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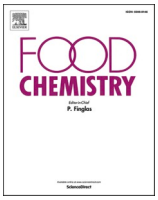



Impact of different salt concentrations on growth, biochemical composition and nutrition quality of *Phaeodactylum tricornutum* and *Spirulina platensis*

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ABSTRACT

The effects of different salt concentrations on the growth, aroma and quality parameters of *P. tricornutum* and *S. platensis* were investigated for the first time.Salt contents were 15, 25, 30 and 35‰ for the *P. tricornutum* and 20, 25, 30 and 35‰ for the *S. platensis* culture. Aroma compounds were analyzed by HS-SPME-GC/MS while the organic acid and sugar composition were studied by HPLC-PDA-RID.The main amino acids were glutamic acid (6.24–12.96 mg/100 g) and alanine (3.67–13.65 mg/100 g) while the prevailing sugars were sucrose (229–1099 mg/100 g) and glucose (192–2322 mg/100 g) in the *P. tricornutum* and *S. platensis* samples.The most prevailing aroma compound was acetic acid (113 μg/kg) in the *P. tricornutum* samples and heptadecane (36941 μg/kg) in the *S. platensis* samples.A significant reduction was found in the amount of aroma compounds, amino acids, sugars and organic acids with increasing salt concentration.The study results are valuable for producing high-quality and flavorful *P. tricornutum* and *S. platensis* products.

1. Introduction

The increase in global population and changing consumer habits have instigated researchers to develop new edible and alternative food and protein sources. The need for protein is mainly met from animal-based sources but interest in alternate protein sources has gained importance recently due to the increase in obesity rate, diseases and antibiotics applied to farm animals (De Jesus et al., 2018; Saranraj & Sivasakthi, 2014). Algae, which can be consumed as an alternative food source, are single-celled and filamentous photosynthetic creatures with cellulosic cell walls and can live both in fresh waters and seas. They have been used as food sources by Mexicans since the Aztec civilization period and the local people living around Lake Chad in central Africa (Gabr et al., 2020). *Spirulina platensis* (*S. platensis*) and *Phaeodactylum tricornutum* (*P. tricornutum*) have been among the alternative sources with their high protein content (30–70%) (Al-Dhabi & Valan Arasu, 2016; Seghiri, Kharbach & Essamri, 2019).

Microalgae are able to produce numerous significant chemical and biological compounds. *P. tricornutum* and *S. platensis* are considered as alternative food sources due to their protein, carbohydrate, vitamin, carotenoid, phycocyanin and fucoxanthin contents, which provide antioxidant, antiobesity and anti-inflammatory effects.

P. tricornutum is a single-celled eukaryotic diatom species belonging to the Pennateae group that can live in the marine conditions although it is a freshwater species. It contains 37% protein, 30% carbohydrates, 20% fat, 6% moisture and 7% minerals on a dry weight basis (Chen et al., 2014). It has color pigments such as chlorophyll *a*, chlorophyll *c* and fucoxanthin. Among these, fucoxanthin is orange in color and is an important pigment found in brown marine algae and diatoms and has been reported to provide anti-inflammatory, anticancer, antiobesity, antioxidant and antidiabetic activities and it is used in numerous applications in the food, pharmaceutical, cosmetic and biofuel industries (Sudhakar et al., 2013; Xia et al., 2013).

S. platensis is a filamentous prokaryotic blue-green alga that attracts great attention due to its high protein, macro and micronutrient contents (70% protein, 15% carbohydrate, 5% fat and 7% minerals). In addition, it contains the pigments of phycocyanin (blue color), chlorophyll (green color) and carotenoid (orange color) (Saranraj & Sivasakthi 2014; Wu et al., 2016). Phycocyanin is a hydrophilic blue pigment found in blue-green algae and has various pharmacological activities such as antioxidant, anticancer and anti-inflammatory effects (Gabr et al., 2020; Ismaiel et al., 2016; Wu et al., 2016). *S. platensis* is consumed as a food supplement promoted as a 'super food' and sold as capsules, flakes or dried powder after dehydrating by spray drying, freeze drying, sun

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drying and hot air drying (Seghiri, Kharbach, & Essamri, 2019).

In recent years, interest in aroma compounds from microalgae has increased due to their various structural properties and interesting biological activities. It has been stated that microalgae produce various aroma compounds such as hydrocarbons, unsaturated aldehydes, dimethyl sulfide and organohalogens (Prestegard et al., 2015; Steinke et al., 2002).

Some physicochemical factors such as temperature, light, salt and nutrient contents of the cultivation medium affect the growth and biochemical composition of the microalgae. Among these, salt is a crucial factor causing stress damage on the growth and metabolism of plants and microalgae altering their metabolic pathways and resulting in an increase or decrease in biologically active compounds (Emad et al., 2010).

This study was conducted to determine how the cell density and pigments of the two most commonly–grown commercially important algae species (*P. tricornutum* that has cell walls and *S. platensis* that lacks cell walls) change during cultivation with different growth medium salt concentrations. Additionally, our objective was to investigate the alterations in their important quality parameters and aroma compounds after harvesting. There has been no study in the literature on the influence of salt contents of the growth medium on the aroma compounds, organic acids, general quality parameters and the growth of *P. tricornutum* and *S. platensis*. In this work, the aim was to comprehensively investigate the effects of different salt concentrations on the growth and important quality parameters of *P. tricornutum* and *S. platensis,* including amino acids, aroma compounds, organic acids and color compositions. The aroma compounds were analyzed by gas chromatography–mass spectrometry (GC–MS) while the amino acids, organic acids and sugar compositions were examined by high-performance liquid chromatography with photodiode array (HPLC-PDA) and refractive index detector (HPLC-RID). The changes in phycocyanin and fucoxanthin pigments were determined by spectrophotometric methods in the study.

2. Materials and methods

2.1. Chemicals

The chemical standards used in the culture medium of the *P. tricornutum* and *S. platensis* were methanol (HPLC grade), ethanol (HPLC grade), sodium hydroxide, acetonitrile (HPLC grade) and amino acid which were obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA). Sucrose, glucose, fructose, acetone and formic acid (HPLC grade) were procured from Merck Company (Darmstadt, Germany). Ultrapure water was used in the preparation of the mobile phases used in HPLC. The standards were prepared daily.

2.2. Cultures of P. tricornutum and S. platensis

P. tricornutum and *S. platensis* cultures were produced under controlled laboratory conditions in the Algal Biotechnology Laboratory of the Faculty of Fisheries of Cukurova University located in Adana, Türkiye. The cultures were conditioned at room temperature at 20 and 25 °C, respectively and grown at a light intensity of 80 μmol photon m^{-2} s^{-1} under laboratory conditions. Continuous illumination was applied and the light intensity was checked by a light meter (Licor, LI-250). Fluorescent lamps (Tekfen, TLD, 36 Watt) were utilized and the cultures placed on the shelves were ventilated by an aquarium air pump (3.5 L/min, 5 Watt). The trial culture groups were kept in six-liter flasks.

Zarrouk medium (Aiba & Ogawa, 1977) and Si-Walne medium (Walne, 1970) were modified and used in the production of the *P. tricornutum* and *S. platensis* cultures, respectively. Sea water was utilized for *P. tricornutum* and pure water was used for *S. platensis*. Optical density measurements were carried out daily while dry matter, chlorophyll *a* and total carotene analyzes were performed every three days from the start of the experiment. In order to determine the most appropriate harvest time, growth curves were calculated based on the optical density measurements in daily samples and harvesting was carried out when the growth entered the stationary phase (18th day for *P. tricornutum*, 30th day for *S. platensis*). The wet biomass was put into 250 mL ground neck flasks and dried with a lab-scale freeze dryer (Teknosem, TRS 4/4V, Istanbul). A vacuum pressure of 0.037 mbar and a temperature of −56 °C was applied during freeze drying without damaging the molecular and physical structure of the sample for about 60 h in reference to the user's guide of the dryer. The final water content of the sample was about 8% (Keskin et al., 2021).

2.3. Applied salt concentrations

Salt concentrations were 15–35‰ for the *P. tricornutum* and 20–35‰ for the *S. platensis* cultures, respectively. These values were determined based on the minimum and maximum salt concentrations at which both species can grow. The optimal salt concentrations are usually accepted as 30‰ and 20‰ for the *P. tricornutum* and *S. platensis* cultures, respectively and these values were used for the control groups in the study. Four culture groups were prepared for each of the two species. Acronyms for the culture samples with different salt concentrations were P15 (15‰), P25 (25‰), P30-C (30‰ control) and P35 (35‰) for the *P. tricornutum* cultures and S20-C (20‰ control), S25 (25‰), S30 (30‰) and S35 (35‰) for the *S. platensis* cultures.

2.4. Analyses conducted during the growth of the cultures

2.4.1. Cell density measurement (Optical density) and dry matter analysis

The optical density (OD_{680}) was measured at 680 nm using a UV–vis spectrophotometer (BMG LABTECH, SPECTROstar Nano, Ortenberg, Germany) by transferring 4 mL of samples from each trial group into a quartz spectrophotometer cuvette (Ismaiel et al., 2016). The amount of dry matter was determined according to the method developed by AOAC (2007) with 10 mL of microalgae culture through glass fiber filter (Whatman GF/C, 1.2 μm, UK).

2.4.2. Chlorophyll a and total carotene analysis

The amounts of chlorophyll *a* and total carotene were determined every three days in reference to the method of Parsons and Strickland (1963). For chlorophyll *a* analysis, a 5 mL sample was filtered through GFC filter paper, which was then placed in 15 mL test tubes. Next, 10 mL of 90% acetone/water solution was added to the test tubes, which were shaken and stored in the dark at a temperature of +4 °C in a refrigerator for 24 h. Following the extraction period, the clear upper portion was collected and the absorption values at 630, 645, 665, and 750 nm were measured using a UV–Vis spectrophotometer (Carry 60; Agilent, Malaysia). The absorption values obtained at 750 nm were used as the baseline correction factor. To determine the total carotene values, the absorption values at 480 nm were measured using a UV–Vis spectrophotometer (Carry 60; Agilent, Malaysia).

2.4.3. Phycocyanin and fucoxanthin analysis

Phycocyanin analysis: The culture was harvested and dried at 55 °C to obtain biomass. For the analysis, one gram of powdered *S. platensis* was weighed and transferred into a glass beaker and then 100 mL of 1.5% $NaNO_3$ solution was added. The mixture was placed in a mixer and kept for 2 h. Then, 5 mL of the samples were taken and transferred into glass tubes followed by centrifugation at 5000 rpm for 5 min. The resulting supernatant was measured at a wavelength of 620 nm using a UV–Vis spectrophotometer (BMG LABTECH, SPECTROstar Nano, Ortenberg, Germany). The amount of phycocyanin was calculated using the equation provided in Boussiba & Richmond (1979).

