Study on the Responson of Biochemical Composition of *S. acuminatus* Biomass during the Spray Drying Process

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Abstract

Spray drying is a very popular method for microalgal biomass drying, however, systematic research on the response of biochemical composition during the process of spray drying has not been addressed so far. This study investigated the influence of the inlet temperature and initial solid content on the biochemical composition of spray dried *Scenedesmus acuminatus* biomass. The fatty acid composition and contents of CHNS, lipid, carbohydrate, protein, starch and pigments were analysed to characterize the quality and bioactivity of dried product. Results showed that the moisture content of dried microalgal powder decreased with the increase of inlet temperature and initial solid content, and the lowest moisture content of 2.37% with a higher drying yield of 84% was achieved at the optimized inlet temperature of 220°C and initial solid content of 16%. The biochemical compositions of CHNS total lipids, carbohydrates, protein, starch and fatty acid were similar with those dried by freeze drying, and were barely altered during the process of spray drying. However, the pigment would be partly degraded as the inlet temperature increased, which could be alleviated by increasing the initial solid content of the microalgal suspension due to the protection of cell aggregates. Thermogravimetric analysis (TGA) further confirmed that spray drying didn't affect the quality of proteins, lipids and carbohydrates, suggesting that spray-drying technique could be applied to *S. acuminatus* for the production of both biofuels and nutritional supplements. These results may serve as a reference for the selection of drying method, utilization of the nutritional components in *S. acuminatus* and selection of biochemical parameters for spray-drying performance evaluation.

1. Introduction

Through highly efficient photosynthesis, microalgae convert carbon dioxide and other nutrients into biological molecules such as lipids, proteins, sugars and pigments [1]. Thus, microalgae are widely used in the production of feed, liquid fuel, food, fine nutraceuticals, and pharmaceutical products and in environmental remediation applications [2]. Following harvesting of the microalgal biomass, the microalgal slurry (5–15% solid content) must be quickly dewatered and dried before it spoils [3].

Currently, the common drying methods are spray drying, rotary drying [4, 5], freeze drying, flash drying [6], solar drying [7], convective drying [8], and vacuum shelf drying [9, 10]. Among these methods, spray drying has become a very popular method for microalgal biomass drying. Spray drying has the advantages of continuous operation, rapid drying within a few seconds [11], and the capability to provide a powdered product requiring no further size reduction [12], which leads to high product quality and enables facile processing of the algal powder. Spray drying has been widely used for different species, such as *Spirulina* [13], *Chlorella* [14], and *Dunaliella* [15, 16] at the production scale and *Nannochloropsis* [17] at the laboratory scale. Palabiyik et al. [17] investigated the changes in the quantities of chlorophyll a and total carotenoids from *Isochrysis galbana* and *Nannochloropsis oculata* biomass dried at different inlet temperatures (150–200°C) using the spray-drying technique. Qi Liu [18] determined the optimal spray-drying parameters for processing *Chlorella* powder and chrysophyte powder through a Box-Behnken experiment and analysed the sensory characteristics and nutritional indicators of bread containing 1% *Chlorella* powder, 1% chrysophyte powder, and a 1:1 commixture. Nirmala et al. [19] reported that the protein, crude fat, carbohydrate, moisture and ash contents of spray-dried *Spirulina platensis* powder were $63 \pm 2\%$, $5 \pm 1\%$, $10 \pm 2\%$, $9 \pm 1\%$ and $10 \pm 1\%$, respectively. The functional properties of the protein were further evaluated, and an attempt was made to correlate the physico-chemical properties to the surface properties of the proteins. Using scanning electron microscopy, Lin, L.P. [20] studied the morphological changes of *Chlorella* and *Spirulina* induced by spray drying. The cellular composition, cellular concentration, feed conditions, temperature, and drying time all affected the morphology of the dried powders from the two different microalgal species. However, there is limited research on the influence of spray-drying conditions on

changes in biochemical composition, resulting in a lack of understanding of the biological activity and quality of microalgal powder after spray drying.

Thus, the objective of this study was to determine the influence of the inlet temperature and initial solid content of the spray-drying process on the moisture content, drying yield. Meanwhile, the contents of CHNS, lipid, carbohydrate, protein, starch, fatty acid composition and pigments under various drying condition were contrasted to characterize the quality and bioactivity of dried product, revealing the resposion of biochemical composition of the products during the process of spray drying. The results may serve as a reference for the selection of drying method, utilization of the nutritional components in *Scenedesmus acuminatus* and selection of biochemical parameters for spray-drying performance evaluation.

