right]^{0,5}$$

Whiteness index (WI) of samples were calculated from the L^*, a^* and b^* values. WI was calculated by the following formula [30].

$$\text{Whiteness Index (WI)} = 100 - \left[(100 - L)^2 + \left(a^2 + b^2\right)\right]^{0.5}$$

2.12. Statistical analysis

Data were subjected to analysis of variance (one-way ANOVA) at 5 % confidence level using Duncan's multiple range test and *t*-test.

3. Results and discussion

3.1. Proximate and amino acid composition

The pH values of *Spirulina*, which had an initial pH of 5.98 ± 0.01, were measured as 3.57 ± 0.07 at the 72nd hour of fermentation. The pH value obtained showed that *Spirulina* was fermented effectively with *L. plantarum* and the recommended pH values (pH below 4.5) for fermented products were reached. The proximate composition of wet unfermented (USp) and fermented *Spirulina* (FSp) and dried unfermented and fermented *Spirulina* with different ratios of MD (USP1:0, USp1:1, USp1:2, FSp1:0, FSp1:1 and FSp1:2) are given in Table 1. The moisture, crude ash, protein and lipid contents of wet *Spirulina* biomass before fermentation were found as 89.21 %, 0.57 %, 8.37 % and 1.62 %, respectively. The ratios of nutritional components of *Spirulina* used in

Table 1
Proximate compositions (%) of spray dried unfermented and fermented *Spirulina* groups.

Groups	Moisture	Crude ash	Protein	Lipid
USp	89.21 ± 0.12^{x}	0.57 ± 0.01^{x}	8.37 ± 0.04^{x}	1.62 ± 0.62^{x}
FSp	88.36 ± 0.02^{y}	0.74 ± 0.02^{y}	8.01 ± 0.01^{y}	2.22 ± 0.25^{x}
USp1:0	2.63 ± 0.12^{c}	6.61 ± 1.84^{b}	71.55 ± 0.01^{c}	11.62 ± 0.22^{c}
USp1:1	2.09 ± 0.25^{b}	3.24 ± 0.92^{a}	34.69 ± 0.03^{b}	5.67 ± 0.35^{b}
USp1:2	1.42 ± 0.21^{a}	2.60 ± 0.26^{a}	22.43 ± 0.02^{a}	3.55 ± 0.30^{a}
FSp1:0	6.37 ± 0.15^{C}	7.70 ± 0.02^{C}	62.94 ± 0.11^{C}	11.63 ± 0.13^{C}
FSp1:1	5.32 ± 0.16^{B}	3.71 ± 0.12^{B}	27.01 ± 0.03^{B}	4.25 ± 0.12^{B}
FSp1:2	3.37 ± 0.19^{A}	2.41 ± 0.05^{A}	16.66 ± 0.03^{A}	1.78 ± 0.21^{A}

USp, unfermented wet *Spirulina*; FSp, fermented wet *Spirulina*; USp1:0, spray dried unfermented *Spirulina* without MD; USp1:1, spray dried unfermented *Spirulina* at a ratio of 1USp:1MD; USp1:2, spray dried unfermented *Spirulina* at a ratio of 1USp:2MD; FSp1:0, spray dried fermented *Spirulina* without MD; FSp1:1, spray-dried fermented *Spirulina* at a ratio of 1FSp:1MD; FSp1:2, spray-dried fermented *Spirulina* at a ratio of 1FSp:2MD.
The values are expressed as mean ± standard deviation, n = 3, Values in a same column followed by different letters (x–y; a–c; A–C) indicate significant differences between groups ($p < 0.05$).

this study were in a similar range with those previously reported [31,32]. Slight differences can be observed due to the components of the culture medium (such as nitrogen, phosphate, and carbon sources), environmental conditions (pH, temperature, light intensity) and harvest time [33]. The fermentation process significantly affected the moisture, crude ash and protein content of *Spirulina* ($p < 0.05$). Moisture and protein content of *Spirulina* after fermentation decreased to 88.36 % and 8.01 %, respectively. However, there was a significant increase (0.74 %) in the ash content of fermented *Spirulina* with the effect of the acidic environment provided by LAB fermentation ($p < 0.05$). A decrease in moisture content after fermentation was also observed in the algae samples of *U. intestinalis*, *C. rupestris* and *F. lumbricalis*, their dry matter increased from 17.95 to 23.69, 34.42 to 36.04 and 32.57 to 33.65, respectively [34]. Although it was reported that there was an improvement in the protein content of fermented foods in general [35], some studies were also noted a decrease or unchanged protein content as in this study [36–38].

Spirulina's high nutritional content makes it a valuable human food. The main problem with using *Spirulina* as an ingredient for industrial foods is mainly related to its moisture content and distinctive taste. The processing of *Spirulina* for improve its functionality, and its dewatering allows storage, preserving and commercial use. For this purpose, fermented and unfermented *Spirulina* produced in this project were spray dried using MD as carrier agent. As seen in Table 1, the moisture content of unfermented and fermented *Spirulina* groups was successfully reduced by spray drying. Agglomeration, sticking and collapse of microparticles can be observed in powdered food products due to high water content [39,40]. Therefore, moisture content in spray-dried samples is the main factor affecting powder stability. In addition, powdered food products with a moisture content of <10 % were accepted as microbiologically safe [41]. Some researchers stated that the drying efficiency increased and the moisture content decreased with the increase in MD ratio in spray dried products [42–45]. Similarly, in this study, it was observed that there was a decrease in the moisture content of the powder obtained by adding MD at increasing rates to the emulsions prepared before spray drying. Moisture contents of USp and FSp groups were determined as 2.63 % and 6.37 %, respectively. It was observed that the addition of MD at a ratio of 1:1 (*Spirulina*:MD) significantly reduced the total moisture content in both the unfermented and fermented groups (2.09 % and 5.32 %, respectively), while the ratio of 1:2 was more effective (1.42 % and 3.37 %, respectively). Therefore, it can be said that the addition of MD was useful for effective spray drying. As expected, the highest protein, lipid and crude ash ratios were observed in the spray dried groups without carrier agent. A proportional change was observed in the protein, lipid and raw ash contents of dried groups depending on the *Spirulina* and MD ratios in the emulsion.

