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Dunaliella microalgae for nutritional protein, an undervalued asset

Yixing Sui and Siegfried E. Vlaeminck*

LinkedIn Yixing Sui: https://www.linkedin.com/in/yixingsui/

Twitter Siegfried E. Vlaeminck: @SigifridoF

Research Group of Sustainable Energy, Air and Water Technology, Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

Website: https://www.uantwerpen.be/en/research-groups/sustainable-energy/

*Correspondence: siegfried.vlaeminck@uantwerpen.be (S.E. Vlaeminck)

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Abstract:

β-carotene production with *Dunaliella* microalgae is established, yet their potential as protein source for food and feed applications seems overlooked. The rich protein content and nutritional tunability of *Dunaliella* make these algae intriguing sources of sustainable protein. It is of societal interest to exploit these promising proteinaceous *Dunaliella* traits.

Dunaliella microalgae: Spotlighted β-carotene and undervalued protein

Microalgae are recognized as promising sources for diversified applications including food, feed and high-value products [1]. Both science and industry have already focused their interests on *Dunaliella* microalgae, especially for their unique feature of hyper carotenogenesis, producing β-carotene as one of the first commercial high-value products from microalgae [2]. The large-scale production of β-carotene from *Dunaliella*, as β-carotene extract or dried biomass, started in the 1980s in Israel, Australia and USA, followed by other countries like India and China [2]. *Dunaliella* has been classified by the U.S. Food and Drug Administration (FDA) as food sources with GRAS (Generally Regarded as Safe) status, and is mostly used for human and animal nutrition, food coloring and cosmetics due to its pro-vitamin and anti-oxidant functions [1]. Among all commercial *Dunaliella* products, they are mostly extracts of β-carotene, accompanied by powders in capsules or tablets. When possible, the residual of biomass allows further usage as glycerol, protein, enzymes, fatty acids, vitamins etc.

Nevertheless, the societal challenge of food and protein scarcity are prompting a need for novel protein sources to sustain the population growth, and microalgae present a resource-efficient and ecological way of producing proteinaceous food and feed ingredients among proteins from other microbes such as bacteria and yeast [3,4]. In this regard, the potential of *Dunaliella* protein has been undervalued. From the 1950s until recently, although *Dunaliella* has been noted for its proteinaceous traits, only scattered studies have been carried out. Even in the established studies, patents and projects (e.g. European project D-Factory), *Dunaliella* protein mostly comes as a by-product used for e.g. animal feeding after β-carotene extraction. These facts leave *Dunaliella* protein rather poorly understood comparing with traditional microalgal protein producers such as *Chlorella* and *Spirulina*. Therefore, it is of great interest to revisit and further explore the potential usage of *Dunaliella* protein.

Dunaliella protein snapshot: Limitations of single-point analyses

The typical way to analyze protein in *Dunaliella* biomass is to assess its protein content and protein composition from snapshot approaches, which are performed under specific conditions and for specific growth phases (mostly in the stationary phase under standard cultivation conditions) [4]. These approaches have shown that the dried biomass of *Dunaliella* consists of 50-80% protein [3,4]. More notably, the essential amino acid (EAA) composition of *Dunaliella*, as indicated by essential amino acid index (EAAI), can reach superior protein quality for human requirement following reference level set by Food and Agriculture Organization (FAO) [5] (Box 1). Compared with soybean and other protein-rich microalgae genera such as *Spirulina* and *Chlorella*, *Dunaliella* also shows either equal or better quantity and quality of proteins [3]. Differently from most other microalgae, *Dunaliella* microalgae lack rigid cellulosic cell wall, so they are more easily digested by both humans and animals. Although the protein profile of *Dunaliella* seems favorable, these snapshot approaches provide incomplete information and neglect the potential variabilities introduced by external cultivation conditions.

Dunaliella protein tunability: Exploring the spectrum

The synthesis of biomass in microalgae strongly depends on nutrient level, salinity, pH, growth phase, and other conditions. Accordingly, studying the tunability of protein quantity and quality in *Dunaliella* can largely broaden the spectrum of snapshot analyses. For instance, nitrogen (N) is an essential element composing protein and amino acids (AA), so its availability can directly affect protein dynamics. High N availability can result in more protein accumulation in *Dunaliella*, provided that other nutrients are neither limiting nor overabundant [6]. Due to its halophilic characteristics, salinity also plays an important role. Around 10% sodium chloride

(NaCl) concentration has been reported to be optimal for both cell growth and protein quantity [4,7]. Although pH level can be influential as well, it is mostly species dependent, with optima ranging from pH 7.5 to pH 8.5 for maximum cell growth and protein quantity [4,7]. Higher light intensity and longer illumination period seem to result in higher protein accumulation in *Dunaliella*, yet they can also lower the light usage efficiency by microalgae, resulting in lower protein yield [5,7,8]. Lastly, the growth phase of microalgae can affect the protein dynamics significantly [4,5]. The exponential phase implies the generation of new cells, which is accompanied by increased protein synthesis for cell reproduction [4,5]. The stationary phase, however, implies a limiting condition which translates into slower cell growth and N assimilation rates, resulting in lower protein quantity [4,5]. It is therefore common to find an increase-decrease pattern of protein level throughout the growth phases [4,5].