2. Materials And Methods

2.1 Solvents and reagents

Organic solvents (acetone, methanol, chloroform, dimethyl sulfoxide, diethyl ether, hexane, etc.) from Merck (Germany) were used. Chemicals for the culture media preparation (NaNO_3 , K_2HPO_4 , Na_2CO_3 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, etc.) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Four pigment standards — lutein, zeaxanthin, chlorophyll b and chlorophyll a — and one fatty acid methyl ester (FAME) standard mixture were purchased from Sigma-Aldrich (USA).

2.2 Microalgal cultivation and harvesting

S. acuminatus (GT-2, State Development & Investment Corp., China) was grown in tubular photobioreactors with a 5 cm light path and a culture volume of 13 m³. Modified BG-11 culture medium with a reduced nitrogen concentration of 16 mg L⁻¹ NO_3^- -N was used to enhance lipid accumulation. The daily maximum solar light intensity inside the greenhouse was approximately 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as measured with a light meter (Li-250A, Lincoln, USA). The average culture temperature was approximately 25°C. During cultivation, air enriched with 2–4 L min⁻¹ pure CO₂ were provided intermittently to maintain the pH between 6.5 and 7.0. The concentration of *S. acuminatus* was 0.2 g L⁻¹ dry weight initially and reached 1 g L⁻¹ within 13 days.

The *S. acuminatus* suspension was harvested by a membrane filtration unit with a filtration area of 180 m², and the microalgal suspension was condensed to a 30 g L⁻¹ slurry. Following centrifugation at 7600 × g with a disc stack centrifuge, 160 g L⁻¹ *S. acuminatus* paste was collected, which was used as the raw material for the spray-drying experiment. *S. acuminatus* suspensions with different concentrations of 80 g L⁻¹, 120 g L⁻¹ and 160 g L⁻¹ were obtained by dilution of the centrifuge-collected paste with the centrifugation supernatant. The viscosities of the *S. acuminatus* suspensions of different concentration were tested using a viscometer (NDJ-9S, Shanghai Pingxuan Scientific Instrument Co., Ltd., China), as shown in Figure S1.

To understand how the drying process affects the quality of the dried powder, a control group was dried to form a powder using a freeze-dryer (FreeZone 10 L, Labconco Corp., USA) at -50°C and 0.021 MPa for 72 h.

2.3 Spray-drying experiment

The spray-drying experiment was conducted with a laboratory-scale mini-spray dryer (B-290, Buchi, Switzerland) equipped with an air compressor (WSC 22140B, Huifeng, China). A fixing feed flow rate of 8 ml min⁻¹ was used, and the atomizing air velocity was 473 L h⁻¹. Therefore, the average residence time of microalgal cells in the chamber was

1.23 s according to the operating manual. Two key factors affecting the spray-drying performance, including the inlet temperature (120°C, 170°C and 220°C) and solid content (8%, 12% and 16%), were evaluated for their influence on the drying performance and changes in the biochemical composition of the spray-dried *S. acuminatus*.

2.4 Analytical methods

2.4.1 Moisture content

The moisture content in the spray-dried biomass was analysed gravimetrically using a heat-generating halogen analyser (MB35, Ohaus, Switzerland). Approximately 1.0 g of the biomass was loaded into an aluminium plate, and the temperature inside the weight chamber was increased to 105°C. The measurement was completed when the weight reading stabilized, which was accurate to 0.001 g. The results were expressed as the weight percent (w/w) [21].

2.4.2 CHNS analysis

The CHNS content in the spray-dried *S. acuminatus* biomass was determined using a CHNS/O analyser (PE2400II, Perkin Elmer Inc., USA) operated at a combustion temperature of 975°C and a reduction temperature of 500°C. The loaded samples (2–7 mg) were weighed with an autobalance (AD-6000, Perkin Elmer Inc., USA), which was accurate to 0.1 µg.