Table 2 shows the amino acid composition of all *Spirulina* groups. Alanine, leucine, arginine and glutamic acid were the major amino acids both unfermented (USp) and fermented (FSp) *Spirulina*. After fermentation there were decreases in the concentrations of serine, glycine, arginine, tyrosine, leucine and phenylalanine ($p < 0.05$). However, significant increases were observed in aspartic acid, histidine and lysine concentrations ($p < 0.05$). Uslu et al. [46] reported that glutamic acid, aspartic acid and leucine were the major amino acids for wet *Spirulina* biomass. Sahin et al. [47] reported that the main amino acids were glutamic acid, glycine, aspartic acid and alanine in unfermented and fermented *Spirulina* with different yeast species (*Debaryomyces hansenii* Y-7426; *Kluyveromyces marxianus* Y-329; *Saccharomyces cerevisiae* Y-369). It was found that the essential and non-essential amino acid concentrations decreased significantly in all fermented groups after fermentation. The same decrease was observed for *Spirulina* fermented with lactic acid bacteria strains by Kurt et al. [32]. Yu et al. [48] studied on the *Spirulina* fermentation with probiotic combinations (lactic acid bacteria, *Bacillus* strains and their mixture). It was stated that the best increase in amino acid concentration was observed in the mixture of lactic acid bacteria and *Bacillus* strains. An increase in the concentration

Table 2
Amino acid compositions (mg/100 g) of spray dried unfermented and fermented *Spirulina* groups.

Groups	Aspartic acid	Glutamic acid	Serine	Glycine	Histidine	Arginine	Threonine	Alanine	Proline	Tyrosine	Valine	Methionine	Isoleucine	Leucine	Phenyl-alanine	Lysine	E/NE
USp	171 ±2.82^{x}	599 ±33.23^{x}	190 ±2.12^{x}	526 ±5.66^{x}	5±0.00^{x}	677 ±10.6^{x}	138 ±6.36^{x}	831 ±4.95^{x}	535 ±26.87^{x}	502 ±4.24^{x}	602 ±56.57^{x}	22.5±2.12^{x}	510 ±0.71^{x}	806 ±4.95^{x}	468 ±3.53^{x}	437 ±2.12^{x}	0.84
FSp	227 ±6.36^{y}	694 ±0.71^{x}	144 ±6.37^{y}	487 ±9.19^{y}	25.5 ±0.71^{y}	615 ±11.31^{y}	122 ±2.12^{x}	739 ±3.53^{y}	476 ±4.24^{x}	341 ±12.02^{y}	521 ±12.02^{x}	15.5±2.12^{x}	506 ±9.19^{x}	784 ±4.94^{y}	430 ±7.07^{y}	485 ±13.43^{y}	0.84
USp1:0	4809 ±38.5^{c1}	9090 ±41.01^{c1}	3739 ±63.64^{c1}	3718 ±48.08^{c1}	1247 ±16.97^{c1}	4133 ±6.36^{c1}	3168 ±36.06^{c1}	5650 ±14.14^{c1}	3268 ±30.40^{c1}	3775 ±44.55^{c1}	4762 ±54.45^{c1}	450 ±16.26^{c1}	4308 ±2.12^{c1}	6192 ±32.53^{c1}	3682 ±20.51^{c1}	6993 ±47.38^{c1}	0.83
USp1:1	2345 ±32.52^{b}	4423 ±40.30^{b}	1311 ±11.31^{b}	1793 ±12.72^{b}	458 ±3.53^{b}	2262 ±1.41^{b}	1105 ±96.87^{b}	2801 ±29.70^{b}	1658 ±8.48^{b}	1856 ±35.35^{b}	2301 ±84.85^{b}	346±6.36^{a}	2067 ±14.85^{b}	3024 ±34.65^{b}	1739 ±28.28^{b}	3191 ±49.50^{b}	0.81
USp1:2	448 ±8.48^{a}	1044 ±15.55^{a}	453 ±19.09^{a}	1224 ±23.33^{a}	94 ±1.41^{a}	1367 ±22.62^{a}	537 ±18.38^{a}	2525 ±65.76^{a}	1574 ±29.69^{a}	1226 ±19.79^{a}	1962 ±32.53^{a}	411±3.53^{b}	1931 ±24.75^{a}	2733 ±45.96^{a}	1499 ±32.53^{a}	1899 ±59.40^{a}	1.26
FSp1:0	4928 ±58.69^{C1}	9112 ±164^{C1}	1727 ±94.04^{C2}	3405 ±45.25^{C2}	163 ±1.41^{C2}	4906 ±22.63^{C2}	1431 ±16.97^{C2}	5406 ±49.50^{C2}	3325 ±33.94^{C2}	3184 ±121^{C2}	4375 ±104^{C1}	330 ±19.09^{C2}	3860 ±78.49^{C2}	5800 ±31.82^{C2}	3058 ±7.78^{C2}	4834 ±27.57^{C2}	0.72
FSp1:1	1577 ±3.23^{B}	3759 ±61.52^{B}	592 ±45.25^{B}	1480 ±31.11^{B}	56.5 ±2.12^{B}	1876 ±8.48^{B}	461 ±1.41^{B}	2404 ±26.16^{b}	1217 ±10.61^{B}	1333 ±33.23^{B}	1752 ±19.09^{B}	294 ±20.50^{B}	1675 ±24.75^{B}	2532 ±27.58^{B}	1424 ±7.78^{B}	2354 ±33.94^{B}	0.81
FSp1:2	975 ±4.95^{A}	2458 ±12.02^{A}	267 ±14.14^{A}	961 ±10.61^{A}	24 ±1.41^{A}	1160 ±21.21^{A}	124 ±2.82^{A}	1547 ±86.97^{a}	833 ±35.35^{A}	779 ±2.83^{A}	1080 ±10.61^{A}	137±8.48^{A}	1092 ±2.83^{A}	1690 ±2.12^{A}	881 ±2.12^{A}	1252 ±18.38^{A}	0.76