Fucoxanthin analysis: The method described by Sudhakar et al. (2013) was employed to quantify the amount of fucoxanthin in the *P. tricornutum* samples. The culture was harvested, and the biomass was

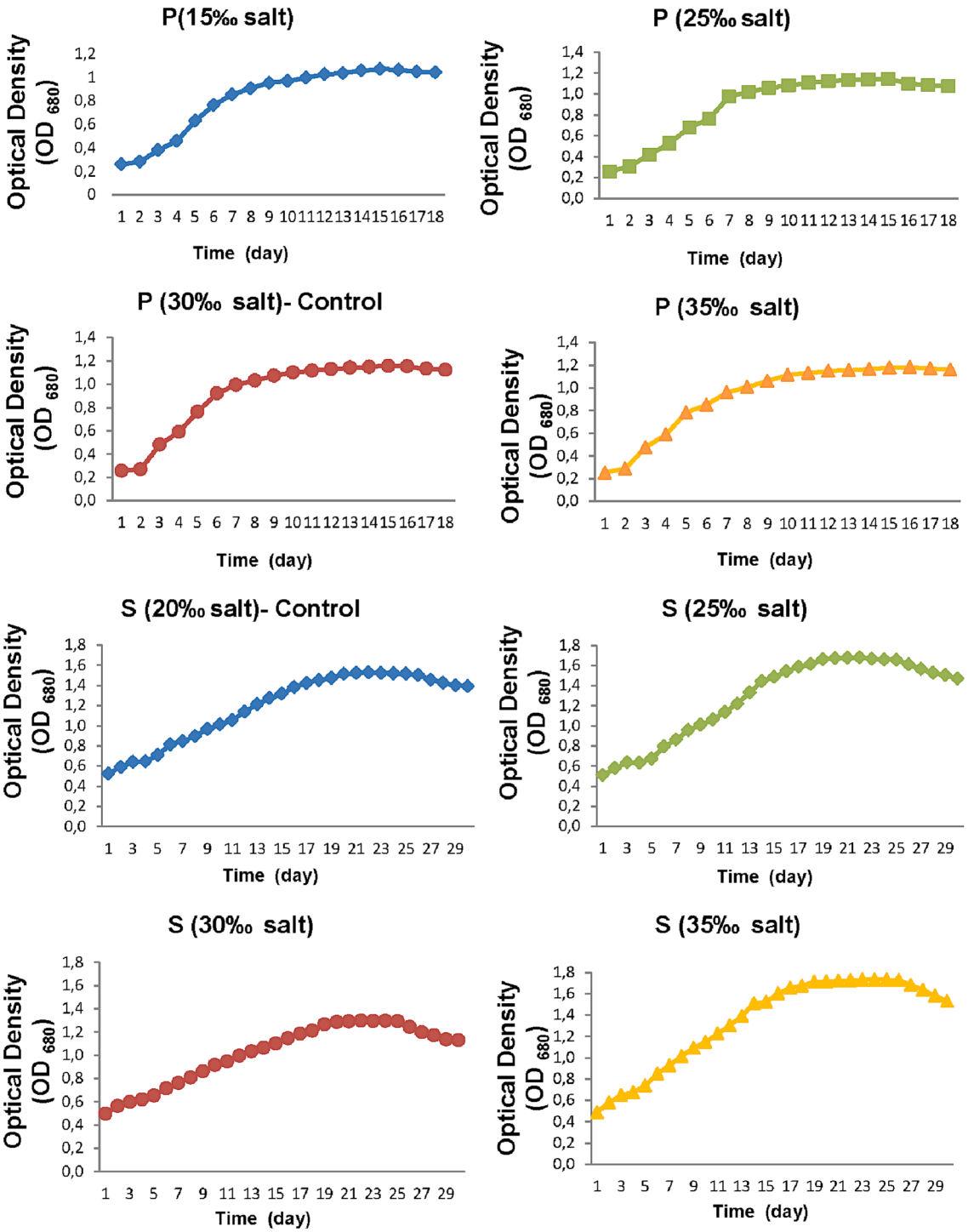

Fig. 1a. Changes in the optical density (cell density) of the *P. tricornutum* and *S. platensis* cultures grown with different salt concentrations (15–35‰) during the experiment.

dried at 55 °C. One gram of the powdered sample was weighed into 15 mL tubes and then 10 mL of 90% ethanol was added. The samples were incubated overnight at 4 °C in a refrigerator and subsequently centrifuged at 8000 rpm for 15 min. The extraction process was repeated three times until the samples became colorless. To prevent light-induced degradation, the samples were stored in amber-colored tubes. The amount of fucoxanthin was calculated using the equation given in Sudhakar et al. (2013).

2.5. Analyses of the dried samples

2.5.1. Total crude protein and dry matter analysis

Total crude protein and dry matter were quantified according to the method of the AOAC (2007).

2.5.2. Color analysis

Color data were quantified by a chromameter (Model CM-5, Konica Minolta, Inc., Japan) based on five color parameters (L*, a*, b*, C, h). Each color parameter is described below (Liang et al., 2004):

L*: brightness (whiteness/lightness/darkness) (between 0 and 100);
a*: redness (positive values) or greenness (negative values);
b*: yellowness (positive values) or blueness (negative values);
C: Chroma value (between 0 and 60) and.
H: hue angle value (red: 0, yellow: 90, green: 180, blue: 270).

2.5.3. Analysis of organic acids and sugars by HPLC-PDA-RID

Dried samples of 1 g of were taken, and 10 mL of solvent (methanol/water: 80/20) was added to it and the mixture was left in a magnetic stirrer for one night at room temperature. The samples were then centrifuged at 4 °C and 6500 rpm for 15 min (Lee and Coates, 2000). The extracts were injected into HPLC with PDA and RID detectors (Shimadzu Prominence-i LC-2030C) to detect and quantify the organic acid and sugar profiles in the study. Formic acid solution (0.1%) was used as carrier phase for organic acids while H_2SO_4 (5 mM) was used at 0.5 mL/min flow rate for sugar profile with BIORAD Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad; Hercules, CA, USA). In order to determine the amounts of the compounds, five different concentrations of solutions were prepared for each standard substance and the compounds were quantified from the calibration curves.

2.5.4. Analysis of the amino acids by HPLC-PDA

The amino acid profile was determined based on the method explained in Zeng et al. (2015) with slight modifications. Dried samples of 0.5 g were hydrolyzed with 10 mL of 0.1 N HCL at 110 °C for 24 h under reflux. Then, 2 mL of the liquid was withdrawn and passed through a 0.45 size filter. A Shimadzu HPLC device (Prominence-i LC-2030C model) was used in the analyses. Samples derived with 3-MPA, OPA and FMOAC were injected into the Agilent Eclipse Plus column C18 (3.5 μm, 4.6 × 100 mm). 20 mM potassium phosphate buffer (45/40/15: acetonitrile/methanol/water) (pH 6.9) was used as mobile phase. The amino acids were detected according to the retention times of the standards and the calculations were performed according to the peak areas at 262 nm for proline and at 338 nm for other amino acids.

2.5.5. Analysis of the aroma compounds by GC-FID and GC–MS

Aroma compounds were determined by gas chromatography-mass spectrometry (GC–MS) with headspace solid-phase microextraction (HS-SPME) in reference to Selli et al. (2008). One milliliter of saturated NaCl and 1 μL of internal standard (2-octanol) were added to the samples. DVB/CAR/PDMS (50/30 μm × 10 mm, 0.6 μL) (Sigma-Aldrich) was employed as SPME fiber. The quantity and identification of the aroma compounds were carried out simultaneously with "Agilent 6890N" gas chromatography and "Agilent 5975B VL MSD" mass spectrometer instruments. Flame ionization detector (FID) (Agilent 7890A) was employed for the amounts of the aroma substances while the separation of the aroma compounds was carried out with a DB-WAX capillary column (60 m × 0.25 mm × 0.4 μm). After the peaks were identified, the amounts of the compounds were computed using the internal standard method (Selli et al., 2008).

2.5.6. Statistical evaluation of the data

The data collected in the study were subjected to one-way ANOVA analysis by utilizing the SPSS statistics package program (Version 22, SPSS Inc., IBM, Chicago, IL, USA). The results were also compared with the literature. The differences between mean values were compared using Duncan's comparison test. Additionally, the correlation based on Pearson correlation coefficients (r) was studied in terms of correlation matrix to evaluate multiple paired comparisons for salt concentrations and dependent variables (optical density (OD_{680}), pigments, protein, amino acids, organic acids, sugar and aroma compounds) using XLSTAT software.

Table 1
Comparison of the optical density, dry matter, chlorophyll *a*, total carotene, fucoxanthin, phycocyanin, protein, dry matter (freeze-dried and powdered) and color of *P. tricornutum* and *S. platensis* grown with different salt concentrations (15–35‰).

		P. tricornutum			
Parameters (18th day)	**Stock culture**	**P15**	**P25**	**P30-C**	**P35**
Optical density (OD_{680})		1.04 ± 0.13^{a}	1.07 ± 0.02^{b}	1.16 ± 0.07^{b}	1.12 ± 0.03^{b}
Dry matter (g/L)		7.54± ±0.51^{a}	7.42 ± 0.53^{a}	9.43 ± 0.73^{b}	5.83 ± 0.26^{a}
Dry matter (%) **		91.74 ± 0.30^{a}	92.22 ± 0.22^{a}	91.58 ± 0.58^{a}	92.10 ± 0.20^{a}
Chlorophyll-a (μg/L)		512.47 ± 82.27^{ab}	329.03 ± 18.76^{a}	530.18 ± 17.84^{b}	440.52 ± 16.54^{ab}
Total carotene (μg/L)		0.10 ± 0.01^{a}	0.10 ± 0a	0.12 ± 0.01a^{b}	0.11 ± 0.01^{a}
Fucoxanthin (mg/100 g)	188.00 ± 0^{d}	146,44 ± 1.72^{b}	137,87 ± 1.49^{a}	174,60 ± 1.70^{c}	141,05 ± 1.58^{a}
Protein amount (%)		21.72 ± 0.41^{a}	24.64 ± 0.50^{b}	26.55 ± 0.16^{c}	29.64 ± 0.15^{d}
Color parameters:					
L*		52.12 ± 0.50^{e}	52.09 ± 0.73^{e}	45.95 ± 0.79^{d}	50.76 ± 0.47^{e}
a*		1.41 ± 0.06^{d}	0.54 ± 0^{c}	5.74 ± 0.25^{f}	2.95 ± 0.14^{e}
b*		24.13 ± 0.61^{e}	22.23 ± 0.60^{d}	26.87 ± 0.34^{f}	18.46 ± 0.24^{c}
C*		24.21 ± 0.56^{d}	22.73 ± 0.10^{d}	25.56 ± 2.20^{d}	18.13 ± 0.78^{c}
h		85.96 ± 0.60^{a}	87.22 ± 1.43a	78.79 ± 0.21^{a}	81.23 ± 0.23^{a}
		S. platensis			
Parameters (30th day)	**Stock culture**	**S20-C**	**S25**	**S30**	**S35**
Optical density (OD_{680})		1.53 ± 0.12^{c}	1.47 ± 0.08^{bc}	1.13 ± 0.04^{a}	1.39 ± 0.08^{b}
Dry matter (g/L)		10.01 ± 0.63^{b}	9.66 ± 0.96^{a}	9.05 ± 0.14^{a}	9.20 ± 0.70^{a}
Dry matter (%) **		91.98 ± 0.14^{a}	92.31 ± 0.32^{a}	92.01 ± 0.01^{a}	92.33 ± 0.19^{a}
Chlorophyll-a (μg/L)		1253.18 ± 123.45^{a}	1104.48 ± 59.54^{a}	1112.38 ± 23.61^{a}	1050.47 ± 14.20^{a}
Total carotene (μg/L)		0.19 ± 0.01^{b}	0.17 ± 0^{ab}	0.16 ± 0.01^{ab}	0.15 ± 0.01^{a}
Phycocyanin (mg/100 g)	362.33 ± 0.13^{e}	312,82 ± 0.3^{d}	198,38 ± 2.09^{c}	163,62 ± 0.50^{b}	157,41 ± 0.96^{a}
Protein amount (%)		55.33 ± 0.21^{d}	53.43 ± 0.25^{c}	52.77 ± 0.19^{b}	51.07 ± 0.33^{a}
Color parameters:					
L*		38.61 ± 0.37^{c}	32.09 ± 0.23^{b}	29.45 ± 0.30^{a}	32.18 ± 0.09^{b}
a*		−4.64 ± 0.31^{a}	−4.80 ± 0.07^{a}	−3.15 ± 0^{b}	−4.43 ± 0.25^{a}
b*		4.13 ± 0.12^{b}	2.26 ± 0.13^{a}	1.62 ± 0.03^{a}	2.74 ± 0.30^{a}
C*		6.43 ± 0.10^{b}	5.21 ± 0.22^{ab}	3.37 ± 0.20^{a}	5.38 ± 0.06^{ab}
h		132.68 ± 6.72^{b}	152.10 ± 1.71^{c}	151.59 ± 0.63^{c}	148.65 ± 0.67^{c}

Different letters (a-b) in the same row show statistical differences ($p < 0.05$).
** Dry matter amount of freeze-dried samples.