2.4.3 Total lipids, carbohydrates, protein, starch and FAMES

Carbohydrates were measured by a phenol-sulfuric acid method [22, 23]. The protein content was analysed using the Bradford method [24], consisting of measurement of the A595 of the samples and standards against a reagent blank and then comparison to a standard curve for quantitation. The starch content was quantified using an assay kit [25] (STA20, Sigma-Aldrich) based on the catalytic hydrolysis of starch into glucose by α-amylase and amyloglucosidase, which involved measurement of the A540 using an ultraviolet spectrophotometer (HACH DR6000, USA) and calculation of the starch content according to the equation $\% \text{Starch} = (\Delta \text{ATEST})(900)/(\Delta \text{ASTD})(\text{mg sample})$.

The total lipids were measured using a gravimetric method [26] after lipid extraction from the biomass using an accelerated solvent extraction system (Dionex 350, Thermo Fisher Scientific, CA, USA). Solvent A (methanol:DMSO, 9:1) and solvent B (hexane:diethyl ether, 1:1) were used to extract the total lipids. The results were expressed as a percentage: total lipid weight/algal weight (w/w).

FAMES produced from *S. acuminatus* oil were quantified using gas chromatography-mass spectrometry (GC-MS) (7890B-5977A, Agilent) with flame ionization detection. The GC column was a silica capillary column (HP-88 column; 60 m×0.25 mm×0.2 µm). All standards and samples were injected in split mode (split/column flow ratio of 20:1). The injection temperature was 250°C. The initial oven temperature of 50°C was held for 2 min, increased at a rate of 25°C min⁻¹ to 175°C, held for 5 min, increased at 7°C min⁻¹ to 210°C, held for 2 min, increased at 2°C min⁻¹ to 230°C, and held for 1 min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV over a scan range of *m/z* 50–650. The injected sample volume was 1.0 µL [27].

2.4.4 Pigment extraction and analysis

Spray-dried *S. acuminatus* powder was extracted using the same Dionex 350 solvent extraction system. A total of 50 mg of algal biomass was extracted with 5 ml of extraction solvent at 1500 psi and 100°C for 3 min. The preheating time was set to 5 min, and the static cycle included two cycles and three methanol extractions. The flush volume at the end of the extraction was 45% of the cell volume, and the purge time was set to 30 s. After extraction, the pigment solutions were collected in a 50 ml brown volumetric flask.

The extracted pigments were determined using high-performance liquid chromatography (HPLC) (Waters Alliance e2695, Waters, USA). A Waters Spherisorb C18 column (250×5 mm, 4.6 μm) was installed. The pigment-extracted solution was subjected to HPLC analysis. The pigments were separated using a solvent mixture of 0.1 M Tris-HCl with a pH of 8.0, pure acetonitrile, methanol, and ethyl acetate [28]. The flow rate was maintained at 1.2 ml min⁻¹, and the sample injection volume was 10 μL .

According to the pigment concentrations in *S. acuminatus*, six pigment working standard solutions were prepared: 0, 0.001 mg ml⁻¹, 0.002 mg ml⁻¹, 0.005 mg ml⁻¹, 0.01 mg ml⁻¹, and 0.02 mg ml⁻¹. Good linear relationships between the mass concentrations and the peak areas of the four pigment standards, i.e., lutein, zeaxanthin, chlorophyll b and chlorophyll a, were observed at retention times of 9.443 min, 9.700 min, 10.343 min and 11.392 min, respectively. The pigment contents in *S. acuminatus* were identified according to the retention times of the standards and quantified using the external standard method. The pigment chlorophyll a was detected at 664 nm, and the other pigments were detected at 445 nm using a diode array detector (2996, Waters, USA).

2.4.5 Morphology and particle size distribution

The spray-dried powder was re-suspended in water, and the morphology of the particles was examined using a microscope (CX31, Olympus Corp., Japan). The particle size distribution of the initial cells and spray-dried powder of *S. acuminatus* was measured using a laser diffraction particle size analyser (Mastersizer 3000, Malvern UK), and the particle size distribution of the initial *S. acuminatus* cells with an average size of 6.95 $\pm$ 0.11 μm is shown in Figure S2.

2.4.6 Thermogravimetric analysis (TGA)

The thermal stability of *S. acuminatus* biomass was measured with a TGA method using a thermogravimetric analyser (TG209C, NETZSCH Gerätebau GmbH, German). Approximately 26 mg of each sample was loaded into a 40 μL Al₂O₃ crucible for each measurement. The temperature inside the chamber was increased to 700°C with a heating rate of 10°C min⁻¹ and a nitrogen gas flow rate of 50 ml min⁻¹.