USp, unfermented wet *Spirulina*; FSp, fermented wet *Spirulina*; USp1:0, spray dried unfermented *Spirulina* without MD; USp1:1, spray dried unfermented *Spirulina* at a ratio of 1USp:1MD; USp1:2, spray dried unfermented *Spirulina* at a ratio of 1USp:2MD; FSp1:0, spray dried fermented *Spirulina* without MD; FSp1:1, spray-dried fermented *Spirulina* at a ratio of 1FSp:1MD; FSp1:2, spray-dried fermented *Spirulina* at a ratio of 1FSp:2MD. The values are expressed as mean ± standard deviation, n = 2, Values in a same column followed by different letters (x–y; a–c; A–C) indicate significant differences between groups ($p < 0.05$). In addition, "1–2" show significant differences between USp1:0 and FSp1:0 in the same column ($p < 0.05$).

of aspartic acid and histidine, and a decrease in the content of serine and arginine were detected, as in the present study. Bao et al. [49] also reported that the main amino acids in unfermented and fermented *Spirulina* were glutamic acid, alanine, arginine and leucine, as found in this study. The researchers stated that there were significant decreases in glutamic acid and alanine content after fermentation. Similarly, in this study, a decrease was observed in alanine, while no significant change was observed in glutamic acid concentration ($p > 0.05$). After fermentation, the decrease in the concentrations of amino acids might be due to their greater assimilation by bacterial metabolism and differences can be seen depending on both the microorganism species and the fermentation time [49,50]. However, no change in the ratio of essential amino acids to non-essential amino acids (E/NE) was observed after fermentation.

Some researchers studied the addition of microencapsulated *Spirulina* to enrich the nutritional content of ice cream, chocolate milk, vegan biscuits, and yogurt and the biochemical compositions of the end products were investigated in these studies [19,51–53]. Faieta et al. [54], De Oliveria et al. [52], and Guarienti et al. [3] studied *Spirulina* microencapsulation by spray drying using different wall materials. These researchers evaluated the microcapsules in terms of proximate compositions, thermal stability and antioxidant properties. However, no data were found on the amino acid composition of unfermented or fermented microencapsulated *Spirulina*. When the unfermented and fermented *Spirulina* biomass were powdered in a spray dryer without using any carrier agents, the main amino acids in both were glutamic acid, lysine, leucine, alanine, aspartic acid, arginine, valine and isoleucine (Table 2). No significant differences were observed among the aspartic acid, glutamic acid and valine amino acids of the USp1:0 and FSp1:0 groups ($p > 0.05$). As it was observed in the wet biomass, differences were seen in the concentrations of all other amino acids (except for threonine, valine, methionine and isoleucine). Although the amino acid concentrations of threonine, valine, methionine and isoleucine were similar in both wet biomass (USp and FSp), lower concentrations were detected in the fermented group after spray drying (between USp1:0 and FSp1:0). It can be thought that these amino acids become more sensitive to heat after fermentation process. The E/NE ratios of USp1:0 and FSp1:0 also reflected this difference (0.83 and 0.72, respectively). However, it was observed that the E/NE ratios were similar (0.81 for both) in USP1:1 and FSP1:1 group dried with MD. In addition, with the increase of MD addition, the E/NE ratio increased from 0.83 to 1.26 and from 0.72 to 0.76 in the USp1:2 and FSp1:2 groups, respectively. Therefore, it can be considered that the addition of maltodextrin when spray-drying unfermented or fermented *Spirulina* can be particularly beneficial for the preservation of essential amino acids. Similarly, Faieta et al. [54] reported that microcapsules containing maltodextrin were more resistant to high temperature in preventing thermal degradation of *Spirulina*.

3.2. Fatty acid composition

The results of fatty acid compositions of the *Spirulina* groups investigated in this study are shown in Table 3. The main fatty acids in wet *Spirulina* biomass (both in unfermented and fermented group) were palmitic (27.39–31.11 %), gamma linolenic (17.41–18.93 %), linoleic (8.11–10.45 %) and palmitoleic (4.36–5.05 %) acids. Similarly, Işık et al. [55] stated that the essential fatty acids of *Spirulina platensis* were palmitic acid (28–37 %), gamma linolenic (11.4–22.2 %), linoleic (20–23 %) and oleic acid (2.6–8.0 %) and the ratios of these fatty acids changed according to the seasons. In this study, it was determined that there was no statistically significant change in the fatty acid composition after fermentation ($p > 0.05$). Although a slight increase was observed in palmitic, palmitoleic, linoleic and gamma linoleic fatty acids, no statistically significant difference was detected. Similarly, it was observed that there was no proportional change in the ratios of fatty acids of the spray dried *Spirulina* groups (except for palmitic and linoleic acids in the fermented ones). Therefore, it can be concluded that the sensitive fatty acids of *Spirulina* were not significantly affected by the applied spray drying inlet temperature. A slight increase was observed in gamma linolenic fatty acid (GLA) with increasing MD addition during spray drying process, but no statistical difference was detected ($p > 0.05$). It is stated that GLA is the main component of phospholipids in the mitochondria of cell membranes and therefore plays a positive role in the treatment of cardiovascular diseases [56]. In addition, it was stated that GLA has a strong immunoprotective effect and turns into prostaglandin, which has an important anti-inflammatory effect in the body [57]. Therefore, the preservation of this fatty acid is important in order to benefit from the pharmaceutical and nutraceutical properties of *Spirulina*.

Table 3
Fatty Acid composition of spray dried unfermented and fermented *Spirulina* groups (%).

