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Effect of pH on *Rhodomonas salina* growth, biochemical composition, and taste, produced in semi-large scale under sunlight conditions

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Abstract

Rhodomonas salina is a microalgal species, belonging to the cryptophytes, and is widely used as aquaculture feed because of its high nutritional profile and phycoerythrin content. This study investigated the effect of pH on the growth, biochemical composition, and taste of *R. salina* when cultivated on a semi-large scale under natural light conditions. Two tubular photobioreactors (200 L) were used for the cultivation of *R. salina* with sunlight as the only illumination source. Two different pH setpoints were applied, 7 and 8.5. Optimal temperature and nutrient conditions were applied, according to previous research findings. The results demonstrated that the productivity of *R. salina* was higher at pH 7, 0.06–0.14 $g_{dry\ weight}\ L^{-1}\ day^{-1}$, compared to pH 8.5, 0.03–0.12 $g_{dry\ weight}\ L^{-1}\ day^{-1}$. It was found that protein and total fatty acid concentrations were higher in the biomass that was produced at pH 8.5, 33.7% and 12.3% of dry weight, respectively, while at pH 7, the protein content was 31.9% and the total fatty acids 8.8% of dry weight. The phycoerythrin concentration, like protein, was higher at pH 8.5, 2.7% of dry weight, compared to pH 7, 1% of dry weight. The free amino acid and nucleotide profile of *R. salina* was affected by the pH, resulting in increased equivalent umami concentration at pH 7. For the sensory evaluation, an expert panel on algae flavors evaluated the effect of pH on the taste of *R. salina*, reporting that the biomass that was produced at pH 7 had more umami flavor than the biomass that was produced at pH 8.5, which was evaluated as more bitter.

Keywords Cryptophyceae · Microalgae production · Photobioreactors · Semi-large scale · Biomass composition · Taste

Introduction

The United Nations reported that "Food production must double by 2050 to meet demand from world's growing population" (United Nations 2017). However, food production is responsible for 26% of global greenhouse gas emissions (Poore and Nemecek 2018). Microalgae have illustrated the potential to meet the need for more sustainable food production, especially after taking into account their ability to capture CO_2 and reduce the environmental impact (Caporgno and Mathys 2018).

Rhodomonas salina (Cryptophyceae) is of great importance in aquaculture because of its lipid profile and phycoerythrin concentration (Guevara et al. 2011; Gonzalez Araya et al. 2012; Arndt and Sommer 2014). Most aquatic animals are not able to synthesize polyunsaturated fatty acids (PUFA) which are crucial for their growth and development (Kanazawa et al. 1979; Langdon and Waldock 1981; Gonzalez Araya et al. 2012). *Rhodomonas salina* is an excellent feed because of its relatively high PUFA levels (> 60% of total fatty acids) and the combination of eicosapentaenoic acid (EPA 20:5n-3, 8% of total fatty acids) and docosahexaenoic acid (DHA 22:6n-3, 3–7% of total fatty acids) (Renaud et al. 1999; Seixas et al. 2009; Coutinho et al. 2020). The increased growth and larvae survival are attributed to the increased concentrations of EPA and DHA in their diets (Thompson et al. 1993; Helm and Bourne 2004; Brown and Blackburn 2013). Moreover, there are many studies that

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correlate the DHA to EPA ratio with the increased performance of larvae (Rodríguez et al. 1997, 1998; Pettersen et al. 2010).

Microalgae can enhance the nutritional value of the human diet with many health-promoting metabolites, such as fatty acids, amino acids, pigments, and antioxidants (Sathasivam et al. 2019). In the last 30 years, many research groups and companies have invested significant amounts of time and money to commercialize the production of microalgae (Chacón-Lee and González-Mariño 2010; Koyande et al. 2019). Despite this high interest in microalgae, they have not yet met the expectations as a major food source.

One of the bottlenecks in the commercialized production of microalgae is the high production cost. Most research that has been conducted in recent years has been carried out on a laboratory scale under constant, controlled conditions. The transition from laboratory to commercial scale production needs to be studied in detail. Norsker et al. (2011) reported that in tubular photobioreactors, a photosynthetic efficiency increase from 3 to 5% can lead to a 35% reduction of the production cost. The cost of pure CO_2 injection represents from 39 up to 71% of the raw material cost, after taking into account the availability of the CO_2 and the separation from flue gas (Acién et al. 2012). In tubular photobioreactors, CO_2 cost is estimated up to 2.7 € kg^{-1} of dry weight, while in raceway ponds, it can reach 4.6 € kg^{-1} of dry weight, due to the poor CO_2 fixation performance of microalgae in open systems (Norsker et al. 2011; Slade and Bauen 2013).

The taste, color, odor, and texture of microalgae biomass have been identified as another potential bottleneck for the use of microalgae in food products. Therefore, currently most commercialized products of microalgae are available as food supplements in the forms of tablets and capsules, or mixed with other products, such as pastes, pasta, or beverages to mask their flavor (Yamaguchi 1996; Liang et al. 2004; Pulz and Gross 2004). Microalgae pigments can also be used in the food industry. For instance, phycocyanin has been used as additive in biscuits and beverages (Dufossé et al. 2005; Koyande et al. 2019). Phycoerythrin, which is a red, water-soluble phycobiliprotein, can serve as a natural colorant in food, gelatin desserts, and dairy products (Dufossé et al. 2005).

We recently showed that *R. salina* is rich in umami flavor, according to the estimated equivalent umami concentration (EUC), which is affected by pH and salinity changes (Latsos et al. 2021a, b). As the next step, this study aims to demonstrate the semi-large scale production of *R. salina* under sunlight conditions and to investigate the effect of pH on biomass productivity, biochemical composition, and taste. Two different pH setpoints were applied, 7 and 8.5. At pH 7, *R. salina* has previously shown optimal growth on small scale, while at pH 8.5, the highest EUC was obtained for *R. salina* (Latsos et al. 2021a, b).

