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Review

New Insights into Duckweed as an Alternative Source of Food and Feed: Key Components and Potential Technological Solutions to Increase Their Digestibility and Bioaccessibility

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Abstract: Sustainability is becoming increasingly important in the world we live in, because of the rapid global population growth and climate change (drought, extreme temperature fluctuations). People in developing countries need more sustainable protein sources instead of the traditional, less sustainable meat, fish, egg, and dairy products. Alternative sources (plant-based, such as grains (wheat, rice sorghum), seeds (chia, hemp), nuts (almond, walnut), pulses (beans, lentil, pea, lupins), and leaves (duckweed), as well as mycoproteins, microalgae, and insects) can compensate for the increased demand for animal protein. In this context, our attention has been specifically focused on duckweed—which is the third most important aquatic plant after the microalgae *Chlorella* and *Spirulina*—to explore its potential for use in a variety of areas, particularly in the food industry. Duckweed has special properties: It is one of the fastest-growing plants in the world (in freshwater), multiplying its mass in two days, so it can cover a water surface quickly even in filtered sunlight (doubling its biomass in 96 hours). During this time, it converts a lot of carbon dioxide into oxygen. It is sustainable, environmentally friendly (without any pesticides), and fast growing; can be grown in indoor vertical farms and aquaculture, so it does not require land; is easy to harvest; and has a good specific protein yield. Duckweed belongs to the family Araceae, subfamily Lemnoideae, and has five genera (*Lemna*, *Spirodela*, *Wolffia*, *Wolffiella*, *Landolita*) containing a total of approximately 36–38 recognised species. Duckweed is gaining attention in nutrition and food sciences due to its potential as a sustainable source of protein, vitamins, minerals, and other bioactive compounds. However, there are several gaps in research specifically focused on nutrition and the bioaccessibility of its components. While some studies have analysed the variability in the nutritional composition of different duckweed species, there is a need for comprehensive research on the variability in nutrient contents across species, growth conditions, harvesting times, and geographic locations. There has been limited research on the digestibility, bioaccessibility (the proportion of nutrients that are released from the food matrix during digestion), and bioavailability (the proportion that is absorbed and utilised by the body) of nutrients in duckweed. Furthermore, more studies are needed to understand how food processing (milling, fermentation, cooking, etc.), preparation methods, and digestive physiology affect the nutritional value and bioavailability of the essential bioactive components in duckweed and in food matrices supplemented with duckweed. This could help to optimise the use of duckweed in human diets (e.g., hamburgers or pastas supplemented with duckweed) or animal feed. More research is needed on how to effectively incorporate duckweed into diverse cuisines and dietary patterns. Studies focusing on recipe development, consumer acceptance, palatability, and odour are critical. Addressing these gaps could provide valuable insights into the nutritional potential of duckweed and support its promotion as a sustainable food source, thereby contributing to food security and improved nutrition. In summary, this article covers the general knowledge of duckweed, its important nutritional values, factors that may affect their biological value, and risk factors for the human diet, while looking for technological solutions (covering traditional and novel technologies) that can be used to increase the release of the useful, health-promoting components of duckweed and, thus, their bioavailability. This article, identifying gaps in recent research, could serve as a helpful basis for related research in the future. Duckweed species with good properties could be selected by these research studies and then included in the human diet after they have been tested for food safety.

Keywords: alternative protein source; duckweed; nutrients; novel food; superfood; bioaccessibility of bioactive compounds; technological treatments

1. Introduction

The population of the world is expected to reach 9.7 billion by 2050, and the global demand for food is estimated to nearly double by that time [1]. Scientists agree that supplying food to this growing population will be a major challenge of the next decade. They highlight the concern of excessive livestock production, which is considered unsustainable for several reasons. This sector is estimated to be responsible for 26% of total ice-free land surface use, 15% of total groundwater use, and 12% of water pollution [2]. Livestock production accounts for approximately 13% of global greenhouse gas (GHG) emissions; consequently, it presumably contributes significantly to climate change [3]. The transformation of natural lands into pastures and croplands leads to a significant decline in biodiversity and to the disruption of ecosystems. Animal farming is considered to be a major contributor to deforestation, which results in an increased level of carbon dioxide in the atmosphere and, hence, in the exacerbation of climate change [4]. Another adverse environmental impact of livestock production is the emergence of antibiotic resistance, which poses a serious threat not only to farm animals but also to surrounding ecosystems and human health due to the overuse of antibiotics [5]. In addition, animal welfare and ethical concerns about intensive animal farming practices are also important issues of agriculture, politics, and economics [6].

In developed countries, plant-based diets have gained increased popularity due to their perceived nutritional and environmental benefits, as well as ethical considerations. In European countries, the estimated percentage of vegetarians ranges between 1% and 10%. The prevalence of these diets is expected to increase in the future [7]; thus, assessing the quality and safety of alternative food sources is a pivotal field of science. Among the three primary macronutrients, the growing demand for proteins has raised concerns about whether traditional sources, such as livestock, can meet this need [1]. As a result, a variety of alternative protein sources have been introduced to consumers, including pulses, algae, insects, plant-based alternative proteins, and cultured meat [8]. The percentage of Google search results for the keyword "alternative protein" shows a slight but constant increase since 2004, while the term "plant-based protein" has gained increasing attention only in the last decade [9]. A systematic review including the results of 91 studies on the consumer acceptance of alternative proteins revealed that pulses and plant-based proteins are much more accepted by consumers than insects and cultured meat [8].