	C16: 0 Palmitic	C16:1 Palmitoleic	C18:0 Stearic	C18:1 Oleic	C18:2ω6 Linoleic	C18:3ω6 Gamma Linolenic
USp	27.39 ± 2.93^{x}	4.36 ± 0.77^{x}	1.85 ± 0.330^{x}	1.83 ± 1.61^{x}	8.11 ± 1.27^{x}	17.41 ± 0.50^{x}
FSp	31.11 ± 1.99^{x}	5.05 ± 0.09^{x}	1.64 ± 0.21^{x}	0.73 ± 0.00^{x}	10.45 ± 0.25^{x}	18.93 ± 0.09^{x}
USp1:0	35.73 ± 0.30^{a}	5.80 ± 0.71^{a}	0.75 ± 0.05^{a}	1.01 ± 0.09^{a}	15.44 ± 0.21^{b}	25.99 ± 0.54^{a}
USp1:1	36.09 ± 0.21^{a}	5.95 ± 0.14^{a}	0.82 ± 0.11^{a}	0.94 ± 0.05^{a}	15.09 ± 0.70^{b}	26.52 ± 0.15^{a}
USp1:2	35.71 ± 0.00^{a}	5.95 ± 0.06^{a}	0.91 ± 0.21^{a}	0.98 ± 0.07^{a}	14.52 ± 0.09^{a}	26.47 ± 0.47^{a}
FSp1:0	38.70 ± 0.77^{B}	6.09 ± 0.02^{A}	1.07 ± 0.22^{A}	0.91 ± 0.07^{A}	13.56 ± 0.21^{B}	23.17 ± 0.11^{A}
FSp1:1	37.17 ± 0.37^{AB}	6.09 ± 0.11^{A}	1.25 ± 0.07^{A}	0.85 ± 0.04^{A}	12.89 ± 0.11^{A}	23.31 ± 0.52^{A}
FSp1:2	36.74 ± 0.37^{A}	6.32 ± 0.110^{A}	1.25 ± 0.07^{A}	0.89 ± 0.04^{A}	12.51 ± 0.11^{A}	24.36 ± 0.51^{A}

USp, unfermented wet *Spirulina*; FSp, fermented wet *Spirulina*; USp1:0, spray dried unfermented *Spirulina* without MD; USp1:1, spray dried unfermented *Spirulina* at a ratio of 1USp:1MD; USp1:2, spray dried unfermented *Spirulina* at a ratio of 1USp:2MD; FSp1:0, spray dried fermented *Spirulina* without MD; FSp1:1, spray-dried fermented *Spirulina* at a ratio of 1FSp:1MD; FSp1:2, spray-dried fermented *Spirulina* at a ratio of 1FSp:2MD.
The values are expressed as mean ± standard deviation, n = 3, Values in a same column followed by different letters (x–y; a–c; A–C) indicate significant differences between groups ($p < 0.05$).

3.3. Organic acid composition

Organic acids are defined as organic compounds with acidic properties. They are also called as carboxylic acids due to the presence of a carboxyl (COOH) group in their structure. Organic acids in fermented products are known to act as markers of total metabolic events and act as natural preservatives, also they are effective on sensory properties [58]. Therefore, determination of organic acid content is considered important in terms of stability, quality and nutritional characterization of the end product. Organic acid composition and amounts of unfermented and fermented *Spirulina* samples are given in Table 4, and organic acid chromatograms are given in Fig. 1. As seen in Table 1, it was determined that the most dominant organic acid in all samples was succinic acid, with amounts ranging from 0.22 to 21.69 g/100 g. Malic acid was found as the second main organic acid in spray dried *Spirulina* samples (0.31 g/100 g for USp1,0 and 0.21 g/100 g for FSp1:0). Vendruscolo et al. [59] detected succinic and malic acids as organic acids in their study on two

Table 4
Organic acid (g/100 g) and C-phycocyanin (%) amounts of spray dried unfermented and fermented *Spirulina*.

	Oxalic acid	Pyruvic acid	Malic acid	Succinic acid	Total	C-phycocyanin
USp	0.03 ± 0.00^{x}	0.01 ± 0.00^{x}	nd	0.22 ± 0.01^{x}	0.26 ± 0.01^{x}	3.580 ± 0.00^{x}
FSp	0.03 ± 0.00^{x}	0.01 ± 0.00^{x}	nd	2.42 ± 0.06^{y}	2.46 ± 0.06^{y}	0.210 ± 0.00 ^{y}
USp1:0	0.13 ± 0.00^{c}	0.06 ± 0.00^{b}	0.31 ± 0.01^{c}	1.26 ± 0.09^{c}	1.76 ± 0.09^{c}	3.473 ± 0.00^{c}
USp1:1	0.09 ± 0.00^{b}	0.02 ± 0.00^{a}	0.18 ± 0.00^{b}	0.73 ± 0.04^{b}	1.02 ± 0.04^{b}	3.446 ± 0.00^{b}
USp1:2	0.07 ± 0.00^{a}	0.02 ± 0.00^{a}	0.14 ± 0.01^{a}	0.45 ± 0.01^{a}	0.68 ± 0.01^{a}	3.453 ± 0.00^{a}
FSp1:0	0.17 ± 0.00^{B}	0.09 ± 0.00^{B}	0.21 ± 0.05^{B}	21.69 ± 0.01^{C}	22.16 ± 0,05^{C}	0.196 ± 0.00^{C}
FSp1:1	0.09 ± 0.01^{A}	0.04 ± 0.01^{A}	0.20 ± 0.00^{B}	16.35 ± 0.04^{B}	16.68 ± 0.04^{B}	0.166 ± 0.00^{B}
FSp1:2	0.09 ± 0.01^{A}	0.03 ± 0.01^{A}	0.07 ± 0.00^{A}	8.83 ± 0.06^{A}	9.02 ± 0.03^{A}	0.123 ± 0.00^{A}

USp, unfermented wet *Spirulina*; FSp, fermented wet *Spirulina*; USp1:0, spray dried unfermented *Spirulina* without MD; USp1:1, spray dried unfermented *Spirulina* at a ratio of 1USp:1MD; USp1:2, spray dried unfermented *Spirulina* at a ratio of 1USp:2MD; FSp1:0, spray dried fermented *Spirulina* without MD; FSp1:1, spray-dried fermented *Spirulina* at a ratio of 1FSp:1MD; FSp1:2, spray-dried fermented *Spirulina* at a ratio of 1FSp:2MD.
The values are expressed as mean ± standard deviation, n = 3, Values in a same column followed by different letters (x–y; a–c; A–C) indicate significant differences between groups ($p < 0.05$).

different microalgae species. Succinic acid is recognized as an important building block chemical [60]. Malic acid is a dicarboxylic acid and an important intermediate in cell metabolism. Besides being the second most important acid in the food industry, it is also used in the pharmaceutical industry and medicine [61]. It is known that microalgae and macroalgae, which are rich in carbon and nitrogen content, can produce succinic and malic acid by using simple sugars such as glucose and fructose [59].