Materials and methods

Strain and pre-culture

The marine cryptophyte *Rhodomonas salina* CCMP1319 was supplied by the Dutch aquaculture industry and pre-cultured in pre-sterilized (20 min at 120 °C) 300 mL Erlenmeyer flasks in 10 times concentrated nutrients of filtered (0.2 µm pore size) f/2 medium (Guillard and Hargraves 1993) with a salinity of 30 g L^{-1}. Cultures were continuously illuminated at a photon flux density (PFD) of 120 µmol photons m^{-2} s^{-1} provided by cool white fluorescent tubes. The temperature in the incubator was maintained at 20 ± 1 °C and air enriched with 5% CO_2 v/v_{air} was supplied in the headspace of the Erlenmeyer flasks.

Experimental setup

Two tubular photobioreactors (LGem MK1-200, LGem, the Netherlands) were used for *R. salina* cultivation, located in Vlissingen, the Netherlands (Fig. 1). An air pump with a 4 L min^{-1} filtered airflow (2 and 0.2 µm pore size) was responsible for the recirculation of the culture in the reactor. The pH was maintained at 7.1 ± 0.3 and 8.5 ± 0.3 for each reactor, respectively, by on-demand CO_2 addition in the airflow of the reactor. The temperature of both reactors was maintained at 21 ± 1 °C by recirculation of hot or cold water over a heat exchanger in the reactor tank. The water was provided by a heat pump (30RQ 017 CHE, Carrier, the Netherlands). Sunlight was the only source of light and was measured by photosynthetically active radiation (PAR) sensor on top of the reactors. The temperature, pH, and light intensity were measured online by a reactor control system (APEX, Fusion, Neptune Systems, USA).

The photobioreactors were operated in chemostat mode with a dilution of 5.5 ± 0.5 L h^{-1}. Dilution took place only for the period when the sunlight was illuminating the reactors, being approximately 11 to 14 h per day, corresponding to a dilution rate of 0.30–0.42 day^{-1}. The dilution was in

Fig. 1 Tubular photobioreactors located in Vlissingen and operated with *R. salina*

line with previous research on cultivation of *Rhodomonas* in outdoor conditions (Oostlander et al. 2020a). The dilution was performed with filtered (0.5 µm and 0.2 µm—SupaPleat Plus, Amazon Filters, UK) saline groundwater with the addition of adequate levels of nutrients by peristaltic pumps. After the addition of nutrients, the groundwater was equal to 10 times concentrated f/2 medium. The photobioreactors were operated from April till July in multiple reactor runs.

Daily measurements

Daily samples were taken from each photobioreactor for the measurement of optical and cell density. The optical density was measured at 750 nm (OD_{750nm}), from which biomass concentration (C_x) was calculated according to Oostlander et al. (2020b). The cell density was measured in Coulter Counter (Multisizer 3, Beckman Coulter, USA) in the size range 7–14 µm.

During the chemostat mode, the volumetric biomass productivity (r_x) was calculated in g L^{-1} day^{-1} according to Eq. 1, where V_H is the harvested volume between two measurements in a Δt period of time and V_R the reactor volume.

$$r_x = \frac{C_x * V_H}{V_R * \Delta_t} \quad (1)$$

Cell composition

The *R. salina* biomass was harvested from the growth medium using the Membrane Algae Filtration (MAF) technology (VITO, Belgium) (Fig. 2). The MAF technology is a submerged membrane filtration (UF membranes) approach where permeate (water and salts) is removed via under pressure resulting in low shear forces on the algal biomass. The latter aspect allows harvesting of fragile cells without inducing cell disruption. The MAF was operated in continuous mode following the dilution rate described in "Experimental setup" section. Each 2–3 days, after dewatering (volume concentration factors up to 100 were reached), the cells were desalted with the MAF till the electric conductivity (EC) was reduced from 38–42 mS cm^{-1} till below 1–2 mS cm^{-1}. The desalted biomass was subsequently freeze-dried (Christ, gamma 1–16 LSC).

Cell disruption was performed in a sonication bath. Subsequently, protein content was determined by the Folin method (Lowry et al. 1951). Fatty acids (FA) were extracted and quantified according to Breuer et al. (2012). The lipids were extracted using a chloroform: methanol (1:1.25 v/v) mixture and methylated in a MeOH solution containing 5% H_2SO_4 for 3 h at 70 °C in a block heater. The fatty acid methyl esters (FAME) were analyzed and quantified by means of gas chromatography mass spectrometry using a Thermo Finnigan TraceGC ultra system/DSQ equipped with a SGE Analytical Science BPX70 column (50 m × 0.32 mm; 0.25 μm) with helium as carrier gas. FAME were identified based on retention time data of known standards. Heptadecanoic triacylglycerol (C17:0 TAG) was added as the internal standard for fatty acid quantification. Total fatty acids (TFA) were calculated as the sum of all individual fatty acids. FA were also expressed as saturated fatty acids (SFA), monounsaturated fatty acids (MFA), PUFA, and DHA/EPA ratio.