2.4.7 Statistical analysis

All experiments were carried out in triplicate. The results were reported as the average values $\pm$ standard deviations. One-way analysis of variance (ANOVA) with a least significant difference (LSD) post hoc test was used to evaluate the statistical significance of the differences between the control and experimental groups. In all data analyses, a *P*-value of 0.05 was considered statistically significant.

3. Results And Discussion

3.1 Influence of the inlet temperature and initial solid content on the moisture content and drying yield

The influence of the spray-drying conditions on the moisture content of the powder was shown in Fig. 1A. The moisture content decreased from 3.95–2.37% as the inlet temperature increased from 120°C to 220°C at an initial solid content of 16%. The same trend was obtained for initial solid contents of 12% and 8%. In addition, an increase in the initial solid content from 8–16% led to a decrease in the moisture content from 3.97–3.22% at 170°C. These results indicated that the moisture content of the spray-dried product was closely associated with both the inlet temperature of the drying air and the solid content of the feed suspension. Namely, a higher inlet temperature and a higher initial solid content was beneficial to obtaining microalgal powder with higher solid content.

The outlet temperature and powder yield under different drying conditions was shown as Fig. 1B. With increasing inlet temperature, the outlet temperature increased significantly for all different treatments, however, the powder yield presented different trends of change. At an initial solid content of 8%, the yield of the microalgal powder increased rapidly from 65–92% when the inlet temperature was increased from 120°C to 220°C, whereas at an initial solid content of 16%, a high yield of 84% was maintained even at lower inlet temperatures. The water loading rate was relatively high at the lower initial solid content (8%), while the driving force was low at the lower inlet temperature (120°C). As a result, *S. acuminatus* biomass cannot be fully dried under at low inlet temperature and initial solid content, and thus, the yield was lower than those at higher inlet temperatures and higher initial solid contents.

The drying yield increased with increasing initial solid content in the feed and increasing inlet temperature, and the highest dry mass production was obtained at an initial solid content of 16%, with a value of 76.8 g powder per hour at an inlet temperature of 220°C. In general, increasing the initial solid content of the feed and increasing the inlet temperature is preferable over other procedural changes to obtain a higher yield [29]. The higher the initial solid content is, the more energy efficient the spray-drying process. However, according to Figure S1, the viscosity of the *S. acuminatus* suspension increased exponentially ($R^2 = 0.98$) with increasing solid content, resulting in difficulty in spraying from the nozzle as the initial solid content exceeded 16%. In our recent study [30], the dewatering cost of membrane filtration increased approximately 20% when an *S. acuminatus* suspension was concentrated from 8–16%. Therefore, the drying performance of an *S. acuminatus* suspension with initial solid contents of 8% and 12% was also studied to explore integration of the dewatering process with the spray-drying process.

3.2 Changes in the biochemical composition of the spray-dried biomass

3.2.1 The CHNS content

Microalgal cells mainly consist of carbon, hydrogen, nitrogen and sulfur. The elemental composition of spray-dried *S. acuminatus* products at different inlet temperatures and initial solid contents was shown in Fig. 2. The C, H, N and S contents of *S. acuminatus* in the freeze-dried biomass were 46.13 ± 0.25 , 6.15 ± 0.54 , 1.74 ± 0.04 , and 0.95 ± 0.06 , respectively. The C, H, N and S contents in the spray-dried biomass for different inlet temperatures and initial solid contents varied over ranges of 43.83–45.59%, 5.06–5.62%, 1.81–2.47% and 0.74–0.85%, respectively. There was no significant difference in the C, H, N and S contents between the spray-dried samples and freeze-dried samples, which demonstrated that spray-drying with these conditions could achieve high-quality products. These were in accord with the literature showing that the carbon content of microalgal biomass ranges between 44.63% and 47.71% and the nitrogen content ranges between 2.33% and 11.29% [31].