Oxalic acid and its salts are organic compounds found as end products of metabolism in plant tissues such as vegetables, seeds and leafy greens. They bind minerals and have been associated with calculus and other health problems [62]. Rusmana et al. [63] found 20–231 ppm of oxalic acid in bekasam, a fish product prepared by lactic acid bacterial fermentation. In this study, the highest oxalic acid content (0.17 g/100 g) was found in the spray dried fermented *Spirulina* group without MD addition (FSp1:0). Pyruvic acid is the simplest monocarboxylic acid found in all living cells. It has an important function as an intermediate product in the breakdown of carbohydrates in higher plants [64]. Gao et al. [65] reported that they determined the pyruvic acid content of 0.020–0.025 g/100 mL in fish sauces produced from Nile tilapia by lactic acid fermentation and that pyruvic acid could be catabolized to minor metabolites. In this study, pyruvic acid was found as 0.01 g/100 g in wet biomass and proportionally higher concentrations (0.02–0.09 g/100 g) in the spray-dried groups. Among the research groups, the highest total organic acid content was observed in spray dried fermented *Spirulina* groups (9–22 g/100 g). Since these organic acids are aroma compounds or their precursors [66], it can be thought that they also contribute to the taste and odour changes of fermented *Spirulina*.

3.4. C-Phycocyanin content

Phycocyanin is a water-soluble pigment-protein complex known to have antioxidant and strong fluorescent properties found in cyanobacteria [67]. Many researchers have suggested that phycocyanin has immune-enhancing, cholesterol-lowering, anti-inflammatory, antiviral and anticancer properties [16,68,69]. However, since cyanobacteria contain many biologically active compounds, it is not entirely possible to attribute the health effects of their consumption to the phycocyanin content alone [67,70]. In this study, c-phycocyanin content was found as 3.58 % in unfermented *Spirulina*, while it was found as 0.21 % in fermented *Spirulina* (Table 4). This remarkable decrease observed after fermentation ($p < 0.05$) was also reported by other researchers [16,22]. De Marco Castro et al. [16] and Niccolai et al. [22] reported that phycocyanin content was significantly reduced after 72 h of lactic acid bacterial fermentation in *Spirulina*. Niccolai et al. [22] suggested that lactic acid bacteria in the medium may have consumed phycocyanin to meet their nitrogen nutrient needs. Liu et al. [14] emphasized that phycocyanin can be converted to phycocyanobilin by proteolysis of lactic acid bacteria.

The c-phycocyanin content in the USp1:0, USP1:1 and USp1:2 groups were 3.473 %, 3.446 %, and 3.453 %, respectively. Within the fermented groups, the phycocyanin content was found as 0.196 %, 0.166 % and 0.123 % in the FSp1:0, FSp1:1 and FSp1:2 groups, respectively. According to these data obtained, although statistical differences were observed between spray dried groups in phycocyanin content, it could be said that these groups generally reflected the contents of wet biomass. Therefore, it could be considered that the spray drying core temperature of powders was lower than the inlet temperature and exposure to this temperature for short periods was effective on the preservation of the

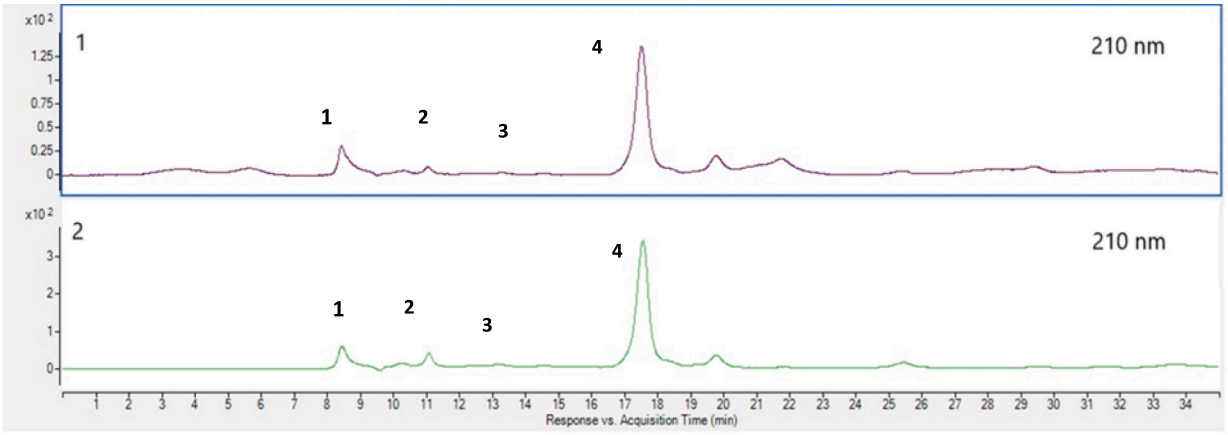


Fig. 1. Organic acid chromatogram (1: Oxalic acid, 2: Pyruvic acid, 3:Malic acid, 4: Succinic acid).

phycocyanin content. Similarly, it was reported that purified phycocyanin could be successfully dried in a spray dryer at an inlet temperature of 110 °C [71]. Jespersen et al. [72] reported that the denaturation temperature of pure phycocyanin was 67 °C according to Differential Scanning Calorimetry results. Fang & Bhandari [73] stated that in spray drying, because the drying time is short (usually 15–30 s), and evaporation occurs on the particle surface, the material remains relatively cold until it becomes dry. The researchers emphasized that in most food ingredients and nutraceuticals, the applied inlet temperature is generally between 150 and 200 °C, and the dry product is exposed to moderate temperatures (50–80 °C) lower than the outlet air. In conclusion, it could be said that fermentation caused a more effective decrease in phycocyanin content than spray drying.

