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Microalgae of interest as food source: Biochemical composition and digestibility

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ABSTRACT

Microalgae are considered a very interesting source for the development of new food products and can be used to enhance the nutritional value of conventional foods, due to their valuable biochemical composition.

The aim of this study was to investigate the biochemical composition, the fatty acid profile and the *in vitro* digestibility of twelve microalgal biomasses (*Arthrospira platensis* F&M-C256; a bloom mainly composed of *Aphanizomenon flos-aquae* from Klamath Lake; *Nostoc sphaeroides* F&M-C117; *Chlorella sorokiniana* F&M-M49; *Chlorella sorokiniana* IAM C-212; *Chlorella vulgaris* Allma; *Tetraselmis suecica* F&M-M33, in nutrient replete medium and starved; *Porphyridium purpureum* F&M-M46; *Phaeodactylum tricornutum* F&M-M40; *Tisochrysis lutea* F&M-M36; *Nannochloropsis oceanica* F&M-M24) of interest as food source.

The three cyanobacteria and the *Chlorella* species presented high protein (50–65%) and low lipid (5–20%) content. A high fiber content (14–17%) was found in *T. suecica* grown in nutrient replete medium, *P. purpureum* and *P. tricornutum*.

Biomasses of marine species contained high concentrations of polyunsaturated fatty acids, mainly C20:5ω3 and C22:6ω3, along with substantial amounts of C16:1ω7, C18:1ω9 and C16:0. The freshwater algae contained high amounts of C18:3ω3 and an even higher amount of C16:0.

A. platensis, *C. sorokiniana* IAM-C212 and *C. vulgaris* showed the highest digestibility, while *T. suecica*, *P. tricornutum*, and *P. purpureum* were the least digestible, likely because of the presence of robust cell walls or of exopolysaccharides that might have limited the action of digestive enzymes.

1. Introduction

The use of microalgae (including cyanobacteria) as food source and food supplements is known since centuries [1]. Microalgae are cultivated for human consumption in many Asian countries, Europe, the USA, and Australia since several decades [2]. Microalgae are also commercialized in the cosmetics industry or as animal feed [3,4]. The microalgae business sector is currently very dynamic with several new companies starting every year. > 150 companies of different sizes, mainly producing *Arthrospira* (spirulina), are present in Europe, mostly in France [5].

Among the microalgal genera largely employed for human consumption there are *Arthrospira, Chlorella* and *Aphanizomenon* due to their high content in essential nutrients and protein [6,7], and *Dunaliella* and *Haematococcus,* which are rich in antioxidant carotenoids [8,9]. *Arthrospira* and *Chlorella* are historically used as food ingredients. The Kanembou in Chad collect spirulina from natural lakes, where special conditions of pH and salinity create a monoalgal bloom [10]. In Myanmar, the natural blooms of spirulina, occurring in small volcanic basins in some periods of the year, are collected, subjected to simple processing and consumed as food [11]. Concerning *Chlorella*, much research was conducted on this genus starting from the 1950s, when it was considered as a potential candidate to combat global food shortage [12]. *Chlorella* is one of the top selling food supplements in Japan and it is produced by > 70 companies worldwide [13]. The Upper Klamath Lake in Oregon (USA) is known for the exploitation of seasonal blooms of *Aphanizomenon flos-aquae*. This cyanobacterial bloom is used to obtain a dietary supplement consumed in the United States and in Europe [14]. *Nostoc* has been used since ancient times for the preparation of traditional dishes in North Asia [15], North America and Northern Europe and as a dietary fiber source to replace fruit and vegetables [16] scarcely available in cold climatic zones. *N. flagelliforme*, called “Facai”,

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is consumed in China and Southeast Asia since ancient times as a health food [17]. *N. commune*, called by the Chinese "Ge-Xian-Mi" and by the Japanese "Ishikurage", is sold at USD $70–120 per kg dry weight as an ingredient for soups and salads or to be fried with meat [18].

The inclusion of *C. protothecoides*, *A. platensis*, *D. bardawil*, and astaxanthin from *Haematococcus* in the GRAS list (FDA, GRAS Notices) also contributed to the diffusion of these microalgae worldwide.

In the EU, novel foods and novel food ingredients are defined as those that "have not been used to a significant degree for human consumption within the Union before 15th May 1997" [19]. With the Regulation (EC) No 2015/2283, the EU, through the European Food Safety Authority (EFSA), guarantees that novel foods and novel food ingredients are subjected to a safety assessment through a unified procedure in order to protect public health [20]. According to the 1997 Regulation, the EU included some microalgae in the list of foods authorized in the EU market (European Union, Novel Food catalogue). The list included *A. flos-aquae* from Klamath Lake, *A. platensis*, *C. luteoviridis*, *C. pyrenoidosa*, and *C. vulgaris*. *Odontella aurita*, *Tetraselmis chuii* and astaxanthin from *H. pluvialis* were successively approved as food or food ingredients after the fulfillment of the novel food regulation procedures [21].

When dealing with novel matrices for food production one of the most important requirements is to demonstrate lack of toxicity in the range of concentrations at which that food or food ingredient is intended for use [20]. This represents one of the main obstacles that have to be faced when applying for a novel food due to long duration and high costs of the *in vivo* experiments. Some microalgae may produce toxins [22]. To have a preliminary picture of their potential toxicity, the microalgal biomasses investigated in this work were tested using two *in vitro* models, human dermal fibroblasts and *Artemia salina* [23]. The biomass from Klamath Lake bloom (mainly *A. flos-aquae*) showed toxicity. *C. vulgaris* Allma, *T. suecica* F&M-M33 and *P. purpureum* F&M-M46 were not toxic, while the remaining algae showed different levels of toxicity especially against fibroblasts, which proved to be a too highly sensitive model [23]. These results encouraged the continuation of the work on toxicity. *A. platensis* F&M-C256 and *T. lutea* F&M-M36 were tested in rats, showing no acute toxicity [24,25].