There exist large unidentified areas preventing an in-depth understanding on how AA dynamics influence protein quality in *Dunaliella*. Only recently, one study demonstrated that EAA levels in *Dunaliella* increase from the exponential towards the stationary phase, despite the increase-decrease pattern of protein quantity [5]. Another study shows a clear benefit of N limitation to boost EAA production in *Dunaliella* despite reduced protein quantity [8]. Both studies indicate the superior quality of *Dunaliella* protein by its essential amino acid index (EAAI) as high as 1.53 (Box 1). These studies show that further insights into dynamics of both protein quantity and quality in *Dunaliella* are required to fully exploit its potential as a protein source.

Dunaliella protein in food and feed applications

From the 1980s until recently, very few studies were published on *Dunaliella* microalgae for human and animal nutrition. The most pronounced benefits of food products containing dried *Dunaliella* biomass, such as bread and pasta, is their enhanced nutritional properties, such as increased proteins and minerals, and improved rheological properties [9,10]. Moreover, *Dunaliella* biomass is better digested by rats (to mimic human digestion) compared with other microalgae and casein, validating its "without rigid cell wall" advantage [11]. In husbandry, the ovarian activity of goats is also improved when fed with *Dunaliella*-supplemented feed, hastening the follicular development [12]. In aquaculture, living *Dunaliella* cells are mostly used as feed ingredients, and sea urchins can efficiently incorporate *Dunaliella* protein into their larvae [13]. These examples show that studies on the practical applications of *Dunaliella* protein are indeed very scattered. Nevertheless, they provide positive prospects for favorable usage in food and feed applications.

Future outlooks and concluding remarks

Single-product exploitation has been the primary focus of microalgal research for decades, even though the remaining biomass after extraction of the main product is still ripe for further valorization [14]. Some microalgae such as *Chlorella* and *Spirulina* producing lower-value products e.g. protein and lipid, are also finding their higher-value paths towards pigments and fatty acids including phycocyanin and linoleic acids. Nevertheless, these exclusive concepts focusing on single product seems less beneficial comparing with a more inclusive strategy: co-production of nutritional compounds in microalgae [14]. As for *Dunaliella*, an emphasis on its protein should not exclude its interesting β-carotene characteristic. Consequently, a new approach to co-produce multiple products of interest (e.g. high quality protein and β-carotene together) in *Dunaliella* biomass may be more advantageous [8]. Similarly, a co-production of some functional proteins can further improve the quality of *Dunaliella* biomass. This biomass can be subsequently used in several applications, bringing proteins with high quality and digestibility, carotenoids with coloring, antioxidant and immune-stimulating functions together, such as poultry feed, pet food, ornamental fish feed and ornamental bird feed [15] (Figure 1). This approach could lead to win-win scenarios where microalgae production efficiency is improved, resulting in products with higher values, and the relatively high production costs of microalgae can be reduced.

The proteinaceous traits of *Dunaliella* present considerable potential for food and feed applications. Nonetheless, the high-value β-carotene production from *Dunaliella* has largely overshadowed its potential as a protein source. It is societally timely to focus on exploiting this digestible high-quality protein, advance its co-production with β-carotene, and nutritionally demonstrate the added value of the optimized products in multiple feed and food applications. Furthermore, as concerned in all microalgal production and biorefinery domains, there still exists various challenges on technological and social-economic aspects, encouraging contributions from future endeavors.

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Box 1. Essential amino acid index (EAAI) determines the protein quality for human food

Essential amino acids (EAA) cannot be synthesized by the human body, so humans rely on external food supplies to provide them. EAAI scores can indicate the quality of a protein source by comparing the ratios of EAA in the protein source to those required by the human body. The following equation is used to calculate the EAAI score:

$$AAI = \sqrt[n]{\frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \times \ldots \ldots \times \frac{aa_n}{AA_n}}$$

where aa_n and AA_n are the EAA content over total protein (mg EAA/g protein) in the sample and the FAO/WHO reference for human requirement, respectively [5]. EAAI values of ≥ 1, 0.95-1, 0.86-0.95, 0.75-0.86 and ≤ 0.75 correspond to superior, high, good, useful and inadequate proteins quality, respectively [5]. For instance, based on the EAA composition of egg and soybean, their EAAI scores are 1.65 and 1.34, respectively [3,5].

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