The so-called "blue foods", derived from aquatic sources, can significantly contribute to sustainable food supply. These include fish and other marine animals, as well as alternative food sources like aquatic vegetables, seaweeds, and microalgae, whether from natural or from artificially cultivated aquatic sources. Proteins from algae, especially species of *Chlorella* and *Spirulina*, are in the focus of scientific attention due to their exceptionally high protein content, rich variety of essential amino acids, high biological utilisation rates, and low allergenicity [10]. Despite their potential, consumers' acceptance of microalgae is limited. Based on recent consumer surveys, Danish researchers concluded that microalgae are recommended to be used in small quantities when incorporated into foods, mainly due to their vivid green colour. Moreover, consumers tend to prefer the taste of freshwater algae over marine species due to the strong fishy flavour of the latter [11]. The study of Onwezen and co-workers (2021) suggests that plant proteins are favoured over microalgae by consumers; hence, plant-based aquatic protein sources may be more suitable as food ingredients [8].

Duckweeds (Lemnoideae) are sustainable sources of nutrients found in still freshwater or wetlands worldwide. These plants are small but have a very high reproductive rate, enabling a time- and cost-efficient production of food with a well-balanced composition [12].

Different species of duckweed have protein contents ranging from 16% to 42% and provide all essential amino acids [13]. They also contain a variety of undigestible carbohydrates, including both cellulose and pectin, which support digestion, regulate blood sugar levels and promote gut health. In addition, duckweed is a rich source of micronutrients and phytochemical compounds, particularly lutein and β-carotene, which reduce the risk of many chronic diseases [13]. However, the utilisation of proteins and other bioactive compounds may be inhibited by several factors, including certain growing conditions [14], cell wall properties [15], secondary metabolites [16], and antinutritive components [17]. Some physical, chemical, and biochemical processes are applicable to enhance the digestibility and bioavailability of food components [18,19]; however, a limited number of studies are available on how these treatments affect the nutritional and sensory properties of duckweeds. The present review focuses on some underexplored issues of duckweed production, with particular attention to their key nutrients and bioactive compounds. The factors limiting the utilisation of these components are also discussed. Moreover, some traditional and innovative technologies are introduced that are potentially applicable to improve the digestibility and bioavailability of nutrients and bioactive compounds.

Duckweed has garnered increasing attention in recent years due to its remarkable versatility and potential for diverse applications. As one of the smallest and fastest-growing flowering plants on Earth, duckweed offers a unique set of characteristics that make it a promising candidate for a wide range of industries and environmental applications. Duckweed can remove excess nitrogen and phosphate from water bodies, making it a promising candidate for use in wastewater treatment. Additionally, the harvested duckweed biomass can be utilised for biofuel production, as certain strains (i.e., *Lemna minor* and *Spirodela polyrhiza*) have been found to be rich in starch [20]. The plant's high protein content and amino acid composition make it a potentially valuable source of nutrition for both human consumption and animal feed [21–23]. Moreover, duckweed has shown promise in the pharmaceutical and dietary supplement industries. Duckweed has also been explored for its potential in phytoremediation, where it can be utilised to remove heavy metals and other pollutants from wastewater [21,24–26]. Particularly, *Lemna* species are well documented for their tolerance to various environmental pollutants, including heavy metals, making them suitable for phyto(bio)remediation [27]. This makes duckweed a valuable tool in addressing environmental challenges and promoting sustainable water management practices.

Despite the numerous benefits of duckweed, large-scale production continues to be a limiting factor for its widespread adoption in various applications [21,24,26]. Nevertheless, the potential of duckweed as a multifunctional plant is evident. With ongoing research and development, the challenges associated with the mass cultivation and harvesting of duckweed are being addressed, paving the way for its increased utilisation in various industries and environmental applications.

The special aim of this review is to explore the nutritional value of all duckweed species in general, in order to ascertain whether they can be used for both human consumption and animal feed. This article will explore the risk factors associated with its consumption and the species of duckweed permitted under current EU legislation. Europe's food consumption structure is characterised by 59% of daily protein intake coming from animal protein sources (meat, fish, milk) and only 41% from plant protein sources. Wheat protein represents more than 50% of the latter, while the intake of leguminous proteins (e.g., soy protein, lupins, beans, peas) represents only 3%. As a result, some cereals (wheat, maize, rice) have become staple foods, leading to geographic homogeneity of foods, dietary monotonicity, and nutritional imbalances, increasing the risk of micronutrient deficiencies, overweight and morbid obesity, and NCDs (non-communicable diseases), including cardiovascular diseases, stroke, cancer, and diabetes.

This makes it increasingly important to explore and investigate plant and other alternative sources of protein that can help meet the protein needs of a growing human population and address nutritional imbalances. Aquaculture sources of protein, like duckweed, with excellent nutritional properties, can offer a proper alternative in the longer term. The amount of the components produced in each clone is a consequence of adaptation to the waters and nutrient sources of the plants' original habitat. This highlights that there is potential for research into how to increase valuable components, i.e., to optimise the growing conditions. Over the past decade, several companies (e.g., Parabel, Hinoman, GreenOnyx and Plantible) have been created to promote duckweed as a food and protein source, while products from *Lemna* and *Wolffia* species have been granted generally recognised as safe status by the US Food and Drug Administration. These activities therefore pave the way for the widespread use of duckweed products for human consumption. Duckweed is heralded as a superfood to be discovered. This has led researchers to investigate the nutritional properties of several species of duckweed (and the foods processed from them). That leads to the realisation of the importance of comparing their nutritional values, paying particular attention to their bioactive properties, to select the species with the best nutritional values to include in our diet. The use of different gentle preservation techniques, cooking methods, and fermentation can also be promising ways of enhancing the biological value of the product. This article collates present information on digestion studies submitted so far to determine the digestibility, bioaccessibility, and bioavailability of some duckweed species. It helps to continue this research and highlights the need to make up for missing studies. By exploiting its applicability in dried form and protein powder concentrate, duckweed can be used in the development of innovative products. In this case, it is also important to ensure proper shelf-life and suitability for consumer needs. The introduction of duckweed and its products as new foods could further expand the range of foods that, in addition to their high nutritional value, also meet future sustainable production principles.