3.2.2 The total lipid, carbohydrate, protein and starch contents

Table 1 listed the total lipid, carbohydrate, protein and starch contents in the *S. acuminatus* biomass at different drying conditions. In the spray-dried biomass, the total lipid, carbohydrate and protein contents were 33.63 ± 1.18 , 37.36 ± 0.81 , and 5.72 ± 0.39 , respectively, and all three components accounted for 72.84–79.95% of the total biomass. Starch and FAMES accounted for 16.67% and 27.78% of the *S. acuminatus* dry weight, respectively. Therefore, the total lipid, carbohydrate, protein and starch contents, as well as the FAME contents, did not change obviously with different inlet temperatures and initial solid contents during the spray-drying process, and these biological components were similar to as those obtained with freeze drying. These results indicated that spray drying didn't change the composition of these biological components under the conditions investigated in this paper.

Table 1
Influence of spray drying conditions on the content of total lipid, total carbohydrates and protein

Content (%)	Conditions	Carb	Lipid	Protein	$\sum(C + L + P)$	Starch	FAMEs
Freeze drying	3days, -50°C	36.21 ± 2.04	33.07 ± 0	8.58 ± 0.25	77.86 ± 2.28	18.94 ± 1.82	27.35 ± 0.27
Spray drying	120°C, 16%	37.06 ± 1.7	35.36 ± 1.6	7.53 ± 0.11	79.95 ± 3.41	17.04 ± 0.83	28.52 ± 0.63
	170°C, 16%	37.86 ± 0.28	34.24 ± 0.1	6.5 ± 0.4	78.6 ± 0.02	18.42 ± 0.32	28.66 ± 0.88
	220°C, 16%	37.68 ± 0.42	34.65 ± 2.5	7.23 ± 0.32	79.56 ± 2.4	17.11 ± 0.34	27.77 ± 0.82
	120°C, 12%	38.28 ± 0.8	33.56 ± 0.44	7.02 ± 0	78.86 ± 0.37	15.42 ± 1.68	28.34 ± 0.13
	170°C, 12%	37.89 ± 0.48	34.73 ± 1.5	6.5 ± 0.15	79.12 ± 0.87	17 ± 1.35	27.21 ± 0.33
	220°C, 12%	36.66 ± 1.07	33.67 ± 1.49	5.65 ± 0.11	75.98 ± 2.46	17.2 ± 0.74	27.42 ± 0.48
	120°C, 8%	36.64 ± 0.66	33.69 ± 0.54	6.16 ± 0.06	76.49 ± 0.18	13.57 ± 0.36	27.72 ± 0.14
	170°C, 8%	38.24 ± 1.89	32.14 ± 0.72	6.58 ± 0.08	76.96 ± 1.09	16.62 ± 1.17	27.21 ± 0.05
	220°C, 8%	35.9 ± 1.72	30.64 ± 2.05	6.3 ± 0.24	72.84 ± 2.29	17.62 ± 0.24	27.18 ± 0.41
Carb = carbohydrate; $\sum(C + L + P) = \sum(\text{Carbohydrate} + \text{Lipid} + \text{Protein})$; FAMEs = fatty acid ethyl ester. Data shown as mean ± standard deviation (n = 3).							

3.2.3 The fatty acid composition

Knothe et al. [32] reported that the FAME content in fuel directly corresponds to the fatty acid composition of the biomass feedstock, and the FAME content in turn determines the properties of the fuel. The FAMEs in *S. acuminatus* lipids mainly consisted of C18:1 (34.18%) and C16:0 (29.65%), and medium-chain fatty acids ($\leq$ C18) were the predominant fatty acids in the biodiesel, as shown in Table 2. The results also showed that unsaturated fatty acids (UFAs) were the dominant components, comprising 68.1–68.7% of the total fatty acids, in the biodiesel, and this result was similar to that obtained by Chen et al.[33]. Fuels rich in monounsaturated fatty acids (MUFAs) would have adequate cetane numbers (CNs), cold flow parameters and viscosities [34], indicating that *S. acuminatus* was an ideal biodiesel feedstock.