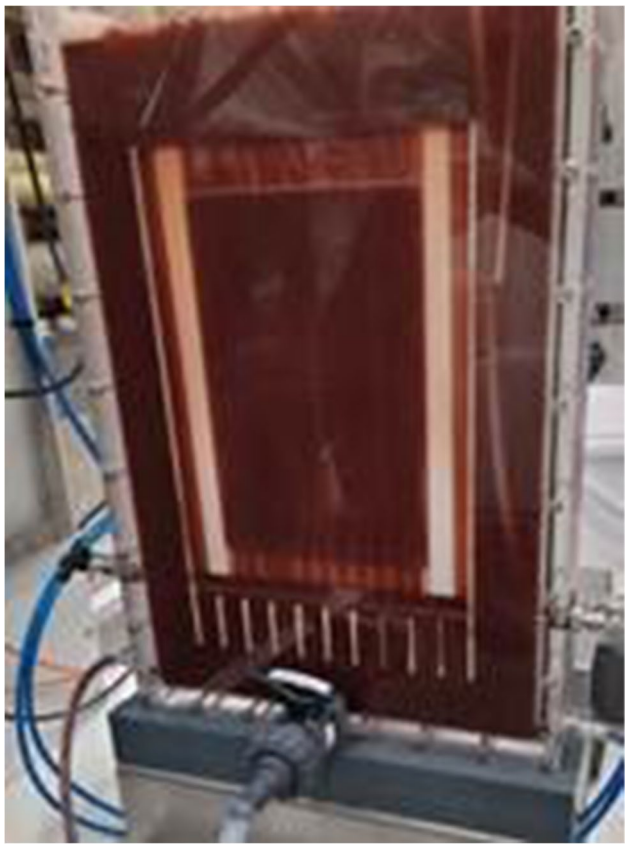

Fig. 2 Membrane algae filtration during the harvesting of *R. salina*

Phycoerythrin was extracted in phosphate buffer by a freeze-thawing process, centrifuged (10 min at 2500 rpm), and analyzed using UV–vis spectroscopy according to Bennett and Bogarad (1973; Lawrenz et al. 2011). Equation 2 was used for the calculation of phycoerythrin (Lawrenz et al. 2011).

$$PE = \frac{A}{\varepsilon d} \times MW \times \frac{V_{sample}}{V_{buffer}} \times 10^6 \quad (2)$$

where A is the absorbance at 545 nm after scatter corrected by subtracting the absorbance at 750 nm, ε is the molar extinction coefficient of phycoerythrin (2.41×10^6 L mol^{-1} cm^{-1}), MW is the molecular weight of phycoerythrin

(240,000 g mol^{-1}), d is the path length in cm, and V_{sample} and V_{buffer} are the volumes of the sample and the buffer, respectively.

Free amino acids (FAA) and nucleotides were extracted and analyzed according to Moerdijk-Poortvliet et al. (2022). In brief, 15 mg of biomass and 5 mL Milli-Q were homogenized and extracted for 15 min at 35 °C followed by centrifugation (3000 rpm, 20 min). A 3 mL sample of supernatant was taken and an acid precipitation step with 600 µL of 37% HCl was performed to remove contaminants. For FAA, an additional purification step was performed using DOWEX 50WX8 cation exchange resin. FAA and nucleotide samples were analyzed by means of high-performance liquid chromatography (HPLC) using a DIONEX Ultimate 3000 HPLC system. For FAA analysis, the HPLC was equipped with an Agilent InfinityLab Poroshell 120 HPH-C18 column (100 × 4.6 mm; 2.7 µm), matching guard column (5 × 4.6 mm; 2.7 µm), and a fluorescence detector (FLD 3100). For nucleotide analysis, the HPLC was equipped with a SIELC PrimeSep D mixed-mode column (150 × 4.6 mm; 5 µm) with a corresponding guard column (10 × 4.6 mm; 5 µm) and an ultraviolet detector (DAD 3000).

Taste activity value and EUC

The contribution of FAA and nucleotides to the overall taste was evaluated with the determination of their taste activity value (TAV). The TAV was calculated as reported by Kato et al. (1989) according to the following equation:

$$TAV = {C_1}/{C_2} \tag{3}$$

where C_1 is the concentration of the taste compounds in mg g^{-1} dry weight, and C_2 is the threshold concentration in mg g^{-1} dry weight, the minimum concentration at which a taste compound can be perceived. When the TAV is more than 1, it is considered that the compound contributes to the overall taste. The taste threshold concentrations that were used are based on Duan et al. (2020).

The umami estimation can be determined based on the concentration of monosodium glutamate (mg MSG g^{-1}), which is equivalent to the umami intensity. The synergy effect between the mixture MSG-like AAs and 5′-nucleotides is represented by Eq. 4 (Yamaguchi et al. 1971):

$$Y = \sum a_i b_i + 12.18\left(\sum a_i b_i\right)\left(\sum a_j b_j\right) \tag{4}$$

where Y is the EUC (mg MSG g^{-1}); a_i is the concentration (mg g^{-1}) of each umami AA (aspartic or glutamic acid); a_j is the concentration (mg g^{-1}) of each umami 5′-nucleotide, inosine 5′-monophosphate (IMP), guanosine 5′-monophosphate (GMP), or adenosine 5′-monophosphate (AMP); b_i is the relative umami concentration (RUC) for each umami AA to MSG (1 for glutamic acid, and 0.077 for aspartic acid); and b_j is the RUC for each umami 5′-nucleotide to IMP (1 for IMP, 2.3 for GMP, and 0.18 for AMP). 12.18 is the synergistic constant based on the concentration of mg g^{-1} used.

Ethical approval

All algae samples, meant for the expert panel, were evaluated on chemical safety, including cadmium (Cd), lead (Pb), mercury (Hg), total and inorganic arsenic (As) and iodine (I), and tested for pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Listeria* spp., *Listeria monocytogenes*, yeast, and molds. Prior to tasting, the safety results were evaluated and approved by the ethical standards of the committee responsible for human experimentation (ECSG-ILVO board).