3. Results and discussions

3.1. Analyses conducted during the growth of the cultures

3.1.1. Optical density (OD_{680}) results

The daily cell density (OD_{680}) changes of *P. tricornutum* and

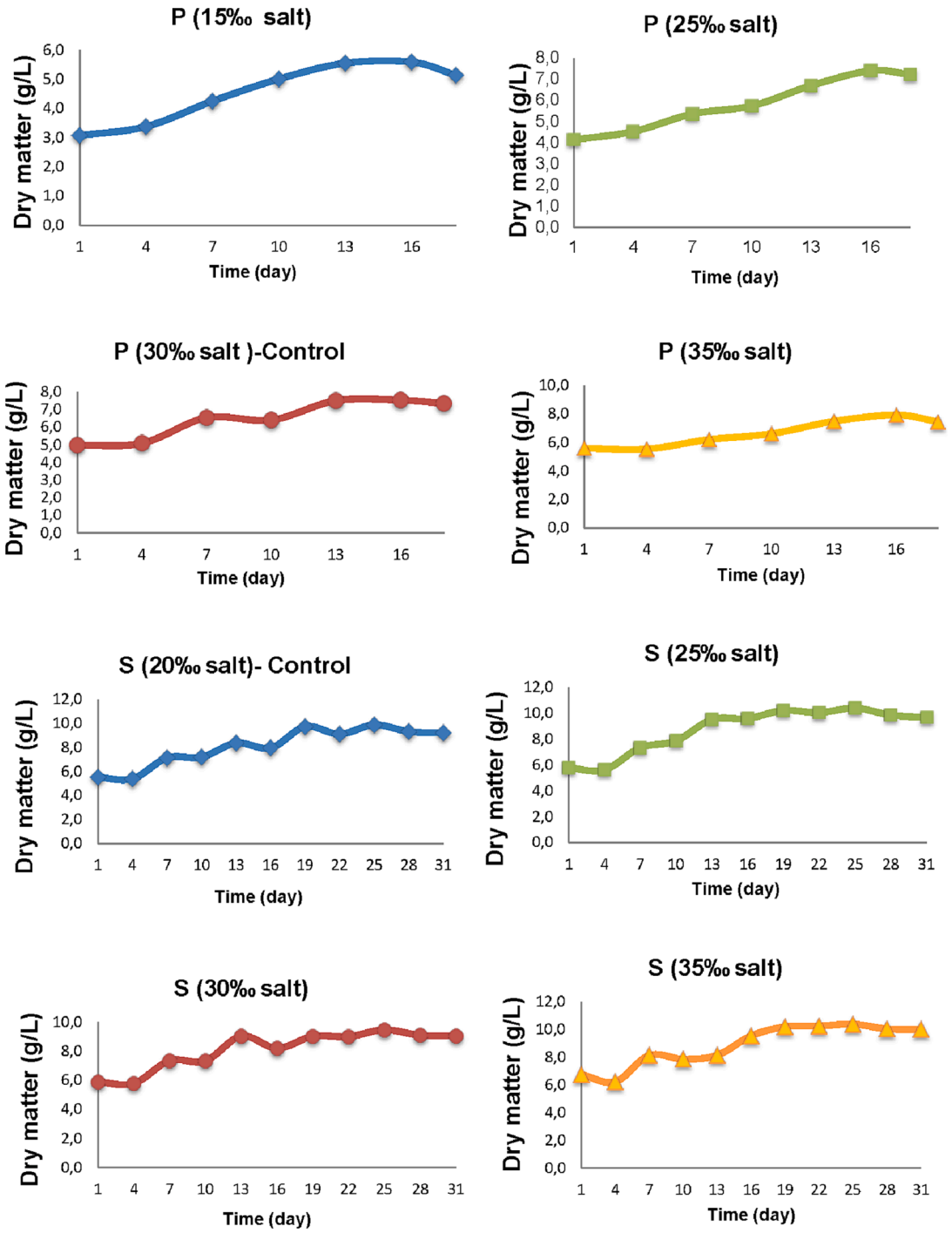

Fig. 1b. Changes in the dry matter (g/L) of the *P. tricornutum* and *S. platensis* cultures grown with different salt concentrations (15–35‰) during the experiment.

S. platensis are presented in Fig. 1a. The growth values, dry matter, chlorophyll *a* and total carotene amounts of the *P. tricornutum* samples grown with different salt concentrations are also given in Table 1.

The trial of the *P. tricornutum* continued for 18 days and the cell density (OD_{680}) on the last day was determined as 1.04, 1.07, 1.16 and 1.12 for P15, P25, P30-C and P35, respectively. In the study, significant differences were observed between the cell density (OD_{680}) values measured on the last day of the study groups ($p < 0.05$). The highest cell density was found in the P30-C sample and the lowest in the P15 sample (Table 1). In a study conducted by Liang et al. (2014), the effect of salinity on large-scale bulk cultures of *P. tricornutum* species was examined at 20‰ and 35‰ salinity and it was reported that 20‰ concentration gave better results in terms of growth rate and biomass distribution.

Regarding the *S.plantensis*, the experiments continued for 30 days and the OD_{680} values which indicate the cell density were determined on the last day of the trial as 1.53, 1.47, 1.13 and 1.39 for S20-C, S25, S30 and S35, respectively ($p < 0.05$). The highest cell density was determined in the S20-C sample (1.53) and the lowest in the S30 sample (1.13) (Table 1). Positive, strong and moderate correlations ($r = 0.99$ and $r = 0.53$) were observed between the salt concentration, OD_{680} and dry matter values of *P. tricornutum* and *S. platensis*, respectively (Fig. 1 in Supplementary material). It was determined that the changes in the salt concentrations had a negative effect on the development of both species.

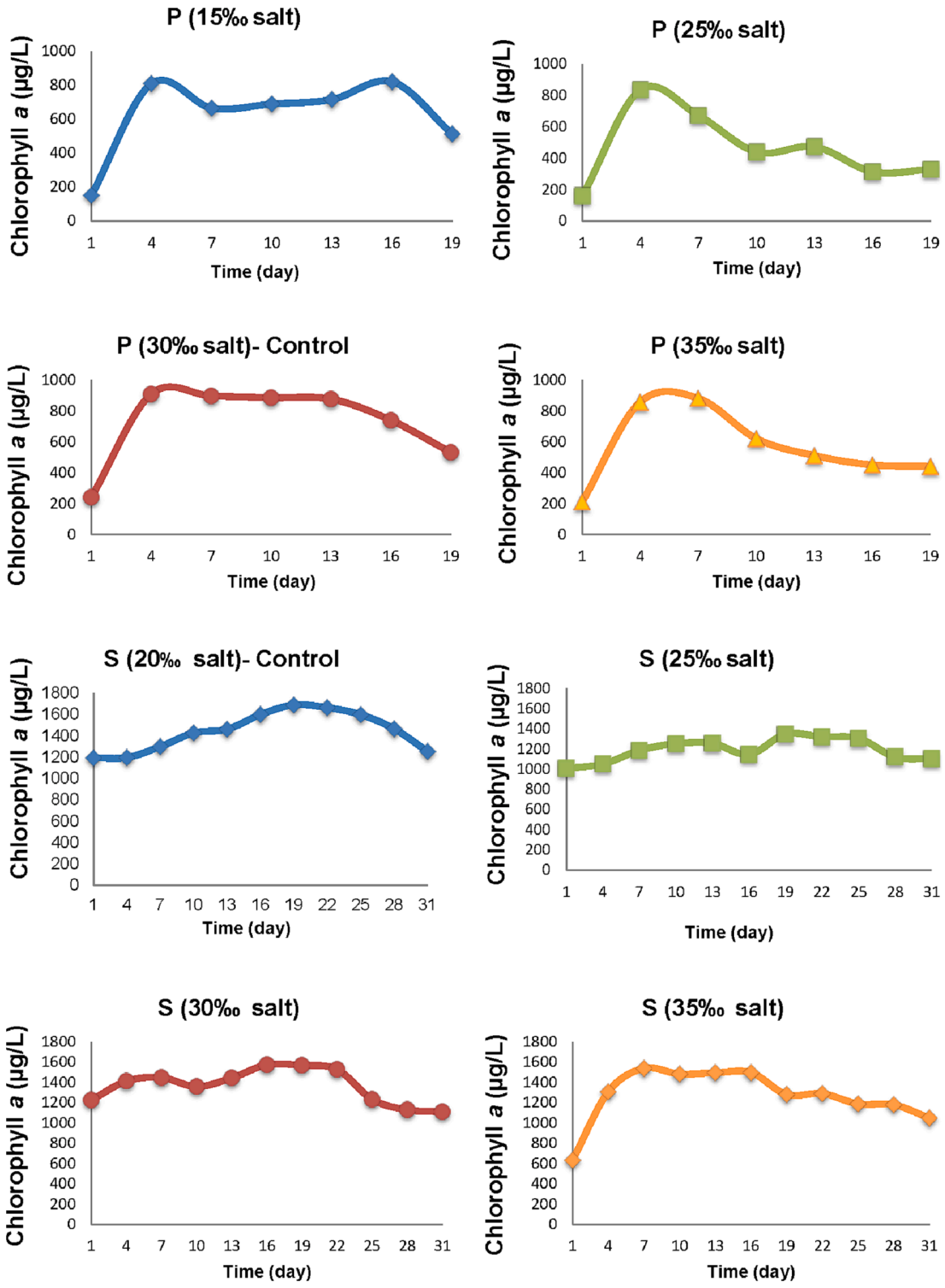

Fig. 1c. Changes in the amount of chlorophyll *a* (µg/L) of the *P. tricornutum* and *S. platensis* cultures grown with different salt concentrations (15-–35‰) during the experiment.

It was observed that salt, which is an important ecological factor for the growth, metabolism and vitality of algae, made it difficult for the cells to adapt to the modified environment and causes a decrease in the growth rate. This can be explained by ionic imbalance, osmotic stress and the damage to the photosynthetic activity (Liang et al., 2014).

3.1.2. Dry matter content results

Dry matter analyzes were performed every three days and the changes during the experiment are shown in Fig. 1b and Table 1. At the beginning of the experiment, the dry matter contents of *P. tricornutum* were 4.98 g/L, 4.13 g/L, 5.59 g/L and 3.08 g/L for P15, P25, P30-C and P35 samples, respectively. On the last day of the experiment, P30-C sample had the highest dry matter content (9.43 g/L) while the P35 sample had the lowest content (5.83 g/L). Thus, increasing salt concentration caused a decrease in the dry matter content (Table 1). However, no significant difference was found between the dry matter