Another problem to deal with when using microalgae as food is the robust cell wall of certain strains, which restricts the access of the digestive enzymes to the cell components [26]. For this reason, information on the digestibility of microalgal biomass is of utmost importance [27]. *In vitro* digestion models are widely used to study the structural changes, digestibility and release of food components under simulated gastrointestinal conditions [28,29]. The most frequently used digestive enzymes (pancreatin, pepsin, trypsin, chymotrypsin, peptidase, α-amylase and lipase) are of porcine, rabbit or human origin [29], or derive from bile salts and mucin [28]. These *in vitro* digestion models have been already adopted to evaluate the digestibility of algal biomasses [30,31].

Determining the biochemical composition and the digestibility is the first requirement to evaluate the potential of novel food sources. In this work, twelve microalgal biomasses belonging to species already approved as food (*A. platensis*, *C. vulgaris* and *A. flos-aquae* from Klamath Lake) and two species not approved but belonging to the same genus already authorized (*C. sorokiniana* and *T. suecica*), together with species used in aquaculture as *Tisochrysis*, *Nannochloropsis* and *Phaeodactylum* [32,33] or in the cosmetic field, as *Porphyridium* [34], were tested for their biochemical composition, fatty acid profile and *in vitro* digestibility to evaluate their potential application as food sources.

2. Materials and methods

2.1. Microalgal strains and biomasses origin

The investigated algal biomasses are listed in Table 1. *A. platensis* F&M-C256, *N. sphaeroides* F&M-C117, *C. sorokiniana* F&M-M49, *T. suecica* F&M-M33, *P. purpureum* F&M-M46, *P. tricornutum* F&M-M40, *T. lutea* F&M-M36, and *N. oceanica* F&M-M24 belong to the Fotosintetica & Microbiologica (F&M) S.r.l. culture collection. Most of the biomasses tested in this study were produced at the facilities of F&M S.r.l. or of the Institute of Bioeconomy of the CNR, both located in Sesto Fiorentino, Florence (Italy). The algae were cultivated in GWP®-II photobioreactors [35] in semi-batch mode, during the spring/summer season (*A. platensis* F&M-C256; *N. sphaeroides* F&M-C117; starved *T. suecica* F&M-M33; *T. lutea* F&M-M36) or during autumn (*C. sorokiniana* F&M-M49; *C. sorokiniana* IAM-C212; *T. suecica* F&M-M33 grown in nutrient replete medium; *P. purpureum* F&M-M46; *P. tricornutum* F&M-M40; *N. oceanica* F&M-M24). The cultures were harvested in the early stationary phase (*N. sphaeroides* F&-C117; starved *T. suecica* F&M-M33; *P. purpureum* F&M-M46) or in the linear growth phase (*A. platensis* F&M-C256; *C. sorokiniana* F&M-M49; *C. sorokiniana* IAM-C212; *T. suecica* F&M-M33 grown in nutrient replete medium; *P. tricornutum* F&M-M40; *T. lutea* F&M-M36; *N. oceanica* F&M-M24) by centrifugation or filtration, and biomasses were frozen, lyophilized and powdered. The powdered biomasses were stored at −20 °C until analysis. Only *A. platensis* biomass was washed with physiological solution during harvesting to remove carbonates. The freshwater strains were cultivated in BG11 [36] and the marine strains in F medium [37]. *A. platensis* was cultivated in Zarrouk medium [38]. *T. suecica* F&M-M33 was also grown in F medium deprived of the nitrogen source. The two commercial products, *C. vulgaris* Allma and Klamath were obtained from Allma Microalgae (Portugal) and from Erbologica S.A.S. (Italy), respectively. In particular, Klamath derives from a natural bloom, mainly composed of *A. flos-aquae*, harvested from the Upper Klamath Lake (Oregon, USA).

Table 1
Algal biomasses tested for biochemical characterization and *in vitro* digestibility.

Strain/biomass	Type of culture medium	Origin of biomass
Arthrospira platensis F&M-C256	Alkaline	In-house cultivation
"Klamath polvere" (hereafter Klamath)	Fresh	Commercial product
Nostoc sphaeroides F&M-C117	Fresh	In-house cultivation
Chlorella sorokiniana F&M-M49	Fresh	In-house cultivation
Chlorella sorokiniana IAM C-212	Fresh	In-house cultivation
Chlorella vulgaris Allma	Fresh	Commercial product
Tetraselmis suecica F&M-M33 (S)	Marine	In-house cultivation
Tetraselmis suecica F&M-M33 (NR)	Marine	In-house cultivation
Porphyridium purpureum F&M-M46	Marine	In-house cultivation
Phaeodactylum tricornutum F&M-M40	Marine	In-house cultivation
Tisochrysis lutea (T-ISO) F&M-M36	Marine	In-house cultivation
Nannochloropsis oceanica F&M-M24	Marine	In-house cultivation

S, starved; NR, grown in nutrient replete medium.