2. Botanical Description and Geographic Occurrence of Duckweed

Duckweeds are plants of the family Lemnaceae, consisting of five genera (*Landoltia*, *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*) and around 36–38 species. The exact number may differ based on taxonomic revisions and newly discovered species (Figure 1). *Wolffia* species are the smallest angiosperm plants in the world, with the shortest doubling time [28]. The diameter of duckweeds ranges between <1 mm (*Wolffia angusta*) and 1.5 cm (*Spirodela polyrhiza*). Depending on the genus, duckweed fronds vary in shape: they can be round to obovate in *Spirodela*, *Landoltia*, and *Lemna*; hemispherical or boat-shaped in *Wolffia*; or sickle-shaped, tongue-shaped, or ovate in *Wolffiella* [29]. They can be found all over the world, except for deserts and lands permanently covered with ice. They show the greatest growth in tropical and temperate areas, but several species survive in extreme conditions as well [30]. Members of the subfamily *Wolffioideae* do not develop roots and take up nutrients on their leaves. In contrast, the widely occurring *Lemna* and *Spirodela* species develop roots and take up nutrients through both their leaves and roots [31]. Duckweed often covers lakes and slow-moving waters densely, thus creating anoxic conditions. Consequently, the massive proliferation of the plant reduces the biodiversity in the habitat [32]. Duckweed species can grow at a broad pH range of 3.0–7.5 and temperatures between 6 °C and 33 °C, with a doubling time of 2–4 days [33,34]. The optimal daily light integral is recommended to be between 5 and 20 mol/m^2, with a nitrogen-to-phosphorus ratio of 15:1 to support maximum growth potential [35]. Under optimal environmental conditions, duckweeds can produce biomass with a productivity of 10–30 tons/ha/year [36]. Due to their considerable biomass production capacity and high protein content, duckweeds are widely utilised as feed and food sources, as well as for biofuel production [22]. In addition, duckweeds, particularly *Lemna* species, are well documented for their tolerance to various environmental pollutants, including heavy metals, making them suitable for phytoremediation [27].

Figure 1. Cultivation types ((**A**) indoor, sterile, controlled circumstances; (**B**) outdoor, semi-sterile circumstances; (**C**) outdoor, in fish pond, under semi-controlled circumstances; (**D**) in the wild) and some already known duckweed species.

3. Duckweed as an Alternative Source of Nutrients and Bioactive Compounds

Primary metabolites (such as protein, carbohydrates, and lipids) and secondary metabolites (such as phenolics, alkaloids, terpenoids, and carotenoids), along with the vitamin and mineral contents, play a distinct role in the plant's overall nutritional profile and its potential benefits for human health, and it is important to understand their potential in order to use them appropriately (Figure 2).

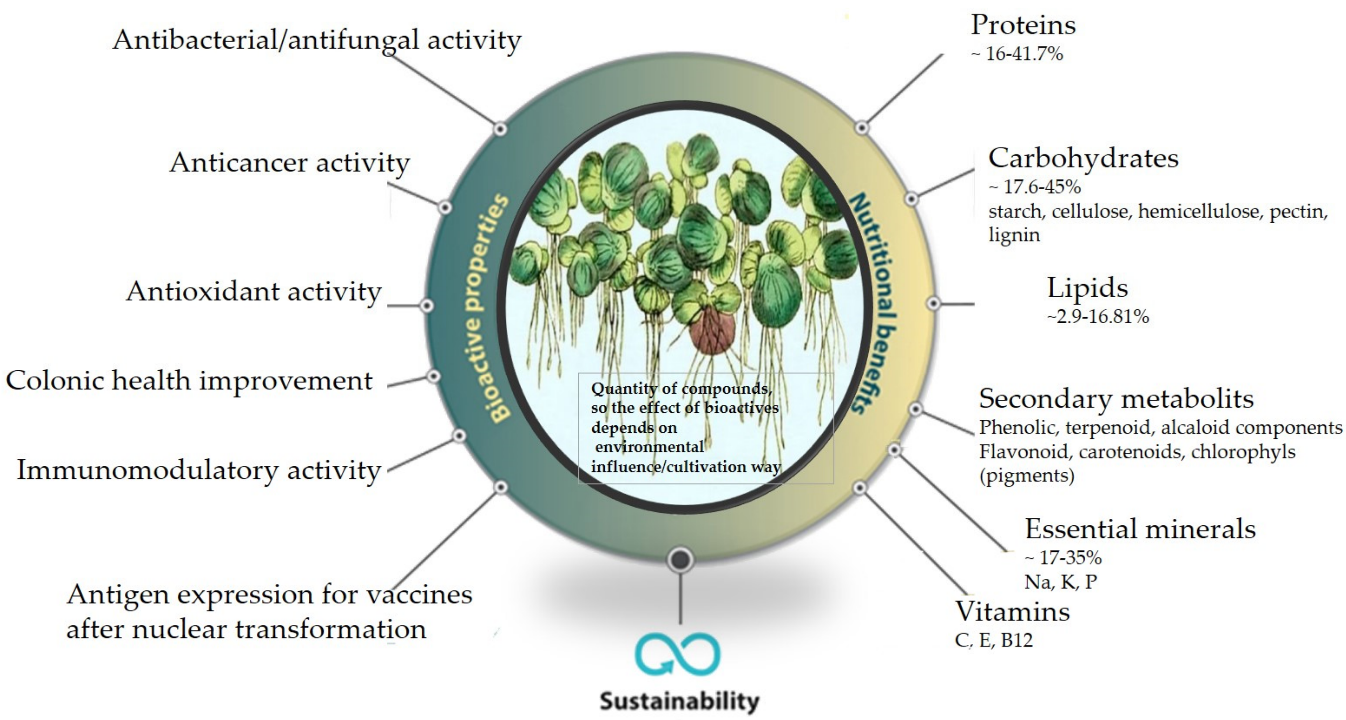

Figure 2. Nutrients with bioactive properties found in duckweed in general.