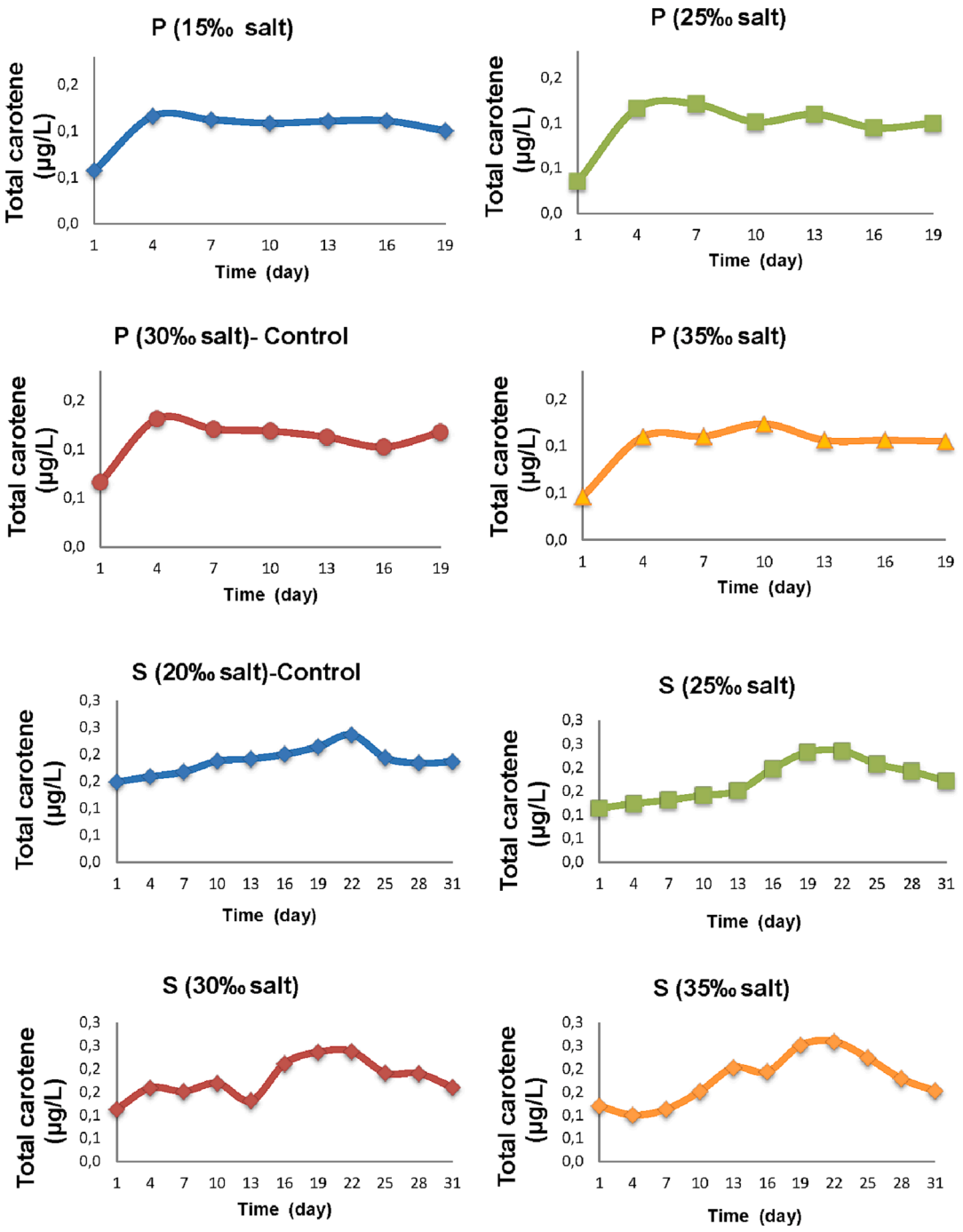

Fig. 1d. Changes in the total carotene content (µg/L) of the *P. tricornutum* and *S. platensis* cultures grown with different salt concentrations (15-35‰) during the experiment.

amounts of the *S. platensis* samples according to different salt contents ($p > 0.05$). At the beginning of the experiment, dry matter amounts for the *S. platensis* were determined as 6.75 g/L, 5.77 g/L, 5.87 g/L and 5.52 g/L for S20-C, S25, S30 and S35 samples, respectively. The amount of dry matter increased during the experiment and its amounts on the last day were 10.01 g/L, 9.66 g/L, 9.05 g/L and 9.20 g/L, respectively (Table 1). Based on these findings, it was determined that the salt concentration had a negative effect on the amount of dry matter. Similarly, in a study on the effect of different salt concentrations on the culture of *Dunaliella salina* and *Chlorella vulgaris* (Shalaby et al., 2010), it was found that high salt concentration decreased the amount of dry matter and cells deaths.

3.1.3. Chlorophyll *a* and total carotene content results

Changes in the daily chlorophyll *a* amounts of the *P. tricornutum* and *S. platensis* samples are shown in Fig. 1c while the chlorophyll *a* and total carotene amounts determined on the last day of the experiments are presented in Table 1.

Regarding the chlorophyll *a* contents, statistically significant differences were observed between the values measured on the last day of the trials in the *P. tricornutum* cultures according to different salt concentrations ($p>0.05$). The highest chlorophyll *a* quantity was observed in P30-C sample (530.18 g/L) while the lowest (329.03 g/L) was in the P25 sample. In contrast, no substantial differences were observed between the chlorophyll *a* values measured in the *S. platensis* samples ($p > 0.05$).

Table 2
Organic acid, sugar and aminoacid compositions of the freeze-dried *P. tricornutum* and *S. platensis* samples grown with different salt concentrations (15–35‰).

		P. tricornutum				*S. platensis*			
		P15	P25	P30-C	P35	S20-C	S25	S30	S35
Organic acids (mg/100 g DW)	α-ketoglutaric acid	226.29 ± 0.70^{bD}	113.86 ± 0.86^{aB}	246.60 ± 1.41^{cE}	342.13 ± 0.70^{dF}	645.53 ± 3.61^{cH}	397.21 ± 2.12^{bG}	85.87 ± 2.82^{aA}	215.04 ± 2.75^{abC}
	Citric acid	125.77 ± 1.41^{cG}	55.75 ± 1.86^{aD}	162.35 ± 2.82^{dH}	96.22 ± 0.70^{bF}	67.87 ± 1.65^{dE}	36.06 ± 0.70^{bB}	48.74 ± 0.28^{cC}	10.52 ± 0.70^{aA}
	Malic acid	nd	nd	17.51 ± 1.41^{aA}	nd	107.09 ± 2.82^{cD}	59.49 ± 0.63^{aB}	67.15 ± 1.41^{bC}	67.73 ± 0.35^{bC}
	Ascorbic acid	7.22 ± 0.14^{bB}	5.13 ± 0.35^{aA}	9.43 ± 0.13^{cC}	11.21 ± 0.70^{dD}	nd	nd	nd	nd
	Succinic acid	36.23 ± 0.48^{bB}	29.05 ± 0.86^{aA}	186.03 ± 1.51^{dF}	61.09 ± 1.43^{cE}	730.41 ± 8.41^{cG}	41.85 ± 1.39^{aC}	41.21 ± 0.70^{aC}	51.18 ± 0.35^{bD}
	Acetic acid	7474.06 ± 0.41^{cF}	6401.60 ± 1.41^{aB}	7584.84 ± 2.12^{dG}	7365.07 ± 7.07^{bE}	7442.73 ± 1.96^{dF}	3620.96 ± 0.70^{aA}	6903.18 ± 3.53^{bC}	7122.25 ± 2.21^{cD}
	Total	***7869.6* ± *4.9^{cF}***	***6605.4* ± *5.4^{aB}***	***8206.7* ± *7.7^{dG}***	***7875.7* ± *10.6^{bE}***	***8993.7* ± *18.5^{aH}***	***4155.6* ± *5.5^{bA}***	***7146.2* ± *8.75^{cC}***	***7466.7* ± *6.4^{dD}***
Sugar (mg/100 g DW)	Sucrose	172.41 ± 0.70^{aB}	168.65 ± 0.21^{dA}	229.58 ± 0^{cD}	209.93 ± 0.01^{bC}	1099.60 ± 0^{dH}	1064.16 ± 0^{cG}	398.25 ± 0^{aE}	923.40 ± 0^{bF}
	Glucose	164.20 ± 0^{bA}	192.48 ± 0.04^{cD}	169.60 ± 0^{aB}	173.86 ± 0^{dC}	2322.13 ± 0^{dH}	2222.69 ± 0^{cG}	1727.64 ± 0.01^{aE}	1993.25 ± 0.01^{bF}
	Fructose	69.75 ± 0^{bB}	92.70 ± 0.07^{dF}	83.17 ± 0^{cE}	71.23 ± 0^{fC}	121.10 ± 0^{dH}	106.82 ± 0^{cG}	74.81 ± 0.03^{bD}	56.58 ± 0^{aA}
	Total	***406.4* ± *0.2^{aA}***	***453.8* ± *0.3^{bB}***	***482.4* ± *0^{dD}***	***454.8* ± *0^{cC}***	***3542.8* ± *0^{dH}***	***3393.7* ± *0^{cG}***	***2200.7* ± *0.04^{aE}***	***2973.2* ± *0^{bF}***
Aminoacids (g/100 g DW)	Aspartic acid	3.76 ± 0.05^{bB}	4.80 ± 0.28^{cC}	3.75 ± 0.12^{bB}	2.11 ± 0.08^{aA}	11.06 ± 0.09^{dG}	5.23 ± 0.05^{aD}	9.11 ± 0.76^{cF}	7.46 ± 0^{bE}
	Glutamic acid	6.10 ± 0.10^{aA}	6.24 ± 0.01^{aA}	6.03 ± 0.16^{aA}	6.17 ± 0.08^{aA}	11.06 ± 0^{bC}	9.07 ± 0.05^{aB}	11.76 ± 0.02^{cC}	12.96 ± 0.07^{dD}
	Asparagine	5.10 ± 1.59^{cG}	3.93 ± 0.15^{bF}	3.68 ± 0.07^{bE}	2.22 ± 0.30^{aB}	3.27 ± 0.02^{dD}	2.29 ± 0.01^{bB}	2.83 ± 0.04^{cC}	1.75 ± 0.20^{aA}
	Serine	3.65 ± 0.18^{aB}	3.50 ± 0.07^{aB}	3.38 ± 0^{aB}	3.66 ± 0.11^{aB}	5.49 ± 0.30^{dD}	4.55 ± 0.02^{cC}	3.29 ± 0.14^{bB}	2.57 ± 0.09^{aA}
	Glutamine	4.51 ± 0.70^{cC}	3.51 ± 0.55^{bB}	3.78 ± 0.06^{bB}	2.52 ± 0.14^{aA}	nd	nd	nd	nd
	Histidine	nd	nd	1.85 ± 0.07^{aA}	nd	nd	nd	nd	nd
	Glycine	3.06 ± 0.53^{cC}	2.93 ± 0.24^{bB}	2.84 ± 0.22^{aA}	2.53 ± 0.40^{aA}	6.35 ± 0.05^{dF}	4.79 ± 0.02^{bE}	4.06 ± 0^{aD}	4.25 ± 0.01^{aD}
	Threonine	2.66 ± 0.35^{aA}	2.47 ± 0.62^{aA}	5.35 ± 0.43^{cD}	3.28 ± 0.19^{bB}	6.94 ± 0.40^{cE}	5.77 ± 0.62^{bD}	5.25 ± 0.04^{bD}	4.98 ± 0.04^{aC}
	Arginine	3.58 ± 0.15^{bB}	3.72 ± 0.03^{bB}	4.26 ± 0.16^{cD}	2.89 ± 0.14^{aA}	4.67 ± 0.04^{dE}	3.64 ± 0.05^{aB}	4.13 ± 0.01^{cD}	3.95 ± 0.04^{bC}
	Alanine	3.21 ± 0.02^{aA}	3.01 ± 0.02^{aA}	3.13 ± 0.03^{aA}	3.67 ± 0.14^{bB}	13.65 ± 0.71^{cE}	10.49 ± 0.02^{bD}	9.16 ± 0.01^{aC}	9.85 ± 0.04^{abC}
	Tyrosine	2.93 ± 0.18^{bcB}	3.04 ± 0.27^{cC}	2.75 ± 0.12^{bB}	1.42 ± 0.45^{aA}	6.32 ± 0.62^{cE}	4.74 ± 0.46^{bD}	2.82 ± 0.05^{aB}	2.06 ± 0.04^{aB}
	Cysteine	3.85 ± 0.15^{cF}	2.39 ± 0.11^{aC}	6.19 ± 0.08^{dG}	2.88 ± 0.09^{bE}	3.25 ± 0.11^{dF}	1.85 ± 0.19^{bB}	0.91 ± 0.03^{aA}	2.68 ± 0.04^{cD}
	Valine	4.05 ± 0^{cD}	4.32 ± 0^{cD}	3.37 ± 0^{bC}	1.63 ± 0.23^{aA}	6.38 ± 0.31^{cF}	4.79 ± 0.23^{bE}	2.72 ± 0.10^{aB}	2.24 ± 0.02^{aB}
	Methionine	3.43 ± 0.17^{bE}	3.32 ± 0.25^{bE}	3.24 ± 0.03^{bE}	2.49 ± 0.61^{aC}	2.75 ± 0.11^{dD}	1.14 ± 0.03^{bB}	0.58 ± 0.01^{aA}	2.36 ± 0.09^{cC}
	Tryptophan	3.42 ± 0.43^{bD}	3.19 ± 0.24^{bD}	3.51 ± 0.01^{bD}	2.25 ± 0^{aB}	7.41 ± 0.34^{dE}	3.63 ± 0.27^{cD}	2.87 ± 0.0.07^{bC}	1.90 ± 0.21^{aA}
	Phenylalanine	2.49 ± 0.62^{aC}	2.40 ± 0.45^{aC}	3.15 ± 0.05^{cD}	2.87 ± 0.62^{bC}	2.50 ± 0.04^{cC}	1.14 ± 0.03^{bB}	0.97 ± 0.03^{aA}	1.32 ± 0.13^{bB}
	Isoleucine	3.47 ± 0.34^{aA}	3.42 ± 0.51^{aA}	3.68 ± 0.71^{bA}	3.23 ± 0.22^{aA}	12.85 ± 0.02^{dE}	10.46 ± 0.09^{cD}	8.94 ± 0.07^{bC}	7.27 ± 0.01^{aB}
	Leucine	2.38 ± 0.26^{aA}	2.58 ± 0.29^{aA}	2.85 ± 0.03^{bB}	2.41 ± 0.18^{aA}	10.96 ± 0.97^{dF}	8.96 ± 0.04^{cE}	4.55 ± 0.04^{bD}	3.71 ± 0.08^{aC}
	Proline	0.80 ± 0.01^{bB}	0.69 ± 0.02a^{bB}	0.53 ± 0^{aA}	1.00 ± 0.03^{cC}	0.85 ± 0.08^{aB}	1.27 ± 0.01^{aD}	1.05 ± 0.02^{aC}	3.40 ± 0.05^{dE}
	Total	***62.5* ± *0^{cC}***	***59.5* ± *0.6^{bB}***	***67.3* ± *0.7^{dD}***	***49.3* ± *1.7^{aA}***	***115.8* ± *0.2^{dH}***	***83.8* ± *0.2^{cG}***	***75.0* ± *0.6^{bF}***	***74.26* ± *0.8^{aE}***