2.2. Biochemical composition

All biomasses were analyzed for protein, carbohydrate, lipid, dietary fiber, ash, and moisture. Elemental analyses were performed using a CHNSO Analyzer (Flash EA, 1112 Series, Thermo Electron Corporation, USA) [39]. Total protein content was calculated as N x 6.25, where N is the nitrogen content determined through the elemental analysis. Carbohydrate was determined following Dubois et al. [40] and lipid following Marsh & Weinstein [41]. Dietary fiber was determined by AOAC Method 985.29 [42]. Moisture and ashes were analyzed following the ISTISAN protocol [43].

2.3. Fatty acid profile

Fatty acids were analyzed following the ISTISAN protocol [43]. The nutritional quality of the lipid fraction was estimated by the Hypocholesterolemic/Hypercholesterolemic ratio (H/H), which was

calculated according to Santos-Silva et al. [44], as follows:

$$H/H = (C18{:}\ 1\omega9 + C18{:}\ 2\omega6 + C20{:}\ 4\omega6 + C18{:}\ 3\omega3 + C20{:}\ 5\omega3 + C22{:}6\omega3)/(C14{:}\ 0 + C16{:}\ 0)$$

2.4. In vitro digestibility

The biomasses *in vitro* digestibility was evaluated by the method of Boisen and Fernández [45], modified as follows.

Microalgae biomasses were grounded, sieved and weighed (1 g, particle size ≤ 1 mm) and transferred in 250 mL conical flasks. To each flask, phosphate buffer (25 mL, 0.1 M, pH 6.0) was added and mixed, followed by HCl (10 mL, 0.2 M) and pH was adjusted to 2.0. To each flask, 3 mL of a freshly prepared 10 g L^{-1} porcine pepsin (0.8 FIP-U/mg, Applichem, Darmstadt, Germany) solution in water were added. The flasks were incubated at 39 °C for 6 h with constant agitation (150 rpm). After, phosphate buffer (10 mL, 0.2 M, pH 6.8) and NaOH solution (5 mL, 0.6 M) were added to each sample and pH was adjusted to 6.8. Then, to each sample 10 mL of a freshly prepared porcine 50 g L^{-1} porcine pancreatin (42,362 FIP-U/g, Applichem, Darmstadt, Germany) solution in ethanol:water (50/50 v/v) were added. The flasks were incubated again at 39 °C, 150 rpm, for 18 h. A reagent blank without sample was also prepared. The undigested residues were collected by centrifugation at 8000 rpm for 30 min and washed with deionised water. This procedure was repeated twice and the final supernatant was filtered on glass-fiber membranes (47 mm Ø, pore 1.2 μm). The pellet and membranes were dried at 80 °C for 6 h, and then at 45 °C until constant weight.

The dry matter (DM), organic matter (OM), crude protein (CP), and carbohydrate (C) *in vitro* digestibility (%) of the twelve biomasses was calculated from the difference between the initial biomass weight and the undigested weight (after correction for the blank) divided by the initial weight and multiplied by 100. Casein (Sigma Aldrich Corp., St. Louis, USA) was used as the reference material with 100% digestibility.

2.5. Statistical and data analysis

All the analyses were performed in triplicate except for dietary fiber. Data are expressed as mean ± standard deviation. For statistical correlation analysis, Pearson's correlation coefficient was used and a significant correlation at the $P < 0.05$ level was considered. GraphPad Prism 6.01 was used for these aims.

3. Results and discussion

3.1. Biochemical composition

The content of the main biochemical components in algal cells varies depending on the investigated microalga and on culture conditions, growth phase and physiological status [14]. The analytical procedure adopted to quantify the different components can lead to differences in the final results. All these factors should be taken into consideration when comparing data from different studies.

Table 2 shows the biochemical composition of the twelve microalgal biomasses tested in this work. Protein was the main component of cyanobacterial biomasses (51–64%). Chlorophyceae also exhibited a high protein content (> 50%), except *C. sorokiniana* IAM C-212 and *T. suecica* (40%). A similar or lower protein content (34–43%) was found in the remaining species (*P. purpureum*, *P. tricornutum*, *T. lutea*, *N. oceanica*). As expected, *T. suecica* grown under nitrogen starvation showed a very low protein content (18%).

The protein content of the examined microalgal biomasses was similar to the value found in the literature for the same species [46–52]. Microalgae, especially cyanobacteria, usually exhibit a higher protein content in comparison to proteinaceous crops such as pea and soybean (21% and 34%) [53,54]. By taking 5 g of *A. platensis* or of *C. vulgaris* per day (the amount usually recommended by the nutrition facts label for commercial *Arthrospira* and *Chlorella*-based products), it is possible to enrich the diet with about 6% of the daily protein requirement [55].

Nitrogen starved *T. suecica* showed the highest content of carbohydrate (37%). In most of the studied microalgae total carbohydrate content varied between 10 and 19%, which is within the range found in the literature for these species [51,52,56,57]. *C. vulgaris*, *T. lutea* and *T. suecica* grown in nutrient replete medium exhibited a carbohydrate content from 6 to 10%, which is significantly lower than that found in the literature [56–58] (Table 2). Carbohydrate content in most of the studied microalgae is far lower than that found in traditional vegetable food/feedstuff (as corn, wheat and soybean, 85%, 84% and 30%, respectively) [59].

The investigated microalgal biomasses exhibited lipid contents varying from 11 to 29%, with the exception of Klamath (6%). In particular, *C. sorokiniana* (both strains), *T. suecica* grown in nutrient replete medium, *T. lutea*, and *N. oceanica* showed a lipid content close to 30%. With the exception of Klamath, the lipid content for the examined algae were comparable to those found by other authors for the same species [50,51,56–58,60] (Table 2).