Sensory evaluation

Descriptive sensory analyses on the algae were performed using an internally trained expert panel on algae flavors. The panel consisted of 8 assessors who are able to distinguish basic tastes and specific taste attributes for *R. salina*. Training of the selected panelists was performed according to ISO8586, by which they were familiarized with the following characteristic tastes: (1) salt; (2) bitter; (3) umami; (4) seafood taste (crab); and (5) earthy (beetroot) taste (Table 1). These tastes were selected and scored using a scale ranging from 0 (absent) to 10 (very strong). Samples were prepared by homogenizing 10 g freeze-dried *R. salina* in 1 L of bottled water (crystalline). Fresh *R. salina* solutions were presented to each panelist in randomly coded and closed 20 mL screw-capped amber colored glass vials.

Data analysis and statistics

Analysis of covariance (ANCOVA) was applied to the productivity data for the comparison of productivity regression lines over light intensity. All the cell composition data and the sensory evaluation results were analyzed by multiple *t*-tests to determine whether pH has a significant effect on

Table 1 List of taste sensory attributes with descriptions selected for quantitative descriptive analysis as a result of preliminary sessions. Between brackets is the used reference

Sensory attributes	Associated taste (reference)
Earthy	Beetroot (pure beetroot juice)
Seafood	Crab ($10g_{dry}$ weight L^{-1} crab powder *Cancer pagurus*)
Salt	Salt (1.19 g L^{-1} NaCl)
Bitter	Caffeine (0.195 g L^{-1} caffeine)
Umami	Monosodium glutamate (0.595 g L^{-1} MSG)

the cellular composition of *R. salina*. Statistical analysis was performed using SPSS 25.0 statistical package (SPSS Inc., USA) and Prism 8.0.2 (GraphPad, USA).

Results

Productivity

Rhodomonas salina had higher daily productivity at pH 7 compared to pH 8.5 (Fig. 3). At pH 7, the productivity was increased in line with the increase of the light intensity, ranging from 0.055 to 0.137 g L^{-1} day^{-1}, in low and high light intensity, respectively. At pH 8.5, the increase of light intensity did not influence the productivity, which varied from 0.034 to 0.120 g L^{-1} day^{-1}. The regression lines of the datasets of the two different pH setpoints did not show any significant difference ($p = 0.104$). It is worth mentioning that there was no productivity data at pH 8.5 in light intensities higher than 33 mol photons m^{-2} day^{-1}. In these light intensities, the algal biomass in the reactor collapsed.

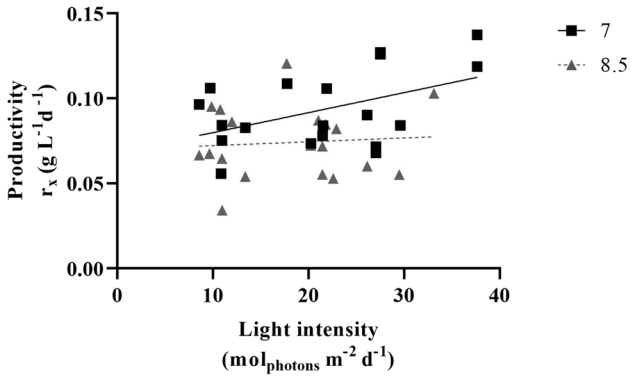

Fig. 3 Biomass productivity of *R. salina* cultivated under sunlight conditions at pH 7 (black squares) and 8.5 (grey triangles)

Cellular composition

The cellular composition of *R. salina* was examined from the biomass that was produced by the photobioreactors after going through the downstream steps that are mentioned in "Materials and methods." The protein concentration of the *R. salina* biomass produced at pH 8.5 was significantly higher than the protein concentration at pH 7, 33.7 ± 0.6% and 31.9 ± 0.2% of the dry weight, respectively ($p = 0.02$) (Fig. 4A). A similar increase was observed at the phycoerythrin content of *R. salina*. At pH 8.5, the phycoerythrin concentration was 2.7% of dry weight, while at pH 7, the phycoerythrin concentration was 1% of dry weight (Fig. 4B). Due to the limited samples ($n = 3$), this difference was not statistically significant ($p = 0.13$).

The main fatty acids of *R. salina* cultivated in both pH setpoints were α-linolenic (18:3n-3), stearidonic (18:4n-3), palmitic (16:0), and linoleic (18:2n-6) (Table 2). However, at pH 8.5, *R. salina* had higher TFA concentration, 12.3% of dry weight, compared to pH 7, 8.8% of dry weight (Fig. 5A). This is a result of increased concentration of saturated, unsaturated, and polyunsaturated fatty acids at pH 8.5. EPA and DHA are also increased at pH 8.5, 0.8% and 0.3% of dry weight, respectively, compared to pH 7, 0.6% and 0.2% of dry weight (Fig. 5B). The DHA to EPA ratio was not