Different letters (a-b, A-H) in the same row show statistical differences ($p < 0.05$).
**(a–d): Statistical analysis was applied separately for *P. tricornutum* and *S. platensis*.
**(A-H): Expresses the statistical difference between *P. tricornutum* and *S. platensis*.
nd: Not detected.

The amounts of chlorophyll *a* observed on the last day were determined as 1253, 1104, 1112 and 1050 g/L for the S20-C, S25, S30 and S35 cultures, respectively. There have been a limited number of research in the literature on the changes in the amount of chlorophyll *a* due to salt stress in *P. tricornutum* and *S. platensis* cultures. Shalaby et al. (2010) stated that salt stress applied in microalgae cultivation caused a decrease in chlorophyll and also in some xanthophylls (neoxanthin and violaxanthin). The findings of the current study were similar in a way that the amount of chlorophyll *a* decreased with higher salt concentration. This reduction in the chlorophyll and biomass may be associated with the inhibition of the photosynthesis mechanism. In previous studies, the quantity of chlorophyll *a* was determined between 0.37 and 3.03 mg/g in *P. tricornutum* (Wu et al., 2016; Rebolloso-Fuentes et al., 2000) while it was higher (0.65–25.0 mg/g) in *S. platensis* cultures (Gabr et al., 2020; Ismaiel et al., 2016).

The changes in the carotene contents of *P. tricornutum* and *S. platensis* grown with different salt contents are shown in Fig. 1d and Table 1. No significant difference was observed between the total carotene amounts on the last day of the trials for the *P. tricornutum* samples ($p > 0.05$). The total carotene amounts were determined as 0.10, 0.10, 0.12 and 0.11 g/L, respectively. In previous studies, the total carotene amounts of *P. tricornutum* varied between 0.18 and 6.14 mg/g (Goiris et al., 2012; Foo et al., 2017). In regards to the *S. platensis* cultures, the total carotene amount was determined as 0.19, 0.17, 0.16 and 0.15 μg/L for the S20-C, S25, S30 and S35 samples, respectively ($p < 0.05$) (Table 1). In previous studies, the total amount of carotene in *S. platensis* varied between 0.5 and 2.4 mg/g (Gabr et al., 2020; Ismaiel et al., 2016). In the current study, a negative and strong correlation ($r = -0.50$ to -0.98) was found between salt concentration, pigments and color parameters of *P. tricornutum* and *S. platensis* (Fig. 1 in Supplementary material). It is thought that increasing the amount of salt causes inhibition on the photosynthetic activities reducing the quantities of the chlorophyll *a* and total carotene.

3.1.4. Phycocyanin concentrations

The phycocyanin amounts of the experimental groups formed with *S. platensis* cultures with different salt contents are given in Table 1. While the amount of phycocyanin was 362.33 mg/100 g at the beginning of the experiment, this value decreased throughout the trial and substantial differences were seen among the experimental groups ($p < 0.05$). The phycocyanin amounts determined on the last day of the tests for S20-C, S25, S30 and S35 samples were 312.82, 198.38, 163.62 and 157.41 mg/100 g, respectively. The highest amount was determined in the control group (S20-C) while the lowest was in the S35 sample. In previous studies with *S. platensis*, the amount of phycocyanin was reported as 9–228 mg/100 g (Gabr et al., 2020; Ismaiel et al., 2016). Ueda et al. (2003) stated that the photosynthesis and protein synthesis capacities of the cells decreased due to the increase in salt content resulting in significant limiting effects on the growth rate and the metabolites produced as a result. The decrease in the amount of phycocyanin obtained in the present study can be explained by this information.

3.1.5. Fucoxanthin concentrations

The fucoxanthin amounts of the *P. tricornutum* experimental groups grown with different salt contents are presented in Table 1. Substantial differences were observed between the experimental groups ($p < 0.05$). While the amount of fucoxanthin was 188 mg/100 g at the beginning of the experiment, the amounts for P15, P25, P30-C and P35 samples were determined as 146.44, 137.87, 174.60 and 141.05 mg/100 g, respectively. The highest amount was observed in the control sample (P30-C) while the lowest amount was in the P25 sample. It was found that the salt concentration negatively affected the photosynthetic mechanism and accordingly, the amount of fucoxanthin decreased. In a study with *P. tricornutum*, it was found that the amount of fucoxanthin varied between 214 and 550 mg/100 g (Wu et al., 2016). Since complex pigment protein fluids cannot be produced in the chloroplasts due to salt stress, it is thought that the biosynthesis of the pigments decreases (Shalaby et al., 2010; Zhao et al., 2014).

3.2. Analyzes conducted on the dried samples

3.2.1. Protein content

Protein amounts of the freeze-dried *P. tricornutum* and *S. platensis* culture samples are presented in Table 1. It was observed that different salt concentrations applied to the culture media caused significant differences ($p < 0.05$) on the protein contents. The protein content of the *P. tricornutum* samples varied between 21.72% and 29.64%. The highest protein content was determined in the P30-C (26.55%) and P35 (29.64%) samples while the lowest was in the P15 sample (21.72%). In previous studies with *P. tricornutum*, protein amounts varied between 36% and 39% (Wu et al., 2016; Rebolloso-Fuentes et al., 2000). In the current study, the protein content of the *S. platensis* samples varied between 51.07% and 55.33%. The highest protein amount was determined in the control sample (S20-C) (55.33%) and the lowest was in the S35 sample (51.07%). Increasing salt concentration led to a decrease in the protein content of the *S. platensis* samples. In the previous studies with *S. platensis*, it was stated that the protein content from 50 to 77% had biological activities with antioxidant, anti-inflammatory, antiviral, antibacterial and neuroprotective effects (Seghiri et al., 2019). In the current study, a strong positive ($r = 0.99$) and negative ($r = -0.97$) correlations were found between the salt contents and protein contents of the *P. tricornutum* and *S. platensis* cultures, respectively (Fig. 1 in Supplementary material). High salinity results in serious damage including impaired metabolism, growth inhibition and loss in quality. Along with the toxicity caused by ions, salinity and osmotic stress result in cell damage, which negatively affects both photosynthesis and protein synthesis (Ueda et al., 2003). It is thought that the biochemical composition of the microalgae is affected by the production parameters (temperature, salt, pH, light, etc.) during the cultivation period as well as their ability to survive in different living conditions. Thus, the decrease in the amount of protein contents obtained in the present study can be explained by this information.

3.2.2. Color

Significant differences were observed between the color parameters of the freeze-dried *P. tricornutum* and *S. platensis* samples grown at different salt concentrations ($p < 0.05$) (Table 1). The highest L* value for the *P. tricornutum* culture was observed in the P15 and P25 samples while the lowest value was determined in the P30-C control sample. For *S. platensis*, the highest L* value was observed in the S35 sample and the lowest value was in the S20-K (control) sample. The findings of the L* values indicate that the color of the *S. platensis* samples was darker than that of the *P. tricornutum* samples. The a* value varied within 0.54–5.74 and from −3.15 to −4.80 in the *P. tricornutum* and *S. platensis* samples while the b* value changed within 18.46–26.87 and 1.62–4.43 in these cultures, respectively. Regarding the other two color parameters, it was seen that the C* value significantly decreased while the h value considerably increased compared to the values of the control groups. The bright yellowish orange color of *P. tricornutum* is due to the orange–colored fucoxanthin pigment found in brown marine algae and diatoms (Xia et al., 2013) while the typical blue-green color of *S. platensis* is due to the chlorophyll *a* and phycocyanin pigment (Wu et al., 2016).

3.2.3. Analysis of the organic acids by the HPLC-PDA

The dried samples of the *P. tricornutum* and *S. platensis* cultures grown with different salt concentrations were analyzed by the HPLC-PDA system for organic acids and the findings are presented in Table 2. The total quantity of the organic acids in the *P. tricornutum* samples varied between 6605 and 8206 mg/100 g. α-ketoglutaric, citric, malic, ascorbic, succinic and acetic acids were identified and quantified (Table 2). The most dominant organic acid was acetic acid in the

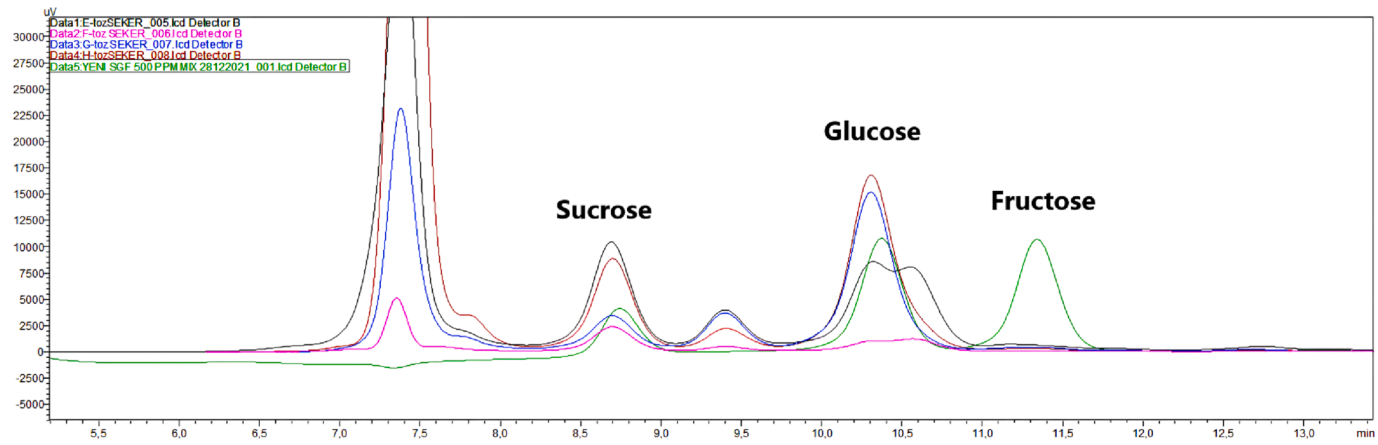

Fig. 1e. Chromatograms for the sugar contents of the *P. tricornutum* and *S. platensis* cultures.

Table 3
Aroma compounds (μg/kg) of *P. tricornutum* grown with different salt concentrations (15–35‰).