The positive effects of high intakes of total dietary fiber (TDF) are related to: i) decreased blood cholesterol and glycaemia; ii) increased volume of fecal bulk and decreased time of intestinal transit; iii) trapping of substances that can be dangerous to the human body (mutagenic and carcinogenic agents); iv) stimulation of the proliferation of the intestinal flora [61,62]. *N. sphaeroides*, *C. sorokiniana* F&M-M49 and all the marine species showed high TDF values (between 9 and 17%). *C. sorokiniana* IAM C-212, *A. platensis*, and *C. vulgaris* were found to contain the lowest amount of TDF (4.4, 5.8, and 5.9%, respectively), which confirms data reported in the literature for these species [63–65]. Only for Klamath the dietary fiber was below detection limit (Table 2). In general, TDF content of most of the microalgae examined in this study was significantly higher compared to that of some cooked cereals, like white rice (0.3%) and oatmeal (1.7%), raw vegetables, such as tomatoes (1.3%) and lettuce (1.0%), and raw fruits such as bananas (1.8%) and pineapple (1.5%) [66]. On the contrary, macroalgae generally show a higher TDF content (33–75%) [67].

Ash content was generally low (< 10%) in freshwater biomasses. Marine biomasses exhibited a higher ash content, reaching 22% in *P. purpureum* (Table 2).

For several of the strains tested in this work previous trials have highlighted the effect of culture conditions on biochemical composition. In particular, the effects of light quality (natural *vs* artificial, white *vs* green, red and blue) were investigated by Abiusi et al. [58] on *T. suecica* F&M-M33 and by Chini Zittelli et al. [68] on *N. oceanica* F&M-M24, the effects of nutrient availability were studied by Guccione et al. [51] on *C. sorokiniana* F&M-M49 and *C. sorokiniana* IAM-C212 and by Rodolfi et al. [69] on *P. tricornutum* F&M-M40, and the effects of temperature and salinity were investigated by Guccione et al. [51] on *C. sorokiniana* F&M-M49 and *C. sorokiniana* IAM-C212.

3.2. Fatty acid profile

Table 3 shows the fatty acid profile of the twelve algal biomasses.

The algal biomasses with the highest ω3 PUFA content (> 3% of biomass dry weight) were *C. sorokininana* F&M-M49 (predominantly C18:3ω3), *P. tricornutum* and *N. oceanica* (mainly C20:5ω3). Among those tested *T. lutea* was the only alga containing C22:6ω3 (Table 3). Concerning the ω6 PUFA, the algae with the highest content (> 2% of biomass dry weight) were *C. sorokininana* (both strains) and *T. suecica* (grown in nutrient replete medium), all containing mainly C18:2ω6, and *A. platensis* containing C18:2ω6 and C18:3ω6 (Table 3). The fatty acid profiles of the investigated microalgae are comparable with those found in the literature for the same species [56–58,65,69]. Studies have

Table 2
Biochemical composition of the twelve microalgal biomasses investigated. Data are expressed as % of algal powder. Except for dietary fiber, results are expressed as averages ± SD (n = 3).

Strain/biomass	Protein (%)	Carbohydrate (%)	Lipid (%)	TDF (%)	Ash (%)	Moisture (%)
A. platensis F&M-C256	63.9 ± 1.0	12.8 ± 0.21	10.7 ± 0.56	5.8	6.1 ± 0.10	7.9 ± 0.20
Klamath	62.4 ± 5.19	18.8 ± 0.15	6.1 ± 0.84	b.d.l.	6.2 ± 0.32	6.8 ± 0.24
N. sphaeroides F&M-C117	50.8 ± 1.45	14.5 ± 0.53	15.1 ± 1.19	11.3	4.0 ± 0.25	7.8 ± 0.28
C. sorokiniana F&M-M49	51.3 ± 0.48	15.5 ± 0.08	22.7 ± 2.00	10.0	5.4 ± 0.11	8.5 ± 0.24
C. sorokiniana IAM C-212	39.9 ± 0.94	10.7 ± 0.90	27.9 ± 1.30	4.4	9.4 ± 0.37	7.5 ± 0.30
C. vulgaris Allma	56.8 ± 2.70	5.9 ± 0.25	16.9 ± 2.83	5.9	9.3 ± 1.47	4.9 ± 0.17
T. suecica F&M-M33 (S)	18.3 ± 0.10	36.8 ± 1.46	22.4 ± 1.15	12.4	14.8 ± 0.47	6.1 ± 0.26
T. suecica F&M-M33 (NR)	40.2 ± 0.51	10.2 ± 0.20	28.5 ± 1.16	17.0	15.7 ± 0.20	7.2 ± 0.14
P. purpureum F&M-M46	34.2 ± 0.10	17.0 ± 1.72	13.1 ± 1.12	15.9	22.0 ± 0.88	10.0 ± 0.39
P. tricornutum F&M-M40	38.8 ± 0.11	11.0 ± 0.70	20.5 ± 0.54	14.1	14.8 ± 0.12	8.0 ± 0.23
Tisochrysis lutea F&M-M36	42.9 ± 0.42	8.6 ± 0.89	27.9 ± 3.25	9.7	11.5 ± 0.27	6.3 ± 0.26
N. oceanica F&M-M24	43.1 ± 0.10	14.3 ± 0.19	28.2 ± 2.04	9.3	12.9 ± 0.84[c]	7.2 ± 0.21

S, starved; NR, grown in nutrient replete medium; b.d.l. below detection limit; TDF, total dietary fiber.

shown that C20:5ω3 and C22:6ω3 are important for proper foetal development (including neuronal, retinal, and immune functions) [70] and may positively affect many aspects of cardiovascular function including inflammation, peripheral artery disease, major coronary events, and coagulation [70,71].