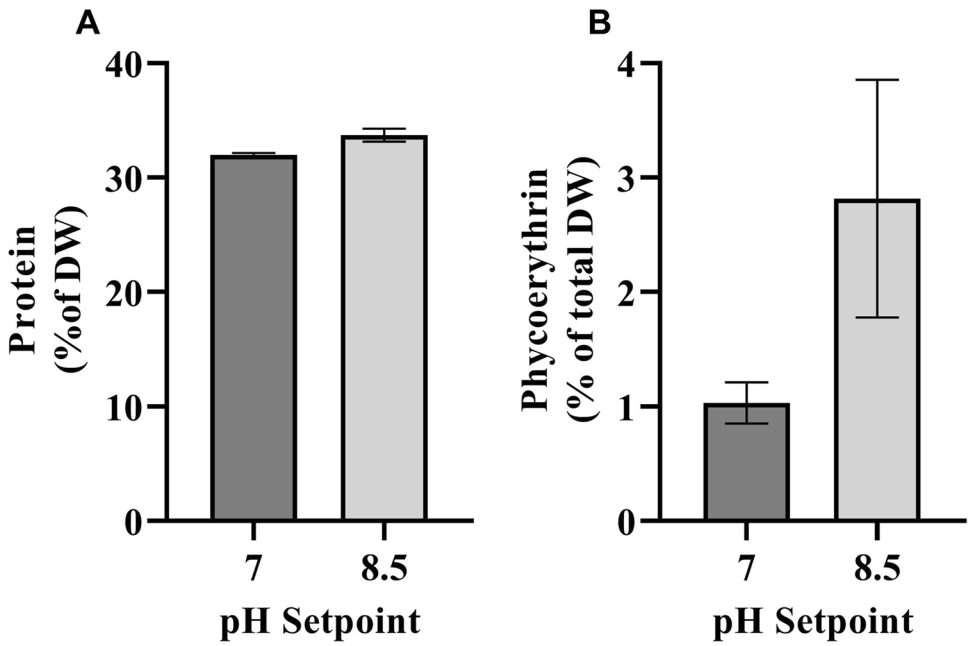

Fig. 4 Protein (**A**) and phycoerythrin (**B**) concentration of *R. salina* cultivated at pH 7 (dark grey) and pH 8.5 (light grey), expressed in % of total dry weight ($n = 3$)

Table 2 Fatty acid content (mg g^{-1} dry weight) of *R. salina* cultivated at two pH setpoints, 7 and 8.5. Data are expressed as the average and standard deviation of three replicates

C	pH 7		pH 8.5	
	Average	STDEV	Average	STDEV
14:0	17.80	2.39	27.61	7.58
12:0	0.15	0.10	0.12	0.02
16:0	28.81	2.10	35.42	0.00
16:1n-7	0.82	0.14	1.09	0.11
16:2n-4	0.24	0.04	0.40	0.11
16:3n-3	0.11	0.09	0.25	0.19
18:0	0.82	0.04	1.45	0.26
18:1n-9	1.88	0.40	2.36	0.40
18:2n-6	10.47	1.43	13.61	1.13
19:0	1.10	0.33	1.59	0.41
18:3n-3	9.16	2.16	15.14	3.44
18:4n-3	7.79	1.57	10.22	1.95
20:1n-9	0.30	0.12	0.75	0.42
20:5n-3 (EPA)	5.58	1.38	7.58	1.97
22:2n-6	0.12	0.03	0.34	0.16
22:5n-6	1.15	0.26	2.07	0.61
22:6n-3 (DHA)	1.91	0.43	2.85	0.97
Total	88.23	8.23	122.85	17.73
SFA	48.69	0.18	66.19	8.24
MUFA	3.01	0.73	4.20	1.21
PUFA	36.53	7.33	52.46	8.28

significantly different between the two pH setpoints, 0.34 at pH 7 and 0.36 at pH 8.5.

Taste active value and equivalent umami concentration

The main free amino acids extracted from *R. salina* were the L-proline, L-glutamic acid, and L-arginine (Table 3). The biomass cultivated at pH 7 had a significantly higher concentration of L-aspartic acid and L-asparagine than at pH 8.5. *Rhodomnas salina* grown at pH 8.5 showed significantly increased concentrations of L-serine, L-glutamine, L-arginine, and L-leucine. The total free amino acid concentration was higher at pH 8.5 in comparison with pH 7, 4.28 and 3.88 mg g^{-1} dry weight, respectively ($p = 0.038$). L-glutamic acid was the only FAA with TAV greater than 1 in the biomass that was produced at pH 7. At pH 8.5, there were two FAA with TAV higher than 1, L-glutamic acid and L-arginine.

All the free nucleotides were significantly increased at pH 7 compared to pH 8.5, except for AMP, which was stable under both pH conditions (Table 4). The increased nucleotide concentration at pH 7, in combination with the increased aspartic and glutamic acid concentration, led to significantly increased equivalent umami concentration, 136 mg MSG g^{-1} of dry weight, compared to 78 mg MSG g^{-1} dry weight at pH 8.5.

Sensory evaluation

Rhodomonas salina biomass that was produced at pH 7 was evaluated by the taste panel with significantly higher umami taste (Fig. 6). *Rhodomonas salina* that was cultivated at pH 8.5 was characterized by a more bitter and earthy taste. Other attributes did not show any significant differences in the evaluation.

Discussion

Productivity

This study investigated the influence of pH in the growth, biochemical composition, and taste of *R. salina* which was successfully cultivated on a semi-large scale under natural sunlight conditions with productivity varying from 0.034 to 0.137 g L^{-1} day^{-1}. The highest productivity that has been reported in continuous culture for *R. salina* is 1.17 g L^{-1} day^{-1} (Latsos et al. 2021a). However, it is noteworthy that this high productivity was obtained in a small scale system (0.4 L) and under constant artificial light conditions

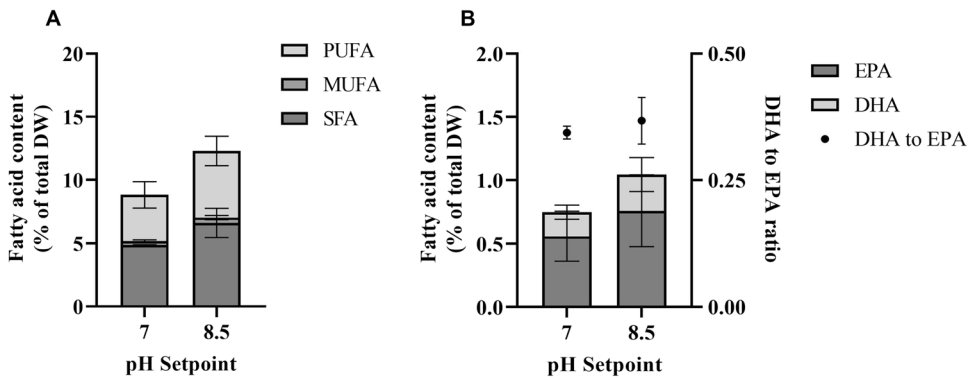

Fig. 5 **A** Saturate (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) and **B** eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and DHA to EPA ratio of *R. salina* at pH 7 (dark grey) and 8.5 (light grey), expressed in % of total dry weight