Chemical groups	LRI	Aroma compounds	P15	P25	P30-C	P35
Nitrogenous Compounds	1391	Piperazine	13.68 ± 0.92^{a}	12.53 ± 0.87^{b}	16.36 ± 1.18^{b}	14.73 ± 5.03^{a}
Alcohols	1648	Cyclobutanol	12.85 ± 1.53^{b}	10.14 ± 0.01^{ab}	19.62 ± 4.60^{c}	7.18 ± 1.98^{a}
	1820	α-phenylethanol	4.03 ± 0.22^{b}	2.34 ± 0^{a}	5.17 ± 0.07^{c}	3.57 ± 0.03^{b}
	1649	Cyclopropyl carbinol	8.78 ± 1.27^{c}	6.20 ± 3.63^{b}	10.65 ± 0.53^{d}	5.22 ± 3.72^{a}
		Total	***39.4 ± 3.7^{c}***	***34.9 ± 4.5^{b}***	***48.8 ± 6.4^{d}***	***30.7 ± 3.7^{a}***
Carboxylic Acids	1480	Acetic acid	109.90 ± 8.39^{a}	111.51 ± 6.47^{a}	113.59 ± 1.39^{a}	111.66 ± 7.64^{a}
	1647	Butanoic acid	12.16 ± 1.21^{a}	15.48 ± 1.28^{b}	18.30 ± 0.79^{c}	14.79 ± 5.05^{b}
	2508	Dodecanoic acid	16.40 ± 0.40^{c}	11.18 ± 0.98^{a}	14.71 ± 0.16^{b}	16.52 ± 0.39^{c}
	1883	Evoninic acid	3.31 ± 1.56^{a}	nd	4.04 ± 1.18^{a}	nd
	954	β-aminobutyric acid	2.80 ± 0.18^{c}	1.80 ± 0.33^{b}	2.70 ± 1.61^{c}	0.70 ± 0^{a}
		Total	***114.6 ± 11.7^{a}***	***139.9 ± 9.1^{b}***	***153.3 ± 5.1^{d}***	***143.7 ± 3.1^{c}***
Aldehydes	984	Pentanal	5.69 ± 0.24^{b}	5.25 ± 0.98^{b}	4.05 ± 0.39^{a}	3.14 ± 0.78^{a}
	1529	Benzaldehyde	81.47 ± 5.94^{c}	29.78 ± 1.60^{b}	25.54 ± 1.04^{a}	17.93 ± 4.07^{a}
	1188	Heptanal	40.63 ± 2.41^{c}	39.22 ± 2.00^{b}	59.13 ± 0.94^{d}	26.11 ± 1.09^{a}
		Total	***127.8 ± 8.6^{d}***	***74.3 ± 4.6^{b}***	***88.7 ± 2.4^{c}***	***47.2 ± 6.0^{a}***
Ethers	2055	Phenyl ether	15.97 ± 0.58^{b}	12.96 ± 0.45^{ab}	11.90 ± 0.41^{a}	11.80 ± 3.36^{a}
		Total	***16.0 ± 0.6^{b}***	***13.0 ± 0.5^{ab}***	***11.9 ± 0.4^{a}***	***11.8 ± 3.4^{a}***
Ketones	1283	2-octanone	38.69 ± 2.77^{b}	39.60 ± 1.07^{b}	50.29 ± 6.69^{c}	18.56 ± 1.62^{a}
	1303	4-cten-3-one	12.02 ± 1.83^{b}	11.87 ± 1.32^{b}	18.68 ± 1.47^{c}	8.60 ± 1.54^{a}
		Total	***50.7 ± 4.6^{b}***	***51.5 ± 2.39^{b}***	***69.0 ± 8.1^{c}***	***27.2 ± 3.6^{a}***
Esters	1194	Ethyl 2-butenoate	16.87 ± 3.50^{b}	16.90 ± 0.26^{b}	18.60 ± 2.15^{c}	12.55 ± 1.26^{a}
	1524	Ethyl β-hydroxybutyrate	52.32 ± 3.92^{c}	39.43 ± 2.93^{a}	66.47 ± 0.81^{d}	43.73 ± 8.04^{b}
		Total	***69.2 ± 7.4^{b}***	***56.3 ± 3.2^{a}***	***85.1 ± 2.9^{c}***	***56.3 ± 9.3^{a}***
Hydrocarbons	1161	6-(1Z,3-butadienyl)-1,4-cycloheptadiene	15.67 ± 0.90^{c}	10.21 ± 2.55^{a}	19.79 ± 0.13^{d}	12.10 ± 0.73^{b}
		Total	***15.7 ± 0.9^{c}***	***10.2 ± 2.6^{a}***	***19.8 ± 0.1^{d}***	***12.1 ± 0.7^{b}***
Aromatic hydrocarbons	1652	Acetophenone	41.25 ± 1.10^{a}	80.36 ± 0.11^{c}	97.17 ± 1.51^{d}	63.93 ± 1.23^{b}
		Total	***41.3 ± 1.1^{a}***	***80.4 ± 0.1^{c}***	***97.2 ± 1.5^{d}***	***63.9 ± 1.2^{b}***
Other compounds	1536	Cyclopentanedecol	9.01 ± 1.48^{c}	6.03 ± 0.03^{a}	10.87 ± 1.79^{d}	7.18 ± 1.67^{b}
	1650	12-Methylaminolauric acid	4.52 ± 0.58^{b}	4.55 ± 0.40^{b}	6.58 ± 0.34^{c}	2.89 ± 0.36^{a}
	2195	α-(1-aminoethyl)-benzenemethanol	4.60 ± 0.42^{b}	4.78 ± 0.22^{b}	6.80 ± 0.01^{c}	1.97 ± 0.53^{a}
Chemical groups	**LRI**	**Aroma Compounds**	**P15**	**P25**	**P30-C**	**P35**
	780	2-Amino-1-propanol	3.63 ± 0.59^{b}	3.68 ± 0.87^{b}	3.70 ± 0.62^{b}	1.51 ± 0.015^{a}
	1551	2,6-Dimethyl-γ-pyrone	4.21 ± 0.26^{b}	2.87 ± 0^{a}	6.75 ± 0.18^{c}	2.90 ± 0.81^{a}
	1490	2-Amino-1-(2-methoxyphenyl) propane	6.99 ± 0.67^{b}	3.48 ± 0.49^{a}	8.71 ± 0.02^{c}	3.76 ± 0.40^{a}
	755	2,3-Epoxy-1-propanol	9.54 ± 0.05^{c}	5.07 ± 0.39^{b}	3.42 ± 0^{a}	3.19 ± 0.48^{a}
	1232	4-Amino-1-pentanol	4.30 ± 0.88^{a}	4.96 ± 0.97^{a}	9.00 ± 0.32^{c}	6.25 ± 0.56^{b}
	1417	2-Methylthiocyclododeca[*c*]thiophene	5.81 ± 1.22^{a}	7.76 ± 0.73^{b}	9.01 ± 0.25^{c}	7.62 ± 1.99^{b}
	1450	Ethyl (2E)-4-[(2-aminopropanoyl)amino]-6-methyl-2-heptenoate	5.92 ± 0.21^{a}	7.37 ± 0.23^{b}	8.87 ± 0.54^{c}	7.36 ± 0.59^{b}
	1680	6-Aza-5,7,12,14-tetrathiapentacene	123.72 ± 6.50^{b}	113.35 ± 7.36^{ab}	154.03 ± 0.43^{c}	66.55 ± 2.99^{a}
	1955	1,2-Benzisothiazole	12.28 ± 4.69^{b}	9.72 ± 2.10^{a}	13.59 ± 1.01^{c}	10.98 ± 3.42^{b}
	1867	Methyl 11-aminododecanoate	3.51 ± 1.21^{ab}	2.76 ± 0.42^{a}	5.83 ± 0.25^{c}	4.67 ± 1.08^{b}
	2060	5-*tert*-butoxy-2-thiophenecarboxylic acid	3.09 ± 0.08^{a}	3.61 ± 0.48^{a}	3.01 ± 0.08^{a}	3.53 ± 0.12^{a}
	2284	Oxaluric acid	8.74 ± 0.27^{a}	8.85 ± 1.10^{a}	16.52 ± 0.18^{c}	10.45 ± 0.62^{b}
		Total	***200.9 ± 9.1^{c}***	***118.8 ± 15.8^{a}***	***266.7 ± 6.0^{d}***	***140.8 ± 15.8^{b}***
		General Total	***698.0 ± 59.9^{b}***	***645.6 ± 45.6^{b}***	***843.4 ± 33.1^{c}***	***533.6 ± 34.3^{a}***

Different letters in the same row (a-d) show statistical differences ($p < 0.05$).
LRI: Linear retention index calculated on the DB-WAX capillary column.
nd: Not detected.

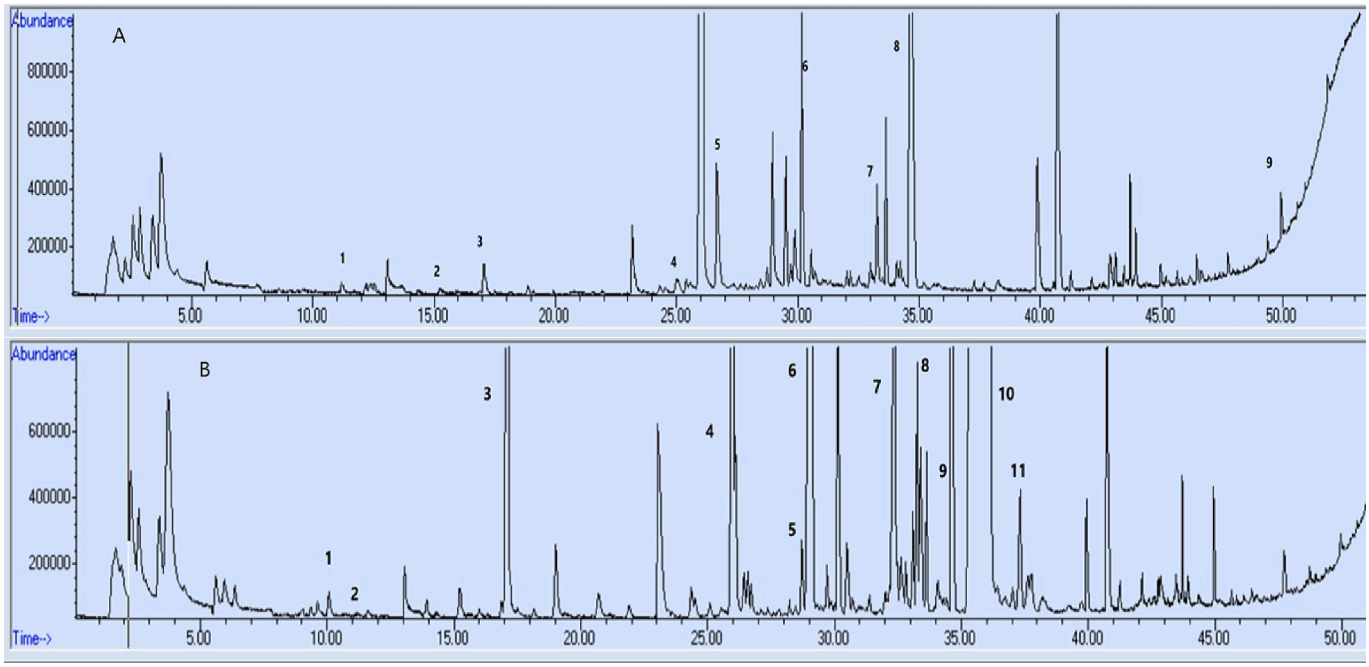

Fig. 2a. (A) Chromatograms for the aroma compounds of the *P. tricornutum* (1: 2-Pentanamine, 2: 1,2-Propandiamine, 3: 2-Octanone, 4: 6-(1Z,3-Butadienyl)-1,4-cycloheptadiene, 5: Acetic acid, 6: Heptanal, 7: Acetophenone, 8: 6-Aza-5,7,12,14-tetrathiapentacene, 9: Dodecanoic acid) (B) Chromatograms for the aroma compounds of *S. platensis* (1: *N*-Methyloctadecylamine, 2: 2-Pentanamine, 3: 2-Octanone, 4: Acetic acid, 5: 2-Ethyl-1-hexanol 6: Pentadecane, 7: Hexadecane, 8: Acetophenone, 9: 6-Aza-5,7,12,14-tetrathiapentacene, 10: Heptadecane, 11: 6(E),8(E)-Heptadecadiene).