The nutritional quality of the lipid fraction of the twelve microalgal biomasses was further evaluated by the Hypocholesteroleminc/Hypercholesterolemic ratio (H/H') (Table 3). The H/H ratio is based on current knowledge of the effects of individual fatty acids on cholesterol metabolism [44]. Nutritionally, high H/H values are considered beneficial for human health [72]. The highest H/H values were found for *T. suecica* grown in nutrient replete medium, *C. sorokiniana* F&M-M49, and in starved *T. suecica* (5.42, 4.66, and 3.68, respectively) (Table 3). The H/H values found for *C. sorokiniana* F&M-M49 and for *P. purpureum* are similar to those reported for marine fish, such as sardine and mackerel, and for vegetable oils, like sesame oil (4.82 and 2.46, respectively) [73,74]. Compared to the microalgae investigated in this study, a significantly higher H/H value was found in flaxseed oil (14.85), used in many food preparations [74], and for the Sacha inchi (*Plukenetia volubilis*) nut, commonly called “Inca Peanut” and considered a potential source of essential fatty acids (20.48) [75].

3.3. In vitro digestibility

The *in vitro* digestibility provides useful information about the nutrient bioavailability of a product [45]. Most of the literature on algae deals with macroalgae [76–78] and only few studies, to our knowledge, focus on the *in vitro* digestibility of microalgae [6,30,79,80].

The *in vitro* digestibility of the twelve microalgal biomasses under investigation was determined by an enzymatic method using pepsin and pancreatin. Digestibility of dry matter (DMD), organic matter (OMD), carbohydrate (CD) and crude protein (CPD) is reported in Fig. 1. *A. platensis* showed the highest DMD (78%). *C. vulgaris*, *C. sorokiniana* IAM C-212, *T. lutea*, *N. sphaeroides* and Klamath showed a DMD higher than 60%. *P. purpureum* was the least digestible (47%) (Fig. 1). Devi et al. [81] and Mišurcová et al. [30] found higher DMD values for *A. platensis* (84% and 94%, respectively) compared to our study (78%). The high digestibility of *A. platensis* can be, at least partially, related to its Gram negative cell wall mainly composed of the peptidoglycan layer and the proteic and lipopolysaccharidic outer membrane. In spite of the similar cell wall, the other cyanobacteria (Klamath and *N. sphaeroides*) showed a lower digestibility, but, still high compared the other tested microalgal biomasses. This may be due to the presence of outer layers of polysaccharides, common among cyanobacteria. The DMD showed by *C. sorokiniana* IAM C-212 (73%) was comparable to that obtained by Mišurcová et al. [30] for *C. pyrenoidosa* (75%). To our knowledge, no literature is available concerning DMD of the other studied microalgae. Digestibility differences appear in some cases at the strain level (see *C. sorokiniana* F&M-M49 and IAM C-212 in Fig. 1). Green algae cell wall typically contains cellulose, hemicellulose, pectic compounds and glycoproteins [82,83], which may confer resistance to the action of digestive enzymes. Also *Nannochloropsis* has a thick, bilayered, cell wall composed of cellulose and algaenans [84] that may reduce digestibility. *Porphyridium* cells are typically covered by polysaccharides [85] that can form stable complexes with proteins and reduce cell access to proteolytic enzymes [86]. In general, our study shows a partial correspondence between cell wall structure and DMD.

The values of OMD and CD reflected those of DMD, except in the case where the starting material was particularly rich in ashes, as for *P. purpureum* (Table 2). In terms of OMD and CD (Fig. 1) *A. platensis* resulted the most digestible (86% and 79%, respectively), on the contrary, *P. tricornutum* and *P. purpureum* confirmed to be the least digestible (51%).

CPD values did not reflect DMD values (Fig. 1). Protein digestibility is an important factor to estimate protein availability [55]. *N. sphaeroides*, *A. platensis* and *C. vulgaris* showed the highest CPD (82%, 81%, 76%, respectively), which compares favourably with the protein digestibility found for beans, oats and wheat (78%, 72% and 77%, respectively) [55]. Most of the other biomasses showed CPD values ranging from 62 to 70%. *N. oceanica* and *C. sorokiniana* F&M-M49 exhibited the lowest protein digestibility values (50 and 55%, respectively). The low CPD of *N. oceanica* is in accordance with the results of Cavonius et al. [87] who reported a low degree of protein hydrolysis of *N. oculata*. Many authors reported diverse CPD values for different species of *Chlorella* (from 70 to 92%) [79,88] and *Arthrospira* (from 70 to 84%) [81,89]. Tibbetts et al. [50] reported higher CPD for *P. tricornutum*, *Nannochloropsis granulata*, *T. chuii* and *Porphyridium aeruginem* (83–97%) compared to the values found in our study. Wild et al. [49] also found higher CPD values for *C. vulgaris*, *C. protothecoides*, *C. sorokiniana*, *N. oceanica*, *N. oculata* and *P. tricornutum* (54–79%).

Studies conducted on brown algae reported an inhibitory action of fiber on pepsin activity with a consequent reduction in protein digestibility [90]. Contrary to Horie et al. [89], no significant ($P > 0.05$) correlation between CPD and fiber content ($r = -0.31$) was found in our study, whereas a significant ($P < 0.05$) negative correlation between DMD ($r = -0.83$), OMD ($r = -0.79$), CD ($r = -0.83$) and fiber content was found (Fig. 2).