One of the key factors contributing to the nutritional value of duckweeds is their ability to efficiently absorb and accumulate nutrients, particularly nitrogen and phosphorus, from the surrounding water. Duckweed biomass has been shown to effectively remove up to 85.6% of nitrogen and phosphate from wastewater, a rate higher than that of other aquatic plants. This efficiency is partially due to the fact that duckweed tends to accumulate large amounts of nitrogen and phosphorus when grown in wastewater, resulting in a higher use-value compared to duckweed gathered from natural water sources [37].

The specific composition and distribution of nutrients varies, influenced by several factors, such as the species, growth conditions, and developmental stage of the plants, as well as the harvesting methods [21,26,38].

Recent research has shown that duckweed can be cultivated under controlled conditions to enhance its production of specific proteins and other beneficial compounds. By manipulating factors such as the availability of nutrients, the growth temperature, light, and the age of the plant, it is possible to optimise the nutritional profile of duckweed.

Interestingly, research has indicated that duckweed's protein production levels can decrease under stress conditions, leading to the accumulation of high amounts of starch, which can be useful for various applications [26].

The secondary metabolite profile of duckweed can vary considerably depending on the specific environmental conditions in which it is growing.

Understanding the underlying mechanisms and regulatory pathways governing nutrient synthesis in duckweed is an area of active research, with potential implications for optimising the production of the valuable primary and secondary metabolites [39,40].

Furthermore, the development of advanced biotechnological tools, such as CRISPR ("Clustered Regularly Interspaced Short Palindromic Repeats") technology, has enabled research scientists to selectively modify the DNA of living organisms. CRISPR—adapted for use in the laboratory from naturally occurring genome editing systems found in bacteria—may enable the targeted manipulation of duckweed's metabolic pathways in the future, potentially leading to the enhanced production of specific probiotic compounds or even more diverse bioproducts [24].

Certain bacterial strains, such as the diazotrophic bacterium *Azotobacter vinelandii*, have been found to form mutualistic relationships with duckweed (bacteria and plants work together indirectly but do not form a close symbiosis), promoting its growth and development [21,24,26,41,42]. These beneficial bacteria live mostly in soil, but also in aquatic environments, and they can fix atmospheric nitrogen, making it available to the duckweed plant while also potentially producing other growth-promoting compounds. *Azotobacter vinelandii* bacteria, as free-living nitrogen-fixing bacteria, are not directly attached to the roots of the duckweed like other nitrogen-fixing bacteria, but the nitrogen that they release into the environment can improve the growth of the duckweed and increase its nutrient contents, especially the protein content [26]. The bacteria's nitrogen fixation process converts nitrogen (N_2) in the air into ammonia (NH_3), which is a source of nitrogen available to plants. The duckweed takes up ammonia or ions from it (e.g., ammonium: NH_{4+}) and uses them in its own metabolic processes to synthesise amino acids. Furthermore, the ability of duckweed to serve as a natural habitat for these bacteria suggests that it could be a valuable source of probiotics for both human and animal consumption, as *Azotobacter vinelandii* seems to exhibit several probiotic-like characteristics [43].

3.1. Protein

The primary nutritional benefit of duckweed consumption lies in its protein content, which varies [13] depending on the harvest location, variety, and weather conditions.

Furthermore, the biological value of the protein in duckweed is enhanced by its well-balanced amino acid profile, with a good representation of essential amino acids [44]. Duckweed proteins have similarities to whey proteins in terms of amino acid composition, making them easily digestible and assimilable by both humans and animals [24]. Duckweed protein contains 2.7% methionine and cysteine, 7.7% phenylalanine and threonine, and 4.8% lysine [44], while whey proteins contain 2.6% methionine and cysteine, 3.5% phenylalanine and threonine, and 9.4% lysine, and both are rich in leucine, threonine, valine, and isoleucine [45]). These similarities suggest that duckweed could serve as an ideal alternative to traditional protein sources, particularly in regions where access to more conventional protein-rich foods may be limited. This property has led to the exploration of duckweed as a viable animal feed supplement as well.

Studies have shown that the contents of lysine, isoleucine, leucine, valine, and phenylalanine in duckweed are 5.9%, 4.29%, 8.42%, 5.46%, and 7.77%, respectively, which are higher than in most cereal crops [46]. The content of threonine is 4.46%, which is higher than the human consumption standard recommended by the FAO (Food and Agriculture Organisation) [46,47].

Wolffia globose and *Wolffia columbiana* are exceptionally rich sources of proteins, while *Wolffia arrhiza* mainly contains carbohydrates. The amino acid compositions of *Wolffia microscopica* and *Wolffiella hyalina* are comparable with the daily requirements for adults recommended by the WHO (World Health Organisation), and they contain sufficient proportions of lysine, sulphur-containing amino acids, threonine, and tryptophan, so they are desirable as human foods [44]. On the other hand, a nutraceutical powder of *Lemna minor* showed an imbalanced amino acid composition as it was deficient in certain amino acids, mainly lysine and methionine [48]. The digestibility of proteins and the bioavailability of essential amino acids are also species-dependent [49] and affected primarily by the amount of antinutritional factors, such as oxalates, phytic acids, and tannins [50].

The duckweed protein produced by RuBisCO is accepted by EU as a good source of essential amino acids, and it has high nutritional value, high in vitro digestibility, and low allergenicity, making it a good candidate for functional foods [51].