P. tricornutum and *S. platensis* samples with the amounts of 7584.74 mg/100 g and 7442.73 mg/100 g, respectively. The highest acetic acid content was observed in the control groups (P30-C and S20-C). In addition to acetic acid, the amounts of α-ketoglutaric, citric and succinic acids were high in both species. The α-ketoglutaric acid is effective in the formation of alanine, glutamine and glutamic acid and it is present in many plants as free and/or as derivatives (Asadi Shahmirzadi et al., 2020). It was observed that the variations in the salt concentration changed the amount of total organic acids. There has been no study in the literature on how salt concentration changes the organic acid composition in the *P. tricornutum* and *S. platensis* cultures; so, the studies on plants were examined. It was stated in one such study that high salt levels cause ionic and osmotic stress in the plant cells disrupting the cell membrane structures (Wang et al., 2021).

3.2.4. Analysis of the sugar compounds by the HPLC-RID

The sugar compositions of the dried the *P. tricornutum* and *S. platensis* samples grown with different salt concentrations determined by the HPLC-RID are given in Table 2 and their chromatograms are shown in Fig. 1e. Glucose, sucrose and fructose were determined in the samples of both species and significant differences were detected in their compositions depending on salt concentrations ($p < 0.05$). The total sugar content varied within 406–482 mg/100 g for the *P. tricornutum* and 2200–3542 mg/100 g for the *S. platensis* samples. The predominant sugar was determined as sucrose for *P. tricornutum* and glucose for *S. platensis*. It was found that the sugar contents were generally higher in the control groups and that the salt quantity reduced the sugar content of *P. tricornutum* and *S. platensis* while the *S. platensis* samples had a higher sugar content than the *P. tricornutum* samples. For the *P. tricornutum* samples, salt concentrations above or below the control level led to a reduction in the total sugar content. In particular, a significant difference was found in sucrose content. The highest glucose and fructose contents were observed in the P25 sample. In the *S. platensis* cultures, the control samples (S20-C) had the richest sugar content while the glucose was dominant in the *P. tricornutum* samples. A higher salt content compared to the control group led to a decrease in the sugar profile and total sugar. In a previous study conducted on *S. platensis*, the total sugar ranged from 309 to 1221 mg/100 g and glucose, fructose, galactose, rhamnose, xylose and ribose were found (Al-Dhabi and Valan Arasu, 2016). In the *P. tricornutum* and *S. platensis* samples in the present study, positive and moderate ($r = 0.10$ and 0.60) and negative and strong ($r = -0.85$) correlations were observed between salt concentrations, organic acid contents and sugar contents, respectively (Fig. 1 in Supplementary material). Since there has been no previous study on the effect of salt on the sugar content of these two species, studies on plants were examined. Under salt stress, Na^+ accumulates in the apoplast in high concentration and that Na^+ can prevent the cell wall from performing its basic functions by disrupting the ionic connections of the structural elements such as pectin in the cell wall structure reducing the amount of sugar (Amirul Alam et al., 2015).

3.2.5. Analysis of the aroma compounds by the GC-FID and GC–MS

In aquatic ecosystems, algae produce numerous volatile organic compounds which include terpenes, furans, sulphides, alkenes, alkanes, aldehydes, alcohols, ketones and esters under the influence of various parameters such as temperature, light, nutrition and abiotic stresses (Bonsang et al., 2010). In the present study, the aroma compounds and their amounts in the dried *P. tricornutum* samples produced with different salt concentrations are given in Table 3 while the corresponding GC–MS chromatograms of the *P.ticornutum* and *S. platensis* samples are displayed in Fig. 2a.

In the *P. tricornutum* samples, a total of 34 aroma compounds including alcohols, ethers, esters, ketones, nitrogenous compounds, hydrocarbons, carboxylic acids, aldehydes and aromatic hydrocarbons were detected and quantified (Table 3). The total amounts of the aroma compounds in the samples varied within 533–843 μg/100 g and it was determined that decreasing the salt concentration increased the total amount of aroma compounds ($p < 0.05$). It was observed that the most prevailing aroma compound in the *P. tricornutum* samples was acetic acid with a sharp odor and the change in the salt content caused significant changes in the quantity of this compound. Acetic acid is representative of a typical sour and pungent aroma with low thresholds

Table 4
Aroma compounds (μg/kg) of *S. platensis* grown with different salt concentrations (20–35‰).

Chemical groups	LRI	Aroma Compounds	S20-C	S25	S30	S35
Alcohols	756	Cyclobutanol	18.02 ± 0.14^{a}	70.34 ± 0.23^{d}	42.23 ± 5.80^{c}	29.57 ± 0.78^{b}
	1499	2-ethyl-1-hexanol	75.82 ± 5.18^{b}	226.80 ± 8.72^{d}	176.04 ± 3.21^{c}	59.32 ± 1.56^{a}
	1885	Benzyl alcohol	59.44 ± 4.73^{a}	103.82 ± 3.64^{c}	83.18 ± 1.33^{b}	50.10 ± 1.32^{a}
	1923	Phenylethyl alcohol	28.49 ± 2.06^{a}	167.10 ± 7.89^{d}	73.26 ± 1.25^{c}	46.06 ± 1.21^{b}
		Total	***181.8 ± 2.1^{a}***	***568.1 ± 20.5^{c}***	***395.2 ± 11.6^{b}***	***185.1 ± 4.9^{a}***
Aldehydes	1277	o-Nitrobenzaldehyde	13.74 ± 0.13^{a}	30.75 ± 0.27^{d}	20.51 ± 0.60^{c}	18.53 ± 0.69^{b}
	979	Pentanal	26.41 ± 1.87^{a}	103.41 ± 3.52^{d}	80.03 ± 0.90^{c}	69.21 ± 1.83^{b}
		Total	***40.2 ± 2.0^{a}***	***134.2 ± 3.8^{d}***	***100.5 ± 1.5^{c}***	***87.7 ± 2.5^{b}***
Carboxylic Acids	1480	Acetic acid	109.56 ± 2.83^{d}	67.67 ± 2.79^{b}	88.80 ± 6.51^{c}	36.71 ± 0.97^{a}
		Total	***109.6 ± 2.8^{d}***	***67. 7 ± 2.8^{b}***	***88.8 ± 6.5^{c}***	***36.7 ± 0.9^{a}***
Ethers	680	2-(4-methoxyphenyl)-1,3-dioxolane	33.42 ± 2.49^{a}	85.83 ± 1.03^{c}	80.09 ± 1.26^{b}	79.08 ± 2.09^{b}
	900	1,1-diethoxy-ethane	35.39 ± 2.23^{a}	103.40 ± 3.02^{c}	74.50 ± 1.32^{b}	79.93 ± 2.11^{b}
		Total	***68.8 ± 4.7^{a}***	***189.2 ± 4.1^{c}***	***154.6 ± 2.6^{b}***	***159.0 ± 4.2^{b}***
Ketones	1283	2-Octanone	2789.83 ± 2.34^{a}	5328.98 ± 1.10^{b}	5254.98 ± 1.82^{b}	7470.99 ± 1.97^{c}
	1303	4-Octen-3-one	41.05 ± 0.29^{b}	162.11 ± 3.89^{c}	196.97 ± 5.47^{d}	20.99 ± 0.55^{a}
		Total	***2830.9 ± 2.6^{a}***	***5991.1 ± 5.0^{c}***	***5452.0 ± 7.3^{b}***	***7492.0 ± 2.5^{d}***
Esters	1441	Ethyl octanoate	nd	1274.57 ± 14.10^{c}	386.25 ± 9.86^{b}	101.93 ± 2.69^{a}
		Total	***nd***	***1274.6 ± 14.1^{c}***	***386.3 ± 9.9^{b}***	***101.9 ± 2.7^{a}***
Hydrocarbons	1454	1-Tetradecene	nd	277.33 ± 1.27^{b}	126.38 ± 5.01^{a}	161.89 ± 4.28^{a}
	256	Pentadecane	1486.46 ± 12.24^{a}	7071.72 ± 3.08^{d}	4708.18 ± 0.21^{c}	3975.40 ± 10.51^{b}
	1811	(5E)-5-Octadecene	114.81 ± 8.26^{a}	426.85 ± 1.70^{b}	169.93 ± 3.11^{a}	232.40 ± 6.14^{ab}
	268	Hexadecane	538.25 ± 4.14^{a}	2547.94 ± 11.30^{c}	1455.85 ± 27.84^{b}	1311.52 ± 34.68^{b}
	900	1-methyl-1-(1-methylethyl)-2-nonyl-Cyclopropane	116.03 ± 9.49^{a}	437.52 ± 5.60^{c}	106.69 ± 1.33^{a}	159.20 ± 4.21^{b}
	1624	*cis*-3-hexadecene	159.91 ± 1.26^{a}	577.28 ± 1.57^{b}	179.88 ± 3.34^{ab}	225.88 ± 5.97^{ab}
	1880	Cyclohexadecane	241.40 ± 2.09^{a}	1157.55 ± 10.37^{c}	246.15 ± 1.38^{a}	384.48 ± 1.01^{b}
	1680	6-Aza-5,7,12,14-tetrathiapentacene	866.84 ± 8.19^{b}	5993.26 ± 30.38^{c}	394.02 ± 7.37^{a}	714.12 ± 1.88^{b}
	1695	Heptadecane	10682.76 ± 73.46^{a}	36941.22 ± 94.62^{c}	30072.92 ± 56.85^{c}	28064.08 ± 74.21^{b}
	1705	8-Heptadecene	820.49 ± 5.71^{a}	2258.61 ± 34.00^{b}	2126.46 ± 40.25^{b}	2084.03 ± 55.11^{b}
		Total	***15027.0 ± 124.9^{a}***	***57689.3 ± 193.9^{d}***	***39586.5 ± 146.7^{c}***	***37313.0 ± 196.0^{b}***
Aromatic hydrocarbons	1652	Acetophenone	524.47 ± 4.88^{a}	4559.18 ± 13.30^{b}	346.37 ± 6.34^{a}	309.65 ± 8.18^{a}
		Total	***524.5 ± 4.9^{a}***	***4559.2 ± 13.3^{b}***	***346.4 ± 6.3^{a}***	***309.7 ± 8.2^{a}***
Phenols	2008	Phenol	45.83 ± 3.52^{a}	139.18 ± 3.64^{d}	79.22 ± 1.16^{b}	91.02 ± 2.40^{c}
		Total	***45.8 ± 3.5^{a}***	***139.2 ± 3.6^{d}***	***79.2 ± 1.2^{b}***	***91.0 ± 2.4^{c}***
Chemical groups	**LRI**	**Aroma Compounds**	**S20-C**	**S25**	**S30**	**S35**
Other compounds	1804	o-Methoxybenzoic acid, 3,5-difluorophenyl ester	53.18 ± 4.60^{a}	235.34 ± 6.49^{b}	81.46 ± 4.05^{ab}	49.11 ± 1.29^{a}
	1724	3-Amino-1-phenylbutane	30.02 ± 2.08^{a}	90.95 ± 3.49^{c}	50.05 ± 1.19^{b}	31.41 ± 0.83^{a}
	1489	3-Amino-2-ethyl-butyric acid	14.73 ± 1.00^{a}	39.76 ± 1.43^{cb}	40.92 ± 5.16^{c}	29.65 ± 0.78^{b}
	1490	(±)-2-Amino-1-methoxypropane	11.26 ± 0.58^{a}	40.47 ± 1.85^{c}	28.45 ± 7.50^{b}	27.43 ± 0.72^{b}
	1756	2-(4-methoxyphenyl)-1,3-dioxolane	33.42 ± 2.49^{a}	85.83 ± 1.03^{c}	80.09 ± 1.26^{b}	79.08 ± 2.09^{b}
	1775	Ethoxy(oxo)acetic acid	28.37 ± 2.26^{b}	nd	18.57 ± 1.44^{a}	567.53 ± 1.50^{c}
	1780	β-(Methylamino)ethanol	342.56 ± 3.37^{b}	nd	108.33 ± 8.07^{a}	nd
	1417	2-Methylthiocyclododeca[*c*]thiophene	29.30 ± 2.11^{a}	79.77 ± 1.99^{c}	48.35 ± 1.14^{b}	51.99 ± 1.37^{b}
	1820	3-hydroxycarbonyl-2,5-dimethylpyrrolidine	33.77 ± 2.64^{a}	109.12 ± 4.27^{c}	39.73 ± 6.98^{a}	57.99 ± 1.53^{b}
	1650	4-Phthalimidobenzoic acid	25.06 ± 2.12^{b}	nd	17.01 ± 1.79^{a}	54.97 ± 1.44^{c}
	1684	1,1-Bis[4-(*dimethylamino*)*phenyl*]-5-(dimethylamino)-isobenzofuran-3-one	17.25 ± 1.22^{a}	nd	21.53 ± 4.38^{b}	nd
	1232	4-Amino-1-pentanol	28.47 ± 2.01^{a}	nd	44.63 ± 7.87^{b}	nd
	1669	6(E),8(E)-Heptadecadiene	157.40 ± 1.26^{a}	501.17 ± 3.05^{b}	314.61 ± 5.94^{ab}	285.83 ± 7.55^{ab}
	1696	4-[2-(methylamino)ethyl]- phenol	73.38 ± 5.88^{a}	nd	114.44 ± 2.88^{ab}	152.82 ± 4.04^{b}
	1790	2-[2-Methyl-2-aminoethyl]benzofuran	30.84 ± 2.21^{a}	269.22 ± 1.92^{c}	73.50 ± 8.63^{b}	73.82 ± 1.95^{b}
	2017	5-(2-Aminopropyl)-2-methylphenol	4.30 ± 0.35^{a}	25.24 ± 0.61^{b}	18.60 ± 0.40^{ab}	13.52 ± 0.35^{ab}
	2029	2,4-Dimethylquinoline	58.80 ± 4.38^{a}	155.13 ± 5.62^{b}	75.64 ± 4.34^{ab}	59.28 ± 1.56^{a}
		Total	***972.1 ± 40.6^{a}***	***1632.0 ± 31.8^{d}***	***1175.9 ± 73.0^{b}***	***1534.4 ± 27.0^{c}***
		General Total	***19783.3 ± 201.5^{a}***	***71744.4 ± 292.8^{c}***	***47762.6 ± 262.9^{b}***	***47310.5 ± 249.4^{b}***