Table 3 Free amino acid content (mg g^{-1} dry weight) of *R. salina* cultivated at two pH setpoints, 7 and 8.5. (*Indicates the significant difference after *t*-test of pairwise comparison between the pH setpoints $*p<0.05$, $**p<0.01$)

Free amino acid	Concentration (mg g^{-1} dry weight)		Significance	Taste threshold (mg g^{-1} dry weight)	TAV		Taste attribute[1]
	pH 7	pH 8.5			pH 7	pH 8.5	
L-aspartic acid	0.19	0.07	**Yes (**)**	1	0.19	0.07	**Umami (+)**
L-glutamic acid	0.78	0.64	No	0.3	**2.60**	**2.12**	Umami (+)
L-asparagine	0.18	0.10	**Yes (*)**	-	**-**	**-**	-
L-serine	0.07	0.11	**Yes (*)**	1.5	0.05	0.07	**Sweet (+)**
L-glutamine	0.08 ±	0.20	**Yes (**)**	-	**-**	**-**	-
L-histidine	0.06	0.05	No	0.2	0.29	0.27	Bitter (-)
Glycine	0.05	0.07	No	1.3	0.04	0.05	Sweet (+)
L-threonine	0.07	0.06	No	2.6	0.03	0.02	Sweet (+)
L-arginine	0.35	0.61	**Yes (*)**	0.5	0.70	**1.21**	**Bitter/sweet (+)**
L-alanine	0.20	0.24	No	0.6	0.34	0.40	Sweet (+)
L-tyrosine	0.13	0.16	No	8.8	0.01	0.02	Bitter (-)
L-cystine	0.03	0.03	No	-	-	-	Bitter/sweet (-)
L-valine	0.11	0.14	No	0.4	0.29	0.35	Sweet/bitter (-)
L-tryptophan	0.02	0.05	No	-	-	-	Bitter (-)
L-leucine	0.21	0.30	**Yes (*)**	1.9	0.11	0.16	**Bitter (-)**
L-lysine	0.17	0.17	No	0.5	0.33	0.33	Sweet/bitter (-)
L-proline	1.17	1.29	No	3	0.39	0.43	Sweet/bitter (+)
Total	3.88	4.28	**Yes (*)**				

[1]Taste attribute (+ = pleasant,— = unpleasant) (Kato et al. 1989; Liu et al. 2019)

Table 4 Free nucleotide content (mg g^{-1} dry weight) and equivalent umami concentration (mg MSG g^{-1} dry weight) of *R. salina* cultivated at two pH setpoints, 7 and 8.5. (*Indicates the significant difference after *t*-test of pairwise comparison between the pH setpoints $*p<0.05$, $**p<0.01$, $***p<0.001$)

Free nucleotides	Concentration (mg g^{-1} dry weight)		Significance
	pH 7	pH 8.5	
Cytidine 5′-monophosphate (CMP)	1.61	1.11	**Yes (**)**
Adenosine 5′-monophosphate (AMP)	0.35	0.36	No
Uridine 5′-monophosphate (UMP)	0.18	0.12	**Yes (**)**
Guanosine 5′-monophosphate (GMP)	1.52	0.95	**Yes (***)**
Inosine 5′-monophosphate (IMP)	1.34	0.92	**Yes (**)**
Total	5.01	3.45	**Yes (**)**
EUC	135.9	77.7	**Yes (***)**

and 24 h light per day. The productivity values obtained in our current study are in line with the values that have been reported in the literature for *R. salina* cultivation in tubular photobioreactors (Oostlander et al. 2020a; Thoisen et al. 2020). Oostlander et al. (2020a) obtained higher productivity values (0.1–0.19 g L^{-1} day^{-1}) for *Rhodomonas* sp., compared to this study, under sunlight conditions, which is the result of higher biomass concentration in the reactors. Thoisen et al. (2020) reported productivity values 0.02–0.1 g L^{-1} day^{-1} for light intensities of 8.6–21 mol photons m^{-2} day^{-1} of artificial light.

The productivity of *R. salina* was higher at pH 7 in comparison with pH 8.5. This result is analogous to the literature, in which many studies have been reported pH 7 as the optimal for the growth and the production rate of *Rhodomonas* (Berge et al. 2010; Latsos et al. 2021a). At pH 8.5, the productivity was lower, while in high light intensities (> 35 mol photons m^{-2} day^{-1}), the biomass in the reactor collapsed. This lower productivity and instability of the reactor at pH 8.5 can be explained by the limited availability of CO_2 (Chenl and Durbin 1994). Latsos et al. (2021a, b) and Hansen (2002) illustrated in their studies that the growth of *R. salina* is reduced at pH 8.5 and 9. At high pH conditions, the concentration of reactive oxygen species (ROS) is also increased, which can overcome the antioxidant defenses of the cells and increase the possibility of cell damage (Liu et al. 2007).