3.2. Carbohydrates

In duckweed, the cell wall contains several carbohydrate components that contribute to its structure and function, but their presence and roles can vary slightly depending on the species and environmental conditions. Duckweed, being a small aquatic plant, has relatively simple and thin primary cell walls that primarily consist of cellulose, hemicellulose, and pectin. Lignin, a complex phenolic polymer typically found in vascular plants for strengthening secondary cell walls (e.g., in wood and xylem tissues), is minimal (negligible) or absent in duckweed due to its aquatic lifestyle and lack of extensive vascular structures. Cellulose is a primary and essential component of the cell wall in duckweed. It forms microfibrils that provide structural support and tensile strength. Hemicellulose is a secondary structural component in the cell wall. It crosslinks with cellulose microfibrils, contributing to the wall's flexibility [52]. Pectin is abundant in the primary cell wall of duckweed, especially in younger or growing tissues. It forms a gel-like matrix that helps maintain cell wall porosity and adhesion between cells, as well as playing a role in water retention [53]. Pectin is known to undergo changes in response to biotic or abiotic stresses, and these changes can have significant implications for plant development, immunity, and industrial applications, such as biomass conversion for biofuel production [54].

Duckweed's fibre content, which can reach up to 45% of its dry weight, makes it a potentially valuable source of this important nutrient [24,55]. Duckweed's fibre content, which includes both soluble and insoluble forms, may offer additional advantages. The high dietary fibre content in duckweed helps maintain gut health, promotes normal digestive function, and contributes to controlling blood sugar levels.

Soluble dietary fibres (in water and from gels), such as pectin and hemicelluloses, are known to have beneficial effects on human health, including the regulation of blood sugar levels and helping to slow down the absorption of fats (weight management), which may be beneficial for individuals with conditions like diabetes or high cholesterol [56]. Furthermore, they help in the promotion of a healthy gut microbiome [13]. These soluble fibres are highly digestible and can be readily absorbed by the human body.

On the other hand, insoluble dietary fibres, such as cellulose and lignin, are less readily digestible but play an important role in maintaining a healthy digestive system (reducing the risk of digestive issues) by adding bulk to the stool and promoting regular bowel movements.

Additionally, more in-depth studies are required to elucidate the potential health benefits and applications of the soluble and insoluble dietary fibres found in these versatile aquatic plants [13,24,26,42]. From a technological perspective, the incorporation of duckweed fibres in food products presents several advantages. The incorporation of duckweed fibres in food products could contribute to the development of novel functional and textural properties. Duckweed fibres could potentially be used as natural thickeners, emulsifiers, or stabilisers, potentially enhancing the mouthfeel and overall quality of food products.

The high energy content of duckweed, primarily in the form of easily fermentable starch, makes it a promising feedstock for biofuel production, further enhancing its value as a sustainable resource [57]. Starch, however, is primarily a storage carbohydrate, not embedded in the cell wall matrix. Instead, starch is stored in plastids (e.g., chloroplasts) within the cell cytoplasm and can accumulate significantly under certain specific stress conditions, such as nutrient deficiency (limited nitrogen or phosphorus availability) or salinity stress [26]. In fact, starch accounts for about 4.0% to 29.8% of duckweed [36], but duckweed grown under specific conditions can contain up to 40% starch by dry weight, highlighting its potential as a renewable energy source [42]. While the carbohydrate profile of duckweed has been well studied, there are still opportunities for further research to optimise its production and unlock its full potential. In summary, according to Pagliuso et al. (2021), the cell wall comprises 20.1% pectin and glucomannan, 35.2% hemicelluloses, 30% cellulose, and 5% lignin, and the fermentable sugars in duckweed's cell walls are glucose, galactose, and xylose. Together, these monosaccharides constitute 51.4% of the cell wall [58].

These findings highlight the functional roles of cell wall polymers versus storage compounds in duckweed's metabolism and its applications in bioenergy and nutrition research.

3.3. Lipids

The lipid content of duckweed ranges from 2.9% to 16.81%. Its fatty acid composition has been extensively studied, providing insights into its nutritional profile [59]. Duckweed is particularly rich in polyunsaturated fatty acids, with linoleic acid (omega-6), linolenic acid (omega-3), arachidonic acid, and saturated fatty acids such as palmitic acid dominating, accounting for about 80% of its total fatty acids.

Duckweed has the potential to prevent inflammatory diseases, cancer, cardiovascular diseases, and other chronic diseases [13,51,60,61]. A study on the fatty acid composition of different duckweed species found that *Lemna minor* had a higher proportion of linolenic acid compared to *Spirodela polyrhiza* [60]. This suggests that the specific duckweed species and cultivation practices can be optimised to enhance the production of desired fatty acids.

The high content of essential polyunsaturated fatty acids in duckweed makes it a potentially valuable ingredient in the development of functional foods and nutraceuticals. Duckweed could be used to fortify various food products, such as vegetable oils, dairy products, and even animal feeds, to increase their nutritional value and provide health benefits to consumers.

3.4. Secondary Metabolites

While their primary metabolites, such as proteins, carbohydrates, and lipids, have been extensively studied, the secondary metabolites of duckweeds have received comparatively less attention.

Secondary metabolites are a diverse range of organic compounds produced by plants that do not directly contribute to their growth, development, or reproduction but, rather, serve various ecological functions, such as defence against herbivores and pathogens, or attraction of pollinators [37].

These compounds can have significant impacts on the interactions between plants and their environment, and understanding their biosynthesis and functions is crucial for developing sustainable agricultural and environmental practices [61,62].

Recent studies have revealed that duckweeds possess a rich array of secondary metabolites, including phenolic compounds (e.g., flavonoids, phenolic acids, tannins), terpenoids, and alkaloids, which may play crucial roles in their adaptability and survival [23]. Furthermore, duckweed contains pigments within its fronds [25].