3.5. Morphology of microcapsules

The morphologies of the sampled *Spirulina* groups were evaluated using scanning electron microscopy (SEM) images. Fig. 2 (a–b) shows SEM images of unfermented and fermented *Spirulina* microencapsulated by spray drying with different ratios of maltodextrin. Faieta et al. (2020) dried *Spirulina* (0.5 % protein concentration on dry basis) with 20 % encapsulant agents (trehalose dehydrate and maltodextrin) by spray drying technique and they found spherical microcapsules because of the amount of coating agent. De Oliveira et al. [52] dried maltodextrin, soy lecithin and *Spirulina* in a spray dryer at a ratio of 5:1:4 m/m, respectively. They reported that the spherical form of microencapsulated *Spirulina* was obtained at this ratio of the coating materials, according to SEM images. Da Silva et al. [19] stated that *Spirulina* dried without coating material had a spiral and partially fragmented structure, while those dried with the addition of MD and citric acid had a more spherical

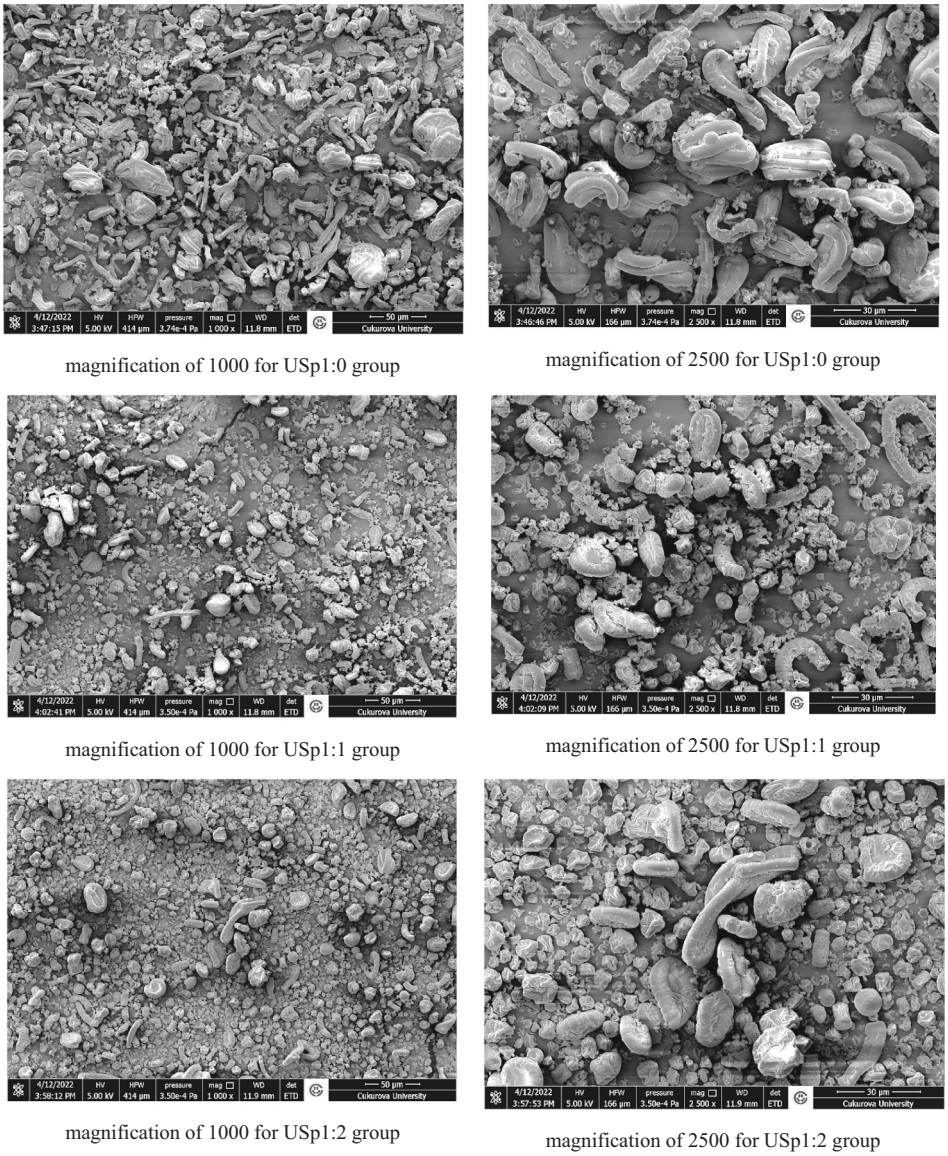
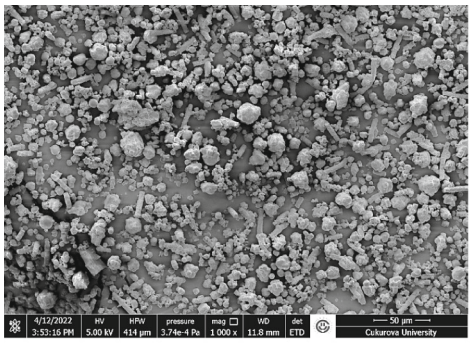
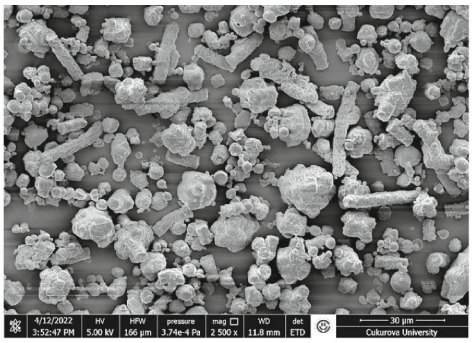


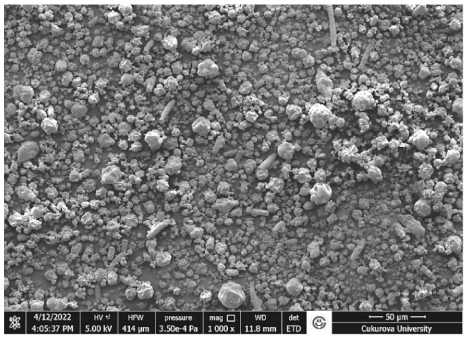
Fig. 2. Scanning electron microscopy (SEM) images of the spray dried unfermented and fermented *Spirulina*.
USp1:0, spray dried unfermented *Spirulina* without MD; USp1:1, spray dried unfermented *Spirulina* at a ratio of 1USp:1MD; USp1:2, spray dried unfermented *Spirulina* at a ratio of 1USp:2MD; FSp1:0, spray dried fermented *Spirulina* without MD; FSp1:1, spray-dried fermented *Spirulina* at a ratio of 1FSp:1MD; FSp1:2, spray-dried fermented *Spirulina* at a ratio of 1FSp:2MD.