Cellular composition

The cell composition of *R. salina* was affected by the different pH's at which it was cultivated. The protein concentration was higher at pH 8.5, which is in line with the literature. Qiu et al. (2017) reported higher protein content in *Chlorella sorokiniana* biomass produced at higher pH. Similar findings

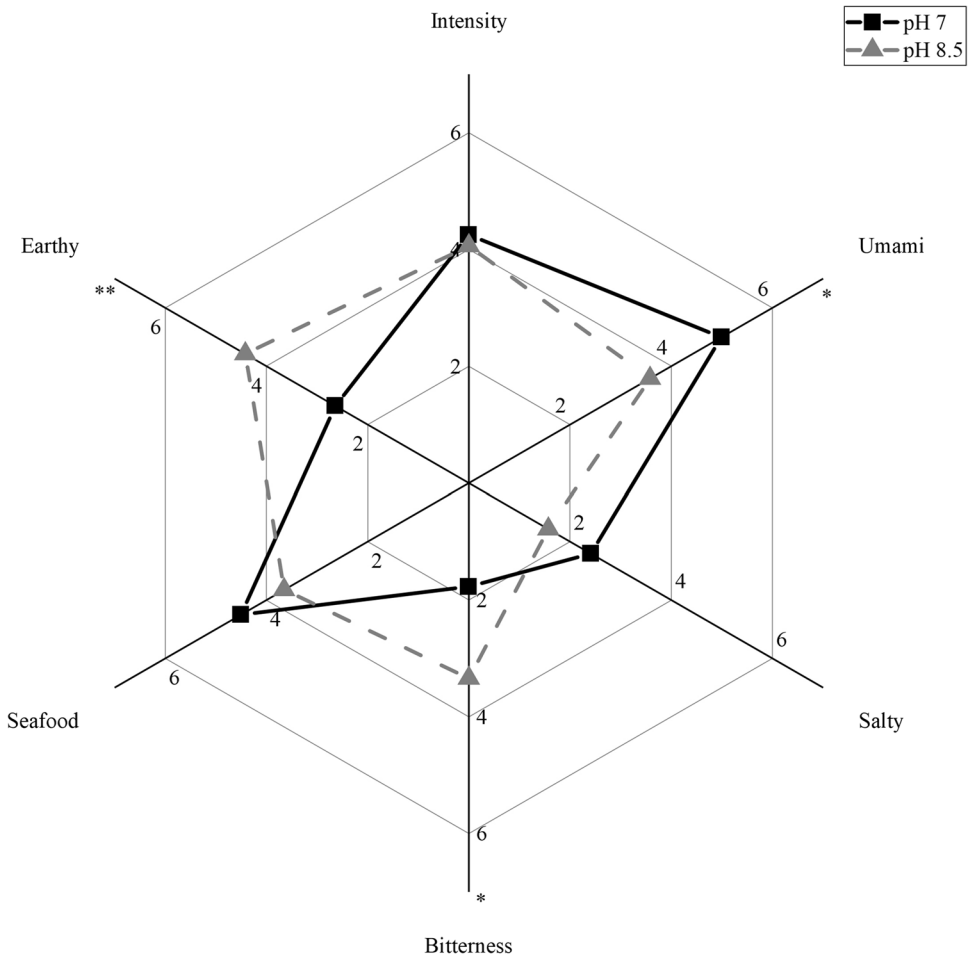

Fig. 6 Descriptive taste panel results of *R. salina* cultivated at pH 7 (dark grey) and pH 8.5 (light grey). These tastes were scored using a scale ranging from 0 (absent) to 10 (very strong). (*Indicates the significant difference after *t*-test of pairwise comparison between the pH setpoints $*p < 0.05$, $**p < 0.01$)

for higher protein concentration at higher pH have been observed for *Spirulina* sp. (Ogbonda et al. 2007). However, *Dunaliella salina* presented lower protein concentration in higher pH (Sui and Vlaeminck 2019). The higher protein content at pH 8.5 can be explained by the higher phycoerythrin content. Phycoerythrin constitutes 12 to 20% of the total protein content (Seixas et al. 2009; Barka and Blecker 2016). The increased phycoerythrin concentration at pH 8.5 is in line with previous literature on *R. salina* (Latsos et al. 2021a). This increase of phycoerythrin can be associated with the antioxidant activity of the pigment as a response to the ROS increase at alkaline conditions (Samarakoon and Jeon 2012; Jung et al. 2016; Gauthier et al. 2020).

The cellular protein content of *Rhodomonas* species varies from 30 to more than 50%, depending on the culture conditions (Renaud et al. 1999; Seixas et al. 2009; Yamamoto et al. 2020). In this study, the protein concentration is lower compared to the literature values. Phycoerythrin concentration of this study is also lower than the reported values in the literature (Seixas et al. 2009; Yamamoto et al. 2020; Latsos et al. 2021b, a). This phenomenon may be explained by the harvesting and desalting steps of our current experiment. In previous studies, it was reported that the experiments were conducted on a laboratory scale and the biomass processing steps did not take as long as the desalting step of our study. Processing of the *R. salina* biomass took several hours, during which time phycoerythrin/protein losses were sometimes observed at the end of the diafiltration. Not only the harvesting and drying steps but also the storage can affect the quality of the biomass (de Farias Neves et al. 2020; Verspreet et al. 2020; Castro-muñoz 2021). The impact of subsequent freezing and freeze-drying, especially on phycoerythrin, required further attention and loss of colors has been observed in some occasions. Marcati et al. (2014) investigated the recovery of B-phycoerythrin from *Porphyridium* using a pilot scale filtration unit, achieving up to 48% recovery of the phycobiliprotein. The microalgal cells may break during the harvesting step in pilot scale, due to the shear force of centrifuge or filtration. However, this did not happen in our experiment and the cells remained intact after the filtration step. The biomass staying out of the culture conditions, such as lower salinity and temperature, and no pH control, could explain the loss of pigmentation and consequently the lower protein concentration. By using a MAF filtration

device which capacity is tailored to harvested amounts (for instance, smaller MAF or higher harvested amounts), the dewatering and desalting time can be reduced.