3.4.1. Phenolic, Terpenoid, and Alkaloid Compounds

Phenolic compounds are known for their antioxidant properties, which can help protect duckweeds from environmental stresses, such as UV radiation and heavy metal contamination [63]. Additionally, phenolic compounds can influence the interactions between duckweeds and other organisms, such as inhibiting the growth of algae and cyanobacteria, which may compete for resources [64]. Furthermore, the presence of flavonoids and tannins in duckweeds has been linked to their ability to withstand oxidative stress and deter herbivores [65,66]. According to existing research, flavonoids have been identified in duckweed, depending on the species and the growing conditions. Components from the flavone subclass of flavonoids isolated from duckweed mainly include apigenin, luteolin, and their derivatives, such as apigenin-7-O-glucoside, apigenin-8-C-glucoside-6-C-glucoside, apigenin-8-C-glucoside-6-C-rhamnoside, luteolin-7-O-glucoside-*C*-glucoside, luteolin-8-C-glucoside-6-C-rhamnoside, and luteolin-6-C-glucoside-8-C-rhamnoside [55,56,67,68]. The diversity of flavonoids gives them pharmacological functions such as natural antioxidant, antimicrobial, anti-inflammatory, anti-tumour, blood sugar-lowering, and blood pressure-lowering effects [24,69], making duckweed a potentially high-value component in the human diet, as well as being applicable in the fields of feed and medicine. Phenolic acids of phenolic components such as isoorientin, vitexin, isovitexin, and hesperetin were also detected in *Lemna perpusilla* and *Lemna minor* [70].

Duckweeds also produce a range of terpenoid compounds, which are a large and diverse class of secondary metabolites. Some terpenoids produced by duckweed have been found to act as repellents, deterring herbivores from feeding on the plant. Terpenoids in duckweeds have been found to exhibit antimicrobial, anti-inflammatory, and insecticidal properties, suggesting their potential role in plant defence mechanisms [61].

Additionally, duckweeds have been found to produce various alkaloids, which can act as deterrents against predators and pathogens [64,65].

3.4.2. Flavonoids, Carotenoids, and Chlorophylls

Duckweeds are known for their diverse pigment composition, which can affect their overall appearance. The plants' pigment profiles may shift in response to environmental factors such as nutrient availability, light intensity, and temperature [71].

Duckweeds are known to possess a variety of pigments, including flavonoids, carotenoids, chlorophylls, which contribute to their characteristic green, yellow, or reddish hues.

Flavonoids are a large group of plant compounds, many of which function as pigments in plants, particularly in flowers, fruits, and leaves. The primary flavonoid pigments identified in duckweeds are derivatives of apigenin and luteolin (mentioned in Section 3.4.1), which contribute to yellow pigmentation. These flavonoids have been identified in various duckweed species, including *Landoltia punctata*, *Wolffiella caudata*, and *Wolffia borealis* [56], and to a lesser extent in *Lemna perpusilla* and *Lemna minor* [70].

Carotenoids, a structurally diverse group of over 700 organic, lipid-soluble pigments, are responsible for the vibrant yellow, orange, and red colours observed in various natural sources, including plants, algae, and some bacteria [72–74]. These pigments not only contribute to the vibrant hues observed in duckweed but also confer a wide range of physiological benefits, such as improvements in survival, growth performance, reproductive capacity, stress tolerance, disease resistance, and immune-related gene expression [75,76]. These pigments play a crucial role in the prevention of human diseases and the maintenance of overall health, owing to their antioxidant properties and their ability to serve as provitamin A nutrients [72–74].

Carotenoids, including astaxanthin, β-carotene, canthaxanthin, fucoxanthin, zeaxanthin, and lutein, have been identified in various duckweed species [40,77]. The presence of carotenoids like astaxanthin, lutein, and β-carotene in duckweeds can have implications for their potential applications in aquaculture and animal feed. These carotenoids are known to play a role in the pigmentation, growth, and health of various aquatic organisms, and their availability in duckweeds may make them a valuable natural source of these compounds [75]. Lutein (a type of xanthophyll that is yellow in colour) is important for the plant's photosynthetic processes and also has antioxidant properties. Zeaxanthin, another type of xanthophyll, is involved in the photoprotective mechanisms of the plant, helping to protect against damage from excess light. Zeaxanthin, in particular, has been shown to improve vision, protect the eyes from intense light damage (preventing cataracts and blindness), and combat systemic inflammation [78]. While not as abundant as lutein and zeaxanthin, β-carotene is another carotenoid found in duckweed (Stomp 2005) [79]. β-Carotene is a provitamin A carotenoid (precursor of vitamin A), meaning that it can be converted into vitamin A in the human body, and it also exhibits potent antioxidant properties (Liu et al., 2020) [24].

Overall, these carotenoids contribute to the plant's ability to absorb light for photosynthesis, protect against oxidative damage, and play a role in the plant's overall health and stress responses. Furthermore, they also offer potential health benefits for animals and humans, such as reducing the risk of degenerative diseases, cardiovascular issues, and cancer [75,80]. Lutein and zeaxanthin are of interest for their potential health benefits, especially for eye health in humans, as they are found in high concentrations in the retina [53]. They can also serve as regenerative resources for astronauts in space environments, exerting antioxidant effects to help protect them from radiation damage [81].

The distribution and abundance of carotenoids in duckweeds can also be influenced by other pigment-related secondary metabolites, such as chlorophylls [82,83]. Chlorophylls are the primary photosynthetic pigments in plants, and they play a crucial role in the light harvesting and energy transduction processes. They are crucial for the photosynthetic processes that power the rapid growth and development of duckweed. These pigments not only facilitate the conversion of light energy into chemical energy but also play a vital role in the plant's ability to thrive in aquatic environments, where light availability can be a limiting factor. Chlorophyll a and chlorophyll b are the two main forms of chlorophyll found in duckweeds, and their relative abundance can affect the overall pigment composition and appearance of the plants [80,84].