The method adopted in this study to determine the protein content is based on the elemental (CHNSO) analysis of biomass [91]. This method represents proteins as crude protein (N x 6.25) [91] and overestimates the real protein content of biomasses rich in non-proteinaceous nitrogen, for example in the case of *A. platensis* F&M-C256, which is particularly rich in nucleic acids [24]. This may justify that the CPD values did not reflect DMD values (Fig. 1).

Table 3
Fatty acid content and profile (% of dry weight) and Hypocholesterolemic/Hypercholesterolemic ratio (H/H) of the twelve microalgal biomasses. Results are expressed as averages ± SD (n = 3).

	Ap	K	Ns	CsM49	CsIAM	CvA	TsS	TsNR	Pp	Pt	Tiso	No
C12:0	–	–	0.20 ± 0.008	1.55 ± 0.03	–	0.55 ± 0.002	–	0.59 ± 0.02	0.14 ± 0.001	0.51 ± 0.03	1.21 ± 0.004	0.35 ± 0.02
C14:0	–	0.25 ± 0.02	0.04 ± 0.03	0.05 ± 0.001	–	0.06 ± 0.004	–	0.03 ± 0.03	0.02 ± 0.02	0.57 ± 0.002	3.09 ± 0.01	0.93 ± 0.03
C16:0	2.56 ± 0.02	1.24 ± 0.02	3.02 ± 0.005	1.74 ± 0.003	1.26 ± 0.02	1.99 ± 0.01	1.33 ± 0.007	0.95 ± 0.005	1.26 ± 0.003	1.27 ± 0.001	1.62 ± 0.005	6.73 ± 0.004
C18:0	0.12 ± 0.01	–	0.15 ± 0.02	0.11 ± 0.002	–	0.26 ± 0.009	–	0.60 ± 0.04	0.04 ± 0.01	0.05 ± 0.008	0.14 ± 0.05	0.35 ± 0.01
Other SFA	0.02 ± 0.01	–	0.13 ± 0.03	0.05 ± 0.009	–	0.14 ± 0.03	–	0.73 ± 0.008	0.04 ± 0.06	0.60 ± 0.1	1.04 ± 0.002	0.24 ± 0.008
Σ SFA	2.70 ± 0.04	1.49 ± 0.05	3.50 ± 0.09	3.50 ± 0.08	1.26 ± 0.02	3.00 ± 0.06	1.33 ± 0.01	2.90 ± 0.1	1.50 ± 0.1	3.00 ± 0.06	7.10 ± 0.09	8.60 ± 0.08
C16:1 ω7	0.29 ± 0.03	–	1.23 ± 0.03	0.15 ± 0.02	0.17 ± 0.01	0.44 ± 0.04	0.04 ± 0.02	0.04 ± 0.005	0.16 ± 0.009	2.42 ± 0.04	0.79 ± 0.01	5.82 ± 0.04
C18:1 ω9	0.18 ± 0.02	–	0.48 ± 0.03	2.52 ± 0.02	0.52 ± 0.005	1.81 ± 0.01	1.95 ± 0.006	1.37 ± 0.02	0.02 ± 0.001	0.90 ± 0.05	2.91 ± 0.02	3.06 ± 0.006
Other MUFA	0.03 ± 0.01	–	0.004 ± 0.01	1.43 ± 0.03	–	0.05 ± 0.03	–	0.49 ± 0.007	0.02 ± 0.008	0.08 ± 0.002	0.10 ± 0.01	0.04 ± 0.02
Σ MUFA	0.50 ± 0.05	–	1.75 ± 0.08	3.90 ± 0.08	0.69 ± 0.02	2.30 ± 0.08	1.37 ± 0.04	1.90 ± 0.03	0.20 ± 0.03	3.40 ± 0.09	3.80 ± 0.04	8.92 ± 0.07
C16:3 ω3	–	0.23 ± 0.01	–	–	0.87 ± 0.03	–	–	–	–	–	–	–
C16:4 ω3	–	–	–	–	–	–	1.02 ± 0.01	–	–	–	–	–
C18:3 ω3 (ALA)	0.01 ± 0.01	1.51 ± 0.04	2.14 ± 0.03	3.44 ± 0.01	1.93 ± 0.003	2.51 ± 0.06	1.76 ± 0.009	1.56 ± 0.04	–	0.07 ± 0.008	1.60 ± 0.005	0.02 ± 0.03
C20:5 ω3 (EPA)	–	–	–	–	–	–	–	0.39 ± 0.007	1.87 ± 0.3	3.97 ± 0.006	–	3.15 ± 0.03
C22:6 ω3 (DHA)	–	–	–	–	–	–	–	–	–	–	1.01 ± 0.01	–
Other PUFA ω3	–	–	–	0.07 ± 0.03	–	–	–	–	–	–	–	–
ΣPUFA ω3	0.01 ± 0.01	1.74 ± 0.04	2.14 ± 0.03	3.51 ± 0.03	2.80 ± 0.04	2.51 ± 0.07	2.78 ± 0.01	1.95 ± 0.05	1.87 ± 0.4	4.04 ± 0.01	2.61 ± 0.02	3.17 ± 0.07
C16:2 ω6	–	–	–	–	0.56 ± 0.05	–	–	–	–	–	–	–
C18:2 ω6 (LA)	1.15 ± 0.02	0.33 ± 0.05	1.08 ± 0.01	2.38 ± 0.001	1.85 ± 0.01	1.85 ± 0.002	1.19 ± 0.06	1.88 ± 0.003	0.35 ± 0.05	0.53 ± 0.004	1.13 ± 0.003	0.28 ± 0.04
C18:3 ω6 (GLA)	1.66 ± 0.01	–	0.03 ± 0.04	0.04 ± 0.06	–	0.01 ± 0.006	–	0.03 ± 0.01	0.01 ± 0.002	0.08 ± 0.007	0.04 ± 0.001	0.07 ± 0.002
C20:2 ω6	–	–	–	0.04 ± 0.008	–	–	–	–	0.03 ± 0.01	0.23 ± 0.001	–	0.04 ± 0.06
C20:4 ω6 (ARA)	–	–	0.03 ± 0.002	–	–	–	–	0.11 ± 0.08	1.06 ± 0.03	0.07 ± 0.01	–	0.48 ± 0.008
Other PUFA ω6	0.01 ± 0.01	–	–	–	–	–	–	–	0.07 ± 0.006	–	–	0.09 ± 0.006
ΣPUFA ω6	2.82 ± 0.05	0.33 ± 0.05	1.14 ± 0.06	2.46 ± 0.008	2.41 ± 0.08	1.86 ± 0.01	1.19 ± 0.07	2.09 ± 0.1	1.52 ± 0.1	0.91 ± 0.03	1.17 ± 0.01	0.96 ± 0.2
ΣPUFA ω3 + ω6	2.83 ± 0.07	2.07 ± 0.08	3.28 ± 0.08	5.97 ± 0.09	5.21 ± 0.1	4.37 ± 0.09	4.87 ± 0.09	3.97 ± 0.2	3.39 ± 0.6	4.95 ± 0.05	3.78 ± 0.04	4.13 ± 0.3
TFA	6.0 ± 0.1	3.56 ± 0.1	8.5 ± 0.2	13.4 ± 0.3	7.16 ± 0.1	9.7 ± 0.2	7.29 ± 0.2	8.5 ± 0.3	5.0 ± 0.7	11.3 ± 0.2	15.1 ± 0.2	21.7 ± 0.5
H/H	0.52	1.23	1.22	4.66	3.41	3.01	3.68	5.42	2.58	3.01	1.41	0.91