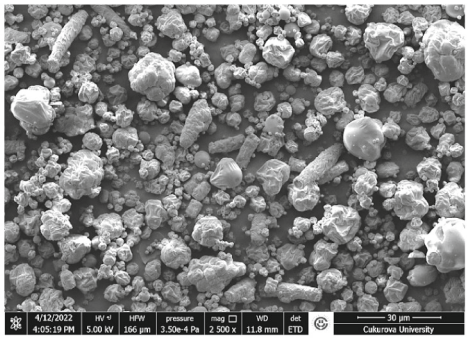
magnification of 1000 for FSp1:0 group



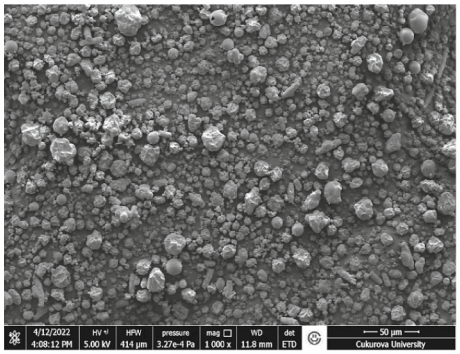
magnification of 2500 for FSp1:0 group



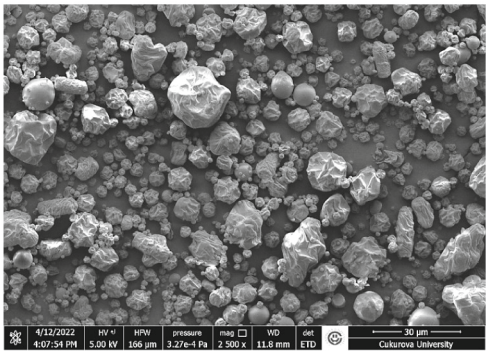
magnification of 1000 for FSp1:1 group



magnification of 2500 for FSp1:1 group



magnification of 1000 for FSp1:2 group



magnification of 2500 for FSp1:2 group

b) SEM images of spray dried fermented *Spirulina*

Fig. 2. (*continued*).

structure. Similarly, in this study, it was observed that when unfermented *Spirulina* was dried without the addition of maltodextrin, it retained its spiral structure (Fig. 2a). However, it was observed that even when fermented *Spirulina* was dried without the addition of MD, showed more spherical shape than unfermented ones. This is thought to be due to lactic acid bacteria that break down the *Spirulina* structure during the fermentation process.

More spherical shaped dried *Spirulina* were obtained as the MD addition rate increased in both groups. However, it can be clearly seen that when *Spirulina* was degraded by the effect of fermentation, it could be coated more effectively with MD (Fig. 2b). Microcapsules should be spherical without cracks or pores for protect the bioactive component in its core from external factors such as oxygen and its release to the surface. Therefore, the most effective microsphere formation in the study was found in fermented *Spirulina* dried with the addition of MD at 1:2 (*Spirulina*: MD) ratio.

3.6. Colour assessment of microencapsules

Colour is one of the most important quality attributes for the food industry, as it has a significant impact on consumer preferences and pleasure. If the colour of the food ingredients is not dominant, it is more compatible with the food which it will be added into. In this study, colour evaluations of unfermented and fermented *Spirulina* were measured with a colorimeter and are presented in Fig. 3. *Spirulina* is characteristically identified by its green - blue colour, which makes it difficult to use as a food ingredient. It was determined that the L^* a^* and b^* colour values of fermented *Spirulina* were quite different from those were dried without fermentation. The L^* value indicates a light dark spectrum ranging from black (0) to white (100). It was determined that L^* value increased from 35.04 to 39.15 in fermented *Spirulina* (FSp1:0). a^* value ($+a^*$ represents red, $-a^*$ represents green) increased from −2.89 to 0.57, the b^* value ($+b^*$ represents yellow, $-b^*$ represents blue colour) increased from 3.32 to 6.06. Accordingly, it can be concluded that there was a decrease in the red and blue colour of fermented