Ultimately, the investigation of pigments in duckweeds has important implications for understanding their photosynthetic efficiency, stress tolerance, and potential applications in areas such as aquaculture and bioremediation. The diverse array of pigments found in duckweed has garnered significant interest in the scientific community, as these compounds have demonstrated a wide range of biological functions, including antioxidant, anti-inflammatory, and antimicrobial properties. As such, the exploration of duckweed as a source of natural pigments and their subsequent application in various industries, such as food, cosmetics, and pharmaceuticals, represents a promising avenue for future research and development.

3.5. Minerals

Duckweed (*Lemnaceae* family) is recognised for its rapid growth and potential as a sustainable food/feed source. Its mineral content includes essential nutrients (~0.2–7%) that are vital for maintaining good health, such as potassium (K), iron (Fe), calcium (Ca), and phosphorus (P), along with trace elements such as manganese (Mn), zinc (Zn), and copper (Cu), which are important for various metabolic processes [78,85].

Duckweed also has the ability to absorb potentially harmful heavy metals from its environment, which makes it useful for bioremediation but raises concerns when used as food or feed. Duckweed can accumulate heavy metals like cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), cobalt (Co), nickel (Ni), selenium (Se), and zinc (Zn) [36]. The concentrations of both nutrient-rich elements and heavy metals in duckweed are highly dependent on the surrounding water quality and the specific cultivation environment. For instance, exposure to contaminated water can lead to significant accumulation of heavy metals, rendering the biomass unsuitable for consumption [27], while duckweed grown in wastewater-fed environments has been found to have higher concentrations of nitrogen and phosphorus compared to duckweed collected from natural water bodies.

The mineral content of duckweed is a crucial factor that contributes to its diverse applications, ranging from phytoremediation, food, and animal feed to biofuel production. Further research is needed to fully understand the mechanisms underlying the mineral accumulation in duckweed and to optimise its cultivation for specific purposes [24,71,86,87].

3.6. Vitamins

The available research suggests that duckweed, like many other plants, is capable of synthesising, and even absorbing and concentrating a variety of essential vitamins, such as vitamin C and vitamin E (especially tocopherol, which is crucial for human health), from its surrounding environment [77].

According to multiple studies, duckweed has been found to provide a new natural plant source of vitamin B12 (cobalamin). The vitamin B12 content of duckweed can vary between 0.5 and 10 μg/100 g dry matter [26,29,88].

The B12 content of duckweed has a particular importance since, traditionally, vitamin B12 has been primarily obtained from animal-based sources, such as meat, eggs, and dairy products, and not from any plant-based materials, thus causing serious B12 deficiency for strict vegetarian individuals, which can be prevented by supplementation with duckweed. The increasing demand for plant-based and vegan diets has sparked interest in exploring alternative sources of this vital nutrient. [29,57,89–91]. Vitamin B12 is essential for the normal functioning of the brain and nervous system and is also involved in red blood cell production; fatty acid, DNA, and RNA synthesis; and in the production of cellular energy [92]. In cases of B12 deficiency, red blood cell counts can be critically reduced and anaemia can occur. This is the reason why B12 is especially known for its role in the treatment and prevention of pernicious anaemia [93]. To ensure that people get enough vitamin B12, the European Union has set a minimum daily intake of B12. The European Food Safety Authority (EFSA) recommends an adequate daily intake (AI) of vitamin B12 of 4 µg/day for the European population and 4.2 to 8.6 µg/day in some less developed countries [94].

In early studies, it was assumed that duckweed, like other plants, could synthesise vitamin B12 through its own metabolic processes. However, recent studies have challenged this notion. Duckweed, being a simple flowering plant, does not possess the necessary biosynthetic pathways to produce vitamin B12, which is a complex molecule typically synthesised by certain bacteria and archaea [21,24,26,42].

4. Human Nutrition and Food Safety Assessment of Duckweed

Duckweed, a common aquatic plant, has gained significant attention in recent years due to its potential as a nutrient-rich food source [95,96]. Duckweeds have traditionally been used to prepare dishes like salads, omelettes, or vegetable curries in some South-East Asian countries (Burma, Laos, and Northern Thailand) [97], although the utilisation of duckweed in home cooking has been relatively limited [85]. It is considered to be a plant-based ingredient for future food and a sustainable alternative protein source that can replace animal meat and is healthier and more affordable [98].

When it comes to the comparative advantages of dried duckweed versus duckweed protein powder, both options hold their own merits. The dried form of duckweed may be preferable in terms of preserving the plant's full spectrum of bioactive compounds. This could be particularly beneficial for individuals seeking a more holistic, whole-food approach to nutrition. On the other hand, the duckweed protein powder offers the convenience of easy incorporation into a wide range of food products, potentially expanding its accessibility and appeal to a broader audience. Ultimately, the choice between dried duckweed and duckweed protein powder may depend on individual preferences and dietary needs. Those seeking a more comprehensive nutritional profile may gravitate towards the dried form, while those looking to boost their protein intake in a versatile manner may find the protein powder more appealing [26,42].

The growing interest in nutrition and diet has led to a reassessment of the food value of duckweed in European countries. This shift in perspective has opened the door for the increased incorporation of duckweed into the cuisine of the EU and beyond, as chefs and home cooks alike explore the culinary versatility of this unique plant. The two main species consumed by humans are *Wolffia arrhiza* and *Wolffia globosa*. Their traditional Thai name is Khai-nam, which was generally regarded as food for poor people [30]. A survey on *Wolffia* consumption in Thailand (Loei Province) showed that the locals still consume Wolffia in traditional ways, as an ingredient in spicy soups and salads [99]. Today, duckweed is increasingly recognised in Western diets as a sustainable alternative protein source that can replace animal meat while being healthier and more affordable [98]. Since Wolffia has a history of consumption in certain parts of the world, it is considered to be a "traditional food from a third country" in the European Union, falling under the novel food regulation. This means that these products should be authorised prior to marketing in the EU.