Different letters in the same row (a-d) show statistical differences ($p < 0.05$).
LRI: Linear retention index calculated on the DB-WAX capillary column.
nd: Not detected.

and high concentrations in *P. tricornutum* (Gao et al., 2020). In addition, 6-Aza-5,7,12,14-tetrathiapentacene, benzaldehyde and heptanal as two aldehydes, acetophenone as one aromatic hydrocarbon, 6-(1Z,3-butadienyl)-1,4-cycloheptadiene and 2-octanone as one hydrocarbon and ketone were determined. In a previous study, the most dominant aroma compound was 6-(1Z,3-butadienyl)-1,4-cycloheptadiene in two different strains of *P. tricornutum* (Bohlin and common accession) and acetophenone and dodecanoic acid were also detected (Prestegard et al., 2015).

In regards to the *S. platensis* samples, a total of 41 aroma compounds

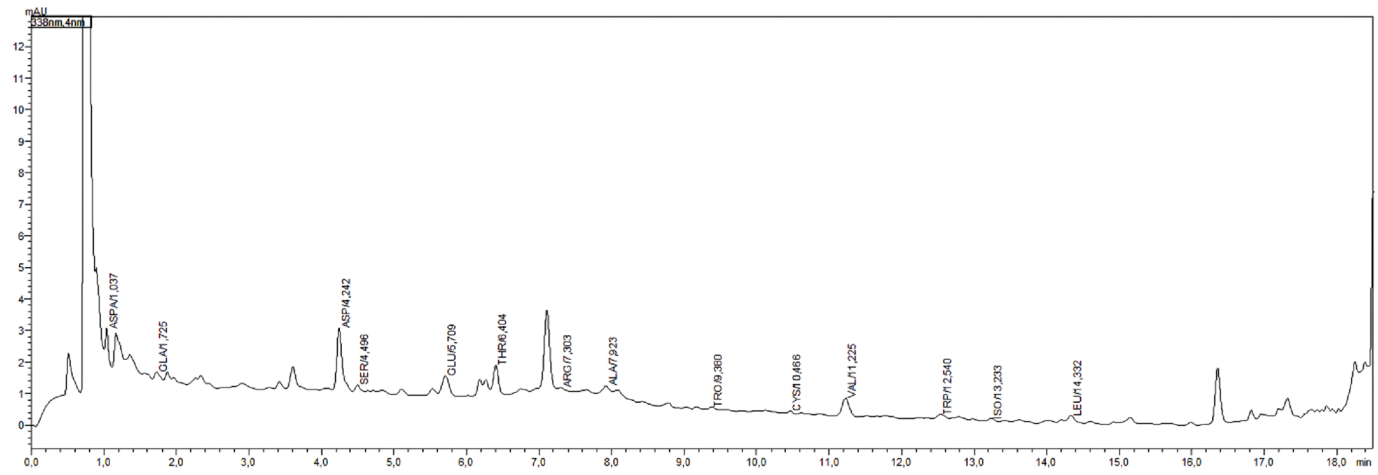

Fig. 2b. Chromatogram for the amino acid contents of the *P. tricornutum* and *S. platensis* cultures.

including alcohols, ethers, ketones, nitrogen compounds, hydrocarbons, carboxylic acids, alkaloids and aromatic hydrocarbons were quantified (Table 4). The total amount of aroma compounds varied between 19,783 and 71744 μg/100 g and it was seen that increasing the salt concentration increased the amount of aroma compounds ($p < 0.05$). The most dominant aroma compound was determined as heptadecane, which is a hydrocarbon, followed by pentadecane and hexadecane. Heptadecane, pentadecane and hexadecane were found in the *S. platensis* samples with high thresholds and concentrations. The aroma of these compounds often becomes detectable at high concentrations or with prolonged exposure. In addition to these, 2-octanone and acetophenone, which are a ketone and aromatic hydrocarbon, were also found in high amounts. Aroma compounds common to *P. tricornutum* and *S. platensis* were acetic acid, acetophenone and 6-aza-5,7,12,14-tetrathiapentacene. A positive and moderate correlation ($r = -0.30$) was observed between the salt concentration and aroma compounds of the *P. tricornutum* and *S. platensis* samples (Fig. 2 in Supplementary material).

Hydrocarbons are usually formed as a result of the decarboxylation of stearic and palmitic acids. Although they have high odor thresholds, their presence imparts an undesirable raw fish flavor to algae and can cause their flavor to deteriorate. Heptadecane and hexadecane, which are classified as hydrocarbons and have a waxy odor, are known to be dominant aroma compounds and exhibit antioxidant, anticancer and anti-inflammatory activity (Aguero et al., 2003; Bonsang et al., 2010; Cuellar-Bermúdez et al., 2017; De Jesus et al., 2018). Heptadecane was identified in both algae and plants and reported to have anticancer, antimicrobial and antioxidant activities (Aydi et al., 2020; Cuellar-Bermúdez et al., 2017). In previous studies, odorous volatiles such as hydrocarbons, 5-hepten-2-one, 6 methyl-, epoxy-β-ionone, 5 methyl-2-hexanone, 2-methylisoborneol, 2-pentylfuran, β-cyclocitral and β-ionone were detected in the *S. platensis* samples (Aguero et al., 2003; De Jesus et al., 2018).

Salts are one of the non-volatile components in the food matrix and can result in a change in the volatility of the aroma substances by chemically and physically interacting with flavoring agents based on their molecular weights and chemical properties influencing the perception of aroma (Tromelin et al., 2010; Tamaru et al., 2018).

3.2.6. Analysis of the amino acids by the HPLC-PDA

The amino acids of the dried *P. tricornutum* and *S. platensis* samples grown with different salt concentrations are presented in Table 2 and their chromatogram is displayed in Fig. 2b.

A total of 19 amino acids were determined in the *P. tricornutum* samples and it was seen that the total amount of amino acids varied within 49–67 g/100 g and decreased depending on the salt concentration ($p < 0.05$). Salt contents above and below the control group caused a significant reduction in the total amount of amino acids. In regards to the amino acid profiles, it was seen that the dominant amino acid was glutamic acid and the highest amount was detected in the P25 sample (Table 2). Histidine was determined only in the control group (P30-C) and was not detected in the samples produced with other salt concentrations.

Regarding the *S. platensis* samples, a total of 17 amino acids were determined and their amounts varied between 74 and 115 g/100 g ($p < 0.05$). The main amino acids were alanine along with aspartic and glutamic acids with the amounts of 13.65, 11.06 and 11.06 g/100 g, respectively. A negative and strong ($r = -0.85$) correlation was observed between salt concentration and amino acid amounts of the *P. tricornutum* and *S. platensis* samples (Fig. 1 in Supplementary material). Alanine is known to be available in the cell walls of some bacteria and in the structures of peptide antibiotics. Other dominant compounds, aspartic and glutamic acid, are the main parts of the proteins and known to have great importance for brain functions. In the current study, the reason for the decrease in the amount of amino acids in both species due to salt stress can be attributed to the increase in osmotic stress due to the restriction of water entry into the cells and the resulting damage to proteins, lipids and nucleic acids (Amirul Alam et al., 2015). It was observed that the dominant amino acids in the *S. platensis* samples were glutamic and aspartic acid with the amounts of 3.78–6.75 g/100 g and 1.89–3.79 g/100 g, respectively (Hizarci Uslu et al., 2009). It was also observed that the amount of proline increased due to the salt stress for both species and its amount varied between 0.53 and 1.0 g/100 g and 0.85–3.40 g/100 g for the *P. tricornutum* and *S. platensis* samples, respectively. It is known that the increase in the proline content under osmotic stress is determined by the balance between the synthesis and degradation and its accumulation capacity is associated with abiotic stress tolerance (Amirul Alam et al., 2015).

4. Conclusion

Salt stress is a crucial factor affecting microalgae growth and biomass yield. This research analyzed the optical density, dry matter, chlorophyll *a*, total carotene, phycocyanin, fucoxanthin, sugar, organic acid, aroma, and amino acids of the dried *P. tricornutum* and *S. platensis* culture samples produced with varying salt concentrations (15–35‰).

The study revealed that the optimal salt concentration was 30‰ for the *P. tricornutum* samples while it was 20‰ for the *S. platensis* samples. When the salt concentration deviated from these optimal levels, the quantities of the optical density, dry matter, chlorophyll *a*, total carotene, phycocyanin, fucoxanthin, sugar, organic acid, aroma, and amino acids decreased. This decline is believed to be caused by the osmotic stress that damages cells and inhibits the photosynthetic mechanism.

It was observed that the salt stress led to a decrease in the total organic acid and sugar content of the experimental groups except for the control groups. Acetic acid was the primary organic acid for the *P. tricornutum* samples while sucrose and glucose were the dominant sugars for the *S. platensis* samples. Glutamic acid was the dominant amino acid in the *P. tricornutum* samples while the *S. platensis* samples contained alanine, aspartic and glutamic acids. Total amino acid levels decreased significantly when the salt concentrations in the growth medium were higher or lower than the control groups.

Salt concentration had also an impact on the aroma by interacting with aroma substances affecting their volatility. Acetic acid, with a pungent odor, was the most common aroma compound found in the *P. tricornutum* samples while heptadecane, a hydrocarbon, was dominant in the *S. platensis* samples.

The results of this study provide insight into the optimal salinity levels for high-quality algae biomass production and the effects of salt on the chemical composition of the *P. tricornutum* and *S. platensis* microalgae. These findings are believed to be beneficial for the production of high-quality and flavorful products from these algae.

CRediT authorship contribution statement

Turkan Uzlasir: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. **Oya Isik:** Formal analysis, Validation, Visualization. **Leyla Hizarci Uslu:** Formal analysis, Validation, Visualization. **Serkan Selli:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. **Hasim Kelebek:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

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

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136843.

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