Table 2
Influence of spray drying conditions on the fatty acid ethyl esters (FAMES) content

Fatty acids (%)	Freeze drying	Spray drying								
	-50°C	120°C,	170°C,	220°C,	120°C,	170°C,	220°C,	120°C,	170°C,	220°C,
		16%	16%	16%	12%	12%	12%	8%	8%	8%
C16:0	8.04 ± 0.09	8.44 ± 0.22	8.52 ± 0.21	8.28 ± 0.27	8.39 ± 0.04	8.12 ± 0.02	8.17 ± 0.13	8.18 ± 0.02	8.05 ± 0.01	7.99 ± 0.02
C16:1	0.34 ± 0.02	0.32 ± 0.01	0.34 ± 0.01	0.32 ± 0.02	0.31 ± 0.01	0.31 ± 0.02	0.29 ± 0.02	0.33 ± 0.01	0.33 ± 0.01	0.29 ± 0.02
C16:2(7,10)	0.62 ± 0.01	0.66 ± 0.03	0.51 ± 0.23	0.64 ± 0.02	0.67 ± 0.02	0.47 ± 0.23	0.64 ± 0.02	0.63 ± 0.01	0.62 ± 0.01	0.63 ± 0.01
C18:0	0.54 ± 0.01	0.58 ± 0.01	0.56 ± 0.01	0.55 ± 0.01	0.56 ± 0.01	0.53 ± 0.01	0.55 ± 0.01	0.55 ± 0.01	0.55 ± 0.01	0.54 ± 0.01
C16:3(7, 10, 13)	0.73 ± 0.01	0.77 ± 0.01	0.78 ± 0.01	0.74 ± 0.02	0.76 ± 0.01	0.74 ± 0.01	0.73 ± 0.01	0.75 ± 0.03	0.72 ± 0.01	0.73 ± 0.01
C18:1-trans	9.31 ± 0.09	9.7 ± 0.22	9.81 ± 0.24	9.53 ± 0.30	9.66 ± 0.03	9.35 ± 0.02	9.41 ± 0.19	9.41 ± 0.06	9.28 ± 0.02	9.26 ± 0.02
C16:4	0.69 ± 0.03	0.7 ± 0.01	0.7 ± 0.01	0.66 ± 0.01	0.69 ± 0.02	0.66 ± 0.04	0.67 ± 0.03	0.69 ± 0.03	0.66 ± 0.01	0.63 ± 0.03
C18-2(9,12)cis	2.85 ± 0.02	2.96 ± 0.08	2.98 ± 0.06	2.79 ± 0.09	2.94 ± 0.02	2.8 ± 0.01	2.76 ± 0.05	2.89 ± 0.02	2.8 ± 0.01	2.71 ± 0.03
C18-3(9,12,15)	3.92 ± 0.05	4.08 ± 0.09	4.14 ± 0.11	3.96 ± 0.13	4.07 ± 0.03	3.92 ± 0.02	3.91 ± 0.05	3.97 ± 0.01	3.88 ± 0.01	3.84 ± 0.02
C18:4	0.31 ± 0.01	0.31 ± 0.01	0.32 ± 0.02	0.29 ± 0.01	0.31 ± 0.01	0.31 ± 0.03	0.29 ± 0.01	0.31 ± 0.01	0.31 ± 0.02	0.32 ± 0.01
Σ SFA	8.58 ± 0.09	9.01 ± 0.21	9.08 ± 0.22	8.83 ± 0.29	8.95 ± 0.05	8.65 ± 0.01	8.72 ± 0.13	8.73 ± 0.02	8.61 ± 0.02	8.62 ± 0.01
Σ UFA	18.77 ± 0.18	19.51 ± 0.42	19.58 ± 0.66	18.93 ± 0.53	19.39 ± 0.08	18.56 ± 0.34	18.7 ± 0.35	19 ± 0.13	18.6 ± 0.06	18.56 ± 0.31
Σ (SFA + MUFA)	18.23 ± 0.19	19.04 ± 0.42	19.23 ± 0.46	18.69 ± 0.56	18.91 ± 0.07	18.32 ± 0.04	18.42 ± 0.32	18.47 ± 0.06	18.22 ± 0.02	18.2 ± 0.28
Σ FAMES	27.35 ± 0.27	28.52 ± 0.63	28.66 ± 0.88	27.77 ± 0.82	28.34 ± 0.13	27.21 ± 0.33	27.42 ± 0.48	27.72 ± 0.14	27.21 ± 0.05	27.18 ± 0.02
SFA = saturated fatty acid, UFA = unsaturated fatty acid, MUFA = monounsaturated fatty acid, FAMES = fatty acid ethyl ester.										
The notation used here to describe FAs is CX: Y, where X is the length of the carbon chain and Y is the number of double bond. Data shown as mean ± standard deviation (n = 3).										