During the authorisation process, the EFSA (European Food Safety Authority) and the EU member states assess the application dossier, and in the event of a positive opinion, the EU Commission authorises the marketing.

Fresh plants of *Wolffia arrhiza* and/or *Wolffia globosa* have been authorised in the EU since 2021 by the Commission Implementing Regulation (EU) 2021/2191.

During the authorisation procedure, the EU member states and the EFSA (European Food Safety Authority) did not submit any safety objection to the marketing of the product. In the technical report, the EFSA (European Food Safety Authority) concluded that the data submitted by the food business operator verify the safe use of the fresh plants of *Wolffia arrhiza* and *Wolffia globosa* cultivated under vertical farming conditions, which must strictly be grown in a safe, contaminant-free environment to make them suitable for human consumption [100]. However, the EFSA (European Food Safety Authority) has remarked that heavy metals and microcystins can accumulate in the culture medium; thus, the presence of these elements in the fresh plants should be controlled. Furthermore, trace elements may come from the use of fertilisers in the cultivation of Wolffia, which can be a safety concern for consumption. Therefore, the Commission specified maximum safety limits for heavy metals (lead: <0.3 mg/kg; arsenic (inorganic): <0.10 mg/kg; cadmium: <0.2 mg/kg; chromium: <1 mg/kg; mercury: <0.10 mg/kg), trace elements (copper: <0.8 mg/kg; molybdenum: <0.3 mg/kg; zinc: <5 mg/kg; boron: <5 mg/kg; manganese: <6 mg/kg), and cyanotoxins (microcystins: 0.006 μg/g), as well as general criteria for microbiological and pesticide contamination [101]. The fresh plants of *Wolffia arrhiza* and *Wolffia globosa* can be marketed as long as they comply with these criteria. In addition to the accumulation of pollutants and microbiological contamination, potential allergenicity is also considered to be a major food safety issue.

On 9 April 2024, the EU Commission authorised the marketing and use of protein concentrates derived from *Lemna gibba* and *Lemna minor* for human consumption [102].

5. Animal Nutrition

The shortage of traditional feed ingredients is becoming an increasingly significant problem for developing countries worldwide. To bridge the gap between feed supply and demand, non-traditional feed sources are coming into focus. Due to their high yield, economic benefits, nutritional value, and positive public perception, duckweed species offer a promising source of protein [12,103–105]. *Spirodela polyrhiza* is of interest as a feed material/component for fish, poultry, and pigs because its lower fibre content makes it easily digestible [44]. Duckweed can be utilised for nitrogen and phosphorus recovery from animal slurry, while also producing high-quality protein feed [106].

A study by Abdullahi et al. (2023) [107] demonstrated that blanched and sun-dried duckweed meals can replace soybean meal in the diet of *Oreochromis niloticus* without negatively affecting protein digestibility or carcass protein levels, with protein digestibility ranging from 75.9% to 79.9% and energy digestibility from 58.4% to 64.5% [108]. Similarly, research with sheep showed that soybean meal can be partially replaced (up to 50%) with duckweed, maintaining economical production while reducing forage costs [109]. While the nutritional value of duckweed-containing feeds varies, stabilisation and optimisation could make duckweed a viable protein source for broiler chickens [110], although Mwale and Gwaze (2013) [111] caution against exceeding 6% inclusion without further studies. In dog diets, duckweed inclusion reduces dry matter, gross energy, and crude protein digestibility, but the addition of exogenous phytase can improve crude protein digestibility at 30% inclusion levels while also enhancing stool consistency [112]. These findings highlight duckweed's potential as a cost-effective and versatile feed ingredient across various species, provided its composition is carefully managed. The variability in digestibility underscores the importance of selecting the right duckweed species and optimising its composition through cultivation and processing for different animal diets. Despite these challenges, duckweed's rapid growth, high protein content, and sustainable production make it a promising feed ingredient, particularly for supporting protein requirements in livestock and aquaculture systems.

Duckweed is a new type of animal feed ingredient, and the proportion of duckweed replacing traditional feed may affect the digestibility and absorption of nutrients by animals. For example, in a study on tilapia, when the substitution rate of duckweed was 20% and the feeding amount was 3%, the weight gain and specific growth rate of the fish increased significantly. Compared to regular feed, the weight gain increased by 57.5% and the specific growth rate increased by 30.3%. However, when the substitution rate exceeded 25%, these indicators began to decrease [113].

6. Factors Influencing the Protein Digestibility and Bioaccessibility of Bioactive Compounds in Duckweed

Duckweed has garnered significant attention for its potential as a source of bioactive compounds with various health benefits [114]. However, the access of these compounds can pose challenges.

Bioavailability and bioaccessibility are two closely related concepts that are often used in the assessment of the absorption and utilisation of nutrients, drugs, and other compounds within the human body. Bioavailability refers to the proportion of a substance that is able to enter the systemic circulation and reach the site of action, whereas bioaccessibility describes the amount of a substance that is available for absorption by the body. *In vitro* and *in vivo* digestion models can be used to provide answers as to whether these degraded components retain their biological activity during their passage through the gastrointestinal tract, and if so, how this bioactive form is utilised: is it absorbed, or can it be utilised in an active form? [115].

One key factor that affects the bioavailability of bioactive compounds in duckweed is the intricate food matrix. The bioaccessibility of a compound, which refers to the amount that is released from the food matrix and available for absorption, is often more relevant than its mere content within the plant. The bioavailability of bioactive compounds is influenced by their chemical structure, interactions with other components during digestion, and the properties of the food matrix [14,114]. For example, the presence of secondary metabolites, such as polysaccharides and organic acids, can interfere with the solubility and separation of proteins during SDS-PAGE [16].

Duckweed, like many other plant-based foods, can have a complex matrix that may hinder the release and absorption of beneficial compounds [96,116]. For example, factors such as crude fibre content and