Ap: *A. platensis* F&M-C256; K: Klamath powder; Ns: *N. sphaeroides* F&M-C117; CsM49: *C. sorokiniana* F&M-M49; CsIAM: *C. sorokiniana* IAM C-212; CvA: *C. vulgaris* Allma; TsS: *T. suecica* F&M-M33 (starved); TsNR: *T. suecica* F&M-M33 (nutrient replete medium); Pp: *P. purpureum* F&M-M46; Pt: *P. tricornutum* F&M-M40; Tiso: *T. lutea* F&M-M36; No: *N. oceanica* F&M-M24; – traces or 0 value.

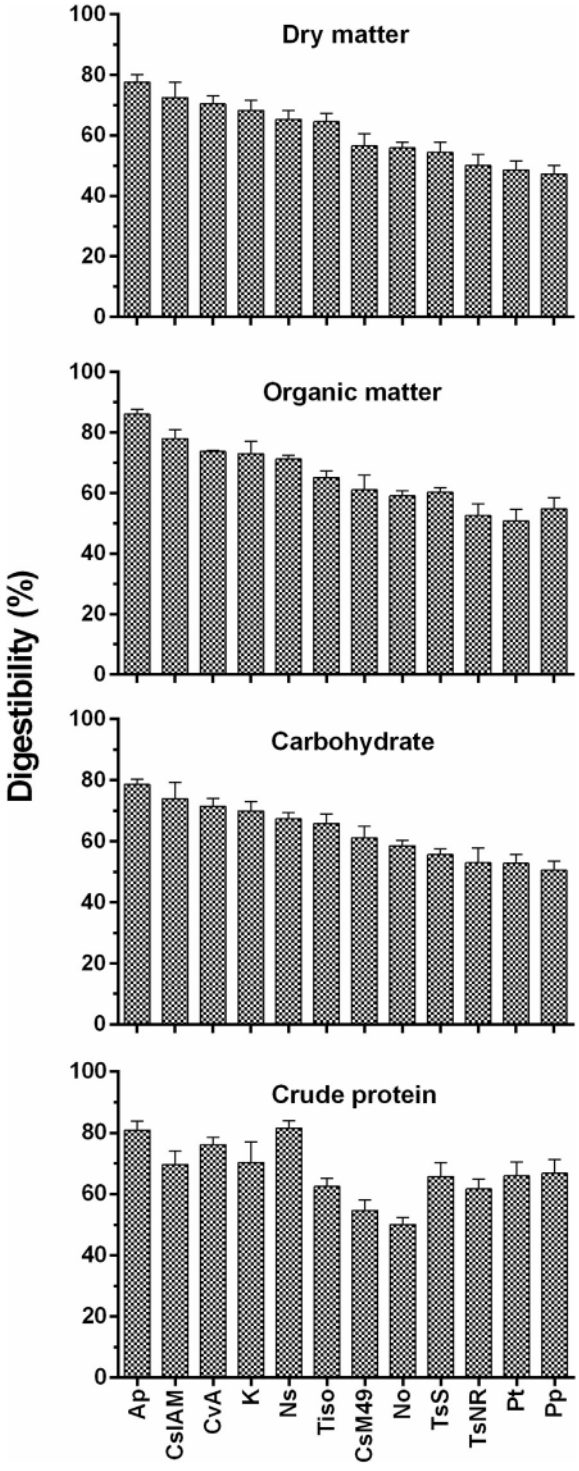

Fig. 1. Dry matter (in order of decreasing digestibility), organic matter, carbohydrate, and crude protein digestibility (expressed as %) of the twelve microalgal biomasses investigated. The analyses were performed in triplicate and data are reported as mean value ± SD. Ap *A. platensis* F&M-C256; K Klamath; Ns *N. sphaeroides* F&M-C117; CsM49 *C. sorokiniana* F&M-M49; CsIAM *C. sorokiniana* IAM C-212; CvA *C. vulgaris* Allma; TsS *T. suecica* F&M-M33 (starved); TsNR *T. suecica* F&M-M33 (nutrient replete medium); Pp *P. purpureum* F&M-M46; Pt *P. tricornutum* F&M-M40; Tiso *T. lutea* F&M-M36; No *N. oceanica* F&M-M24.