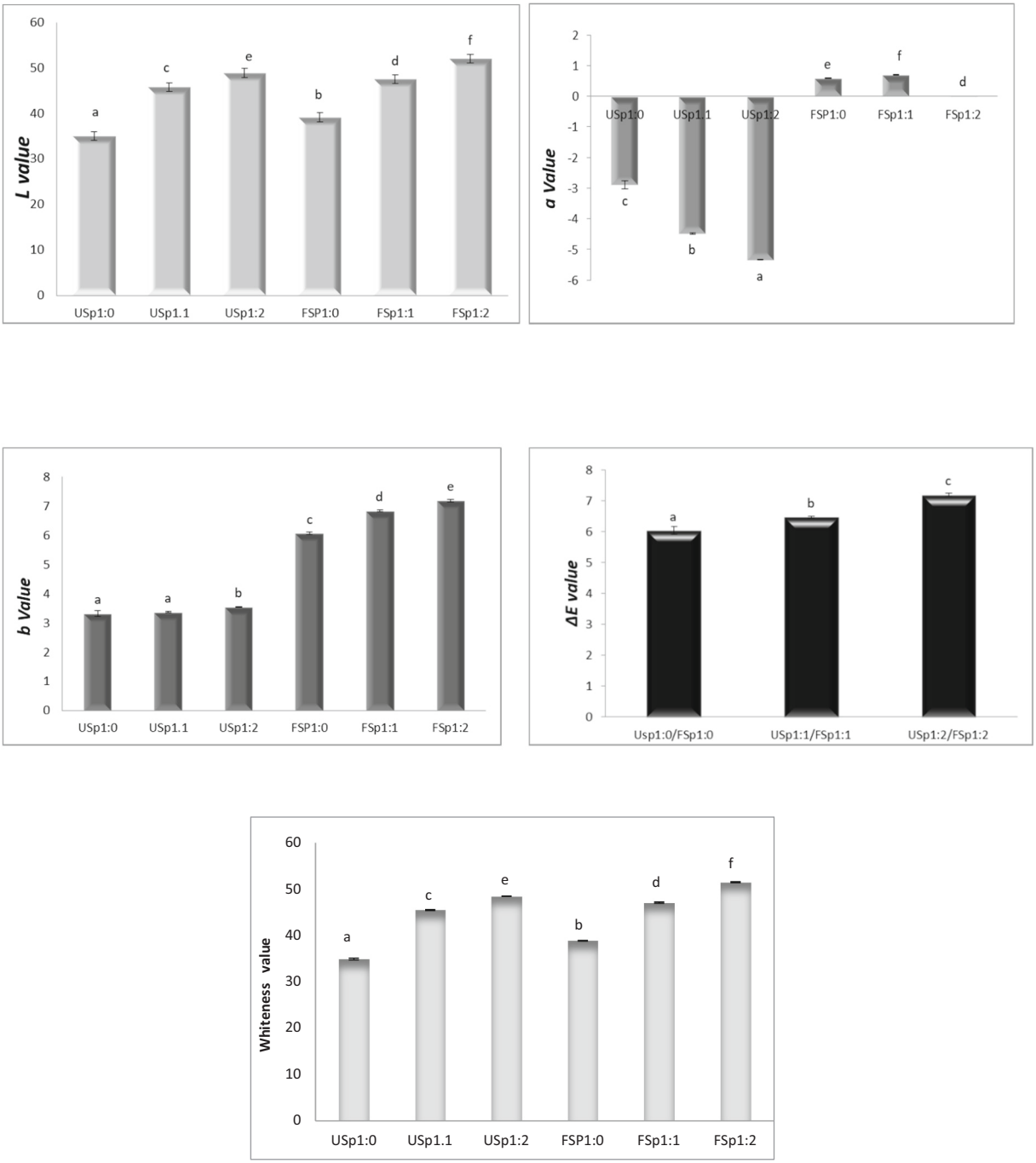


Fig. 3. Colour evaluations (L^*, a^*, b^*, ΔE and Whiteness values) of spray dried unfermented and fermented *Spirulina*. Results are means of six replicates (error bars represent standard deviation). Bars with the different letters (a–f) indicate significant differences ($P < 0.05$).
USp1:0, spray dried unfermented *Spirulina* without MD; USp1:1, spray dried unfermented *Spirulina* at a ratio of 1USp:1MD; USp1:2, spray dried unfermented *Spirulina* at a ratio of 1USp:2MD; FSp1:0, spray dried fermented *Spirulina* without MD; FSp1:1, spray-dried fermented *Spirulina* at a ratio of 1FSp:1MD; FSp1:2, spray-dried fermented *Spirulina* at a ratio of 1FSp:2MD.

Spirulina. Similarly, Martelli et al. [74] stated that the colour of *Spirulina*, which they applied thermal sterilization and fermentation, changed from green-blue to dark green brownish. It can be thought that the reason for this change may be the degradation of pigments such as phycocyanin, xanthophylls, carotenoids and chlorophyll in the structure of *Spirulina* by fermentation and heat application. When the research groups were compared in terms of total colour difference (ΔE*), the results were found to be around 6–7 (Fig. 3). Mahy et al. [75] emphasized that if the colour differences is >2.3, it would be visible. However, they also stated that if the colour difference is >10, it will not be worthwhile to compare the colour because it will be totally different from the original.

Whiteness values calculated from the L^*, a^* and b^* of USp:0, USp1:1, USP1:2, FSp:0, FSp1: 1 and FSp1:2 groups were found to be 34.89, 45.49, 48.47, 38.85, 47.08 and 51.49, respectively. The highest whiteness values were observed with increasing concentrations of maltodextrin addition (Fig. 3). Therefore, it can be concluded that among all groups, the most compatible colour characteristics for adding to other foods were in the FSp1:2 group with a whiteness value of 51.49.

4. Conclusion

After fermentation, the dry matter, crude ash, total organic acid content, and lysine essential amino acid increase in *Spirulina* were the advantages of fermentation, although no improvement was observed in terms of other nutritional components. The most important disadvantage was that fermentation decreased the phycocyanin content of *Spirulina*. It should be remembered, however, that not all of the health benefit effects of *Spirulina* are due to its phycocyanin alone. Moreover, the fermented *Spirulina* were coated more effectively with the wall material thanks to the change in their morphological structures. The use of maltodextrin in unfermented and fermented *Spirulina* provided more effective drying and also showed positive effects on colour. In addition, it was recorded that increasing rates of maltodextrin during spray drying provides the preservation of essential amino acids and gamma linolenic fatty acid. Therefore, it can be concluded that microencapsulation of unfermented or fermented *Spirulina* with maltodextrin has the potential to provide great advantages for incorporation into other foods.

Compliance with ethics requirements

This study does not contain any studies with human participants or animals performed by any of the authors.

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CRediT authorship contribution statement

GÖ: Conceptualization, Project administration, Investigation, Methodology, Writing–original draft. LU: Investigation, Methodology, Formal analysis. MD: Investigation, Methodology, Formal analysis. YS: Formal analysis, Writing–original draft. TU: Formal analysis, Writing–original draft. EK: Investigation, Methodology, Writing-review, editing. All authors read and approved the final manuscript.

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.

Data availability

Data will be made available on request.

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