At a fixed feed rate of 8 ml min⁻¹, there were no significant changes in the carbohydrate, protein, lipid, starch or FAME contents when the inlet temperature ranged from 120°C to 220°C and the initial solid content varied from 8–16% during spray drying. These results implied that this spray-drying technique could be applied to *S. acuminatus* for the production of both biofuels and nutritional supplements.

3.3 Influence of spray-drying conditions on the pigment content

The chlorophyll a, chlorophyll b, lutein and zeaxanthin contents in spray-dried *S. acuminatus* under different drying conditions were shown in Fig. 3A, B, C, and D, respectively. At an initial solid content of 16%, the chlorophyll a content significantly decreased as the inlet temperature increased: from 1.24 mg g⁻¹ at an inlet temperature of 120°C to 0.74 mg g⁻¹ at 170°C and then to 0.41 mg g⁻¹ at 220°C (Fig. 3A). Similar temperature-dependent trends of chlorophyll a were also observed at initial dry weights of 12% and 8%. Interestingly, at the same inlet temperature, chlorophyll a decreased with decreasing initial dry weight. The highest chlorophyll a content (1.24 mg g⁻¹) was achieved at the inlet temperature of 120°C and the initial solid content of 16%.

Figure 3B showed the influence of inlet temperature and initial solid content on the content of chlorophyll b. Chlorophyll b also decreased obviously as the inlet temperature increased from 120°C to 220°C, however, the initial solid content did not have a significant effect on the chlorophyll b content. The trends of lutein with the inlet temperature for various initial solid content were identical to that of chlorophyll a, but there was a smaller range of variation (Fig. 3C). As shown in Fig. 3D, there were no significant changes in the zeaxanthin content under different drying conditions potentially because zeaxanthin was somewhat resistant to changes in heat and light but direct sunlight and high temperature applied for long periods negatively impact its amenability to spray drying [35].

In summary, with an increase in inlet temperature from 120°C to 220°C, the contents of the three pigments significantly decreased 10–50% as a result of pigment degradation at high temperatures. When the inlet temperature was maintained at 120°C, the contents of all pigments except chlorophyll b decreased obviously with the reduction in the algal biomass from 16–8%. A possible reason for this result is that as the algal biomass in the raw material increases, more agglomeration of the product occurs, resulting in protection of the pigments by the agglomerates. The results suggested that when spray drying was used for microalgal-based pigment production, the pigment content might be a useful indicator for optimization of the spray-drying process at the industrial scale, and the pigments could be protected through the formation of large particles.

The particle size distributions of *S. acuminatus* powder dried with different initial solid contents at 120°C were shown in Fig. 4A. The fraction of large particles in the dried *S. acuminatus* powder increased obviously as the initial solid content increased. Namely, aggregates formed more easily with a high initial solid content during the spray-drying process due to the high viscosity of the sprayed slurry. Figure 4B showed a microscopy image of spray-dried *S. acuminatus* powder at an initial dry weight of 16%. In addition to normal single *S. acuminatus* cells, more and larger aggregates were generated with increasing initial solid content, which agreed well with the size distribution result.

Under the same drying conditions, the increase in particle size of spray-dried products at the higher initial dry weight may be caused by the increased droplet size as a result of the higher viscosity [36]. The viscosities of *S. acuminatus* slurries with 8%, 12% and 16% initial solid content were 3.83 cP, 10.22 cP and 108.65 cP, respectively, which also indicated that the change in viscosity, rather than the initial solid content, affected the size distribution of *S. acuminatus* in the dried biomass. The shrinkage ratio decreased with increased viscosity, implying that the droplets produced by spraying slurries with higher initial solid contents and consequently higher viscosity may form a crust at an earlier stage of drying because they were more easily saturated, preventing further shrinkage upon drying [37]. As a result, the degradation of pigments inside the aggregates was reduced at higher initial solid contents, which contributed to the higher pigment content in the dried biomass.