The large differences in digestibility values found in our work for the twelve biomasses compared to the literature may also be related to differences in terms of strains and in the protocols adopted to evaluate the *in vitro* digestibility [29,30,76].

4. Conclusions

The large biodiversity of microalgae available in nature and in culture collections still needs evaluation in terms of useful properties and safety before its exploitation in the food and feed sector. To obtain food algae-based products and co-products, advancing our knowledge on the biochemical composition and digestibility of microalgae is the first key requirement. More microalgal species should be considered for the approval as novel food, thus contributing in the long term to a wider inclusion of these microorganisms in the human diet.

The present study provides information on the biochemical composition, the fatty acids profile and the *in vitro* digestibility of twelve microalgal biomasses that could be exploited to produce new functional foods.

Higher crude protein contents and *in vitro* digestibility were found in cyanobacteria (in particular *A. platensis* F&M-C256), *C. sorokiniana* F&M-M49 and *C. vulgaris* Allma. Marine species were less digestible and contained higher concentrations of polyunsaturated fatty acids (mostly eicosapentaenoic acid and docosahexaenoic acid) along with substantial amounts of palmitoleic, oleic and palmitic acids. Freshwater algae showed high concentrations of α-linolenic acid and an even higher amount of palmitic acid.

It is worth pointing out that to fully assess microalgae nutritional quality, also vitamin, mineral and bioactives content, and bioavailability should be determined and that it is possible to substantially modify the biochemical composition and the digestibility of algal biomass by changing culture conditions (mainly growth medium composition and the source of light).

Declaration of Competing Interest

M.R. Tredici and L. Rodolfi have a financial interest in F&M S.r.l. The other authors declare no conflicts of interest.

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Authors' agreement to authorship and submission

All the authors agreed to the authorship and submission of the manuscript to Algal Research for peer review.

Declaration of authors' contributions

AN, GCZ, MRT: study conception and design; AN, GCZ: cultivation of microalgae, biochemical composition analyses and *in vitro* digestibility; all the authors participated in data analyses, discussion of results, writing and revising the manuscript.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

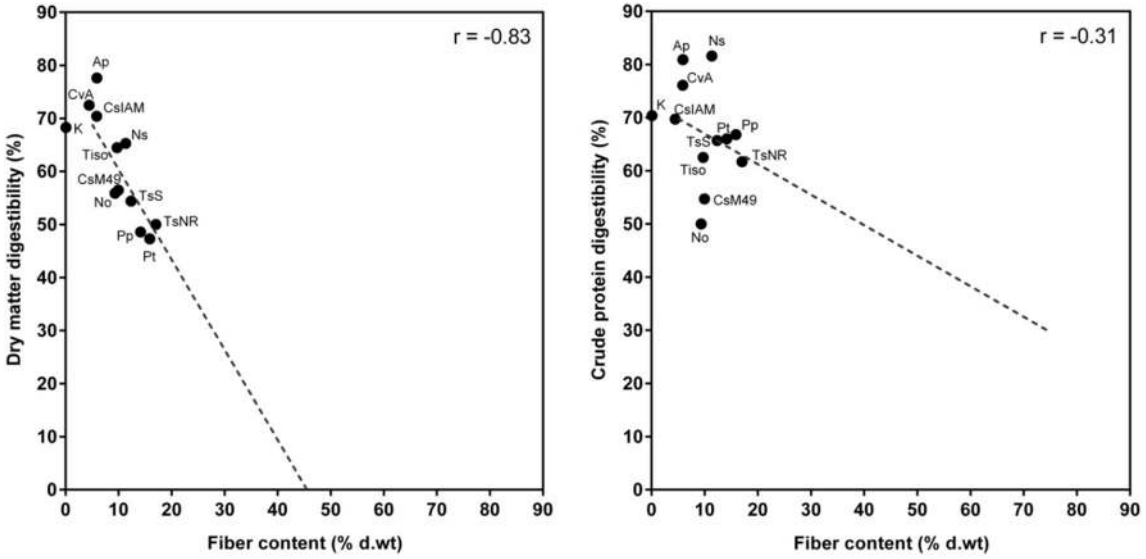

Fig. 2. Linear correlation between fiber content and DMD (left) and CPD (right) ($n = 12$; significance level $P < 0.05$). Ap, *A. platensis* F&M-C256; K, Klamath; Ns, *N. sphaeroides* F&M-C117; CsM49, *C. sorokiniana* F&M-M49; CsIAM, *C. sorokiniana* IAM C-212; CvA, *C. vulgaris* Allma; TsS, *T. suecica* F&M-M33 (starved); TsNR, *T. suecica* F&M-M33 (nutrient replete medium); Pp, *P. purpureum* F&M-M46; Pt, *P. tricornutum* F&M-M40; Tiso, *T. lutea* F&M-M36; No, *N. oceanica* F&M-M24.

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