The major fatty acids of *R. salina* cultivated in both pH setpoints were α-linolenic (18:3n-3), stearidonic (18:4n-3), palmitic (16:0), and linoleic (18:2n-6) acids. This observation is in line with the literature results for *Rhodomonas* species (van Houcke et al. 2017; Coutinho et al. 2020; Latsos et al. 2020). The increase of the pH from 7.0 to 8.5 induced a 1.4-fold increase of SFA, MUFA, and PUFA of *R. salina*. There is extensive literature that supports the increase of the lipid content in many microalgae with the increase of pH. For instance, *Tisochrysis lutea*, *Chlamydomonas reinhardtii*, and *Chlorella* sp. increased their lipid content, especially the PUFA concentration, as a defense mechanism against the alkaline pH stress (Paliwal et al. 2017; Ochoa-Alfaro et al. 2019; Almutairi et al. 2020).

Taste active value and equivalent umami concentration

The main free amino acids in *R. salina* were L-proline, L-glutamic acid, and L-arginine. These results are in agreement with the literature for *R. salina* (Seixas et al. 2009; van Houcke et al. 2017; Latsos et al. 2021a). The increase of pH appears to positively affect the total free amino acid concentration, a finding that has been observed previously (Latsos et al. 2021a). However, the free 5′-nucleotide concentration, and therefore the EUC, was significantly higher at pH 7. This contradicts the study of Latsos et al. (2021a) in which the EUC showed a peak at pH 8.5. There is lack of knowledge on how microalgae change their free amino acid and nucleotide profile as a reaction to changes in cultivation conditions. An explanation might be the scale difference of the experiment, considering that the research of Latsos et al. (2021a) was conducted in 400 mL photobioreactors. There is extensive literature on how scale-up can affect the composition of microalgae (Torzillo and Vonshak 2013; da Silva and Reis 2015; Borowitzka 2016; Borowitzka and Vonshak 2017). In our study, the sunlight was used as only illumination source and, as a result, the light intensity was not stable compared to the laboratory experiment. The fact that in the outdoor scale-up culture, the illumination is not continuous but has a day/night period can affect circadian rhythms and biological functions such as photosynthesis, pigments synthesis, and nitrogen fixation (Corellou et al. 2009; Sorek et al. 2013; Braun et al. 2014). Another explanation could be the desalting step, which took longer in this study than at laboratory scale, and the biomass might overstayed in low salinity, resulting to osmoregulation and changes in biochemical composition.

Sensory evaluation

The expert panel associated the changes in free amino acid and nucleotide profile with the taste of *R. salina*. The biomass that was produced at pH 7 was evaluated with a higher umami taste than the biomass that was produced at pH 8.5. This result is in accordance with the EUC that was calculated and can be explained by the higher L-aspartic acid, L-glutamic acid, GMP, IMP, and AMP at pH 7. *Rhodomonas salina* cultivated at pH 8.5 was characterized as more bitter than at pH 7. The bitterness may be attributed to the increased L-arginine, L-leucine, L-tryptophan, and L-tyrosine, amino acids that are associated with the bitter taste (Kato et al. 1989; Nishimura and Kato 2009). Specifically, the TAV of L-arginine was greater than 1 at pH 8.5, while at pH 7, it was 0.7. The increased fatty acid concentration at pH 8.5 may have some effect on the bitter taste as well. For instance, linoleic acid, which is 1.3 times higher in *R. salina* at pH 8.5, is associated with a bitter flavor (Ledahudec and Pokorný 1991; Khan et al. 2019). Moreover, microalgae under pH stress are known to accumulate polyphenols (Gauthier et al. 2020). These molecules, even though not measured in our study, may also explain the bitter taste at pH 8.5 because bitterness is a major sensory attribute of several common foods and beverages rich in polyphenol compounds (Drewnowski and Gomez-Carneros 2000; Soares et al. 2013).

Conclusion

In this study, *R. salina* was successfully cultivated on semi-large scale (200 L) at two different pH setpoints, under natural sunlight conditions. The productivity was in general agreement with previous research at the pilot laboratory scale. Our results show that the increase of pH from 7 to 8.5 has a significant effect on the productivity and the quality of *R. salina*. The productivity was higher at pH 7 than at pH 8.5. However, there is still potential for improvement of the (semi-)large scale production after considering the higher productivity values obtained at the laboratory scale and indoor conditions. The increase of pH to 8.5 induced the accumulation of lipids and phycoerythrin as a response to the pH stress. The change of the quality of *R. salina* due to pH settings affected the taste of the biomass, characterized by a higher umami taste at pH 7 and a higher bitter taste at pH 8.5. Our study provides the framework for future studies to assess the connection of the biomass composition of microalgae with their taste.

Another aspect of the study was to observe how the quality of *R. salina* can be manipulated. In many cases, contradictory conditions are required for the production of biomass

and synthesis of target metabolites. The cultivation of microalgae in two distinct stages can offer a solution to this problem. In the first step, optimal growth conditions are provided to achieve high biomass productivities. This step is followed by the second stage, where cells are exposed to stress conditions in order to accumulate target metabolites.

Author contribution C. Latsos: conceptualization, implementation, investigation, formal analysis, writing original draft, writing — review and editing. J. van Houcke: conceptualization, writing — review and editing, supervision. L. Bastiaens: formal analysis, writing — review and editing. S. van Roy: formal analysis, writing — review and editing. B. Coleman: formal analysis, writing — review and editing. J. Robbens: formal analysis, writing — review and editing, supervision. T. Moerdijk: formal analysis, writing — review and editing. E. Wassenaar: implementation, formal analysis, writing — review and editing. K. R. Timmermans: conceptualization, writing — review and editing, supervision.

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Availability of data and material Full data is available after a request of the reviewers.

Declarations

Conflict of interest The authors declare no competing interests.

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