3.4 Analysis of the thermal decomposition process

The TGA and the rate of weight loss-derivative thermogravimetry (DTG) curves of both the freeze-dried and spray-dried samples (16% solid content, dried at 220°C) were shown in Fig. 5. The rate of temperature increase was set at 10 K min⁻¹ under a N₂ atmosphere. Three individual stages were distinguished during the combustion process [38]. The

first stage extended from room temperature to 120°C and corresponded to moisture evaporation, resulting in nearly 5% weight loss. The second stage extended from 120°C to 485°C and was attributed to the release and combustion of organic compounds, leading to approximately 14% solid residue formation. During the second stage, three strong peaks at approximately 250.8°C, 300.5°C and 385.6°C were observed, which were attributed to the combustion of lipids, carbohydrates and proteins, respectively. The third stage extended from 485°C to 700°C, with the TGA curve decreasing slowly but the DTG curve remaining almost horizontal. The weight loss was much smaller than that observed in stage two, which could be a result of the continued decomposition of carbon through further breakage of C-C and C-H bonds [39]. Similar results for other microalgal species have been reported in the literature [40–43].

The outlet temperatures were below 130°C, which is below the degradation temperatures of nutritional ingredients indicated by the TGA results. In addition, both freeze drying and spray drying produced almost the same TGA curve (Fig. 5), suggesting that spray drying had no noticeable influence on the gravimetric curve of the dried biomass compared to freeze drying. As the temperature remained below the wet bulb temperature of the drying gas until drying was almost complete [44], the spray-drying conditions did not affect the quality of proteins, lipids or carbohydrates, confirming previous conclusions.

Thus, spray drying can achieve the rapid drying of microalgal biomass and has no effect on the biological components except pigment, and the degradation of pigments would be alleviated by increasing the initial solid content of the microalgal suspension. Additionally, for biodiesel and feed production, higher inlet temperatures and relatively higher dry weight concentrations were recommended to obtain lipids, carbohydrates and proteins in higher yields. However, for pigment production, reducing the inlet temperature and increasing the solid content during spray drying will yield high-quality pigment products.

4. Conclusions

This study investigated the influence of the inlet temperature and initial solid content of the spray-drying process on the moisture content, drying yield, and biochemical composition of the products. Results showed that the moisture content and drying yield presented obvious difference under various spray-drying conditions, namely, the moisture content of dried microalgal powder decreased with the increase of inlet temperature and initial solid content, while drying yield showed an opposite trend. The lowest moisture content of 2.37% with a higher drying yield of 84% was achieved at the inlet temperature of 220°C and the initial solid content of 16%. Comparison of the biochemical composition with freeze drying demonstrated that there were almost no significant differences on the total lipid, carbohydrate, protein, starch and fatty acids for spray-drying microalgal powder. However, the contents of chlorophyll a, chlorophyll b and lutein decreased with increasing inlet temperature and increased as the initial solid content increased, which indicated that pigments were sensitive to the spray-drying conditions. Pigment degradation could be alleviated by increasing the initial solid content, resulting from the protection of cell aggregates. Thermogravimetric analysis (TGA) also proved that spray drying didn't affect the quality of proteins, lipids and carbohydrates. These results may help us to understand the response of biochemical composition of microalgal biomass during the process of spray drying, and provide a reference for the selection of drying method, utilization of the nutritional components in *S. acuminatus* and selection of biochemical parameters for spray-drying performance evaluation.

Declarations

Author contributions

Haiyang Zhang conducted all the related analytical work and drafted the manuscript. Ting Gong, Jing Li and Bo Pan conducted the spray-drying experiment. Xuezhi Zhang and Ming Duan provided suggestions on the manuscript preparation and finalized the revised manuscript. Qiang Hu provided critical comments on the manuscript revision.

Ethics Approval and Consent to Participate

All authors agree with the content and all give explicit consent to submit the paper.

Conflict of interest

The authors declare no conflict of interest.

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Figures

Figure 1

Influence of spray drying conditions on the moisture content(A), outlet temperature and yield(B).

Figure 2

Influence of spray drying conditions on the CHNS distribution

Figure 3

Influence of spray drying conditions on the products pigments content: chlorophyll a (A), chlorophyll b (B), lutein (C), zeaxanthin (D).

Figure 4

Particle size distribution of the spray dried products(A) and Microscopic images of spray dried *S. acuminatus* powder(B)

Figure 5

The Thermo Gravimetric Analysis (TGA) of products: Freeze drying (-50°C for 3 days, A), Spray drying (220°C 16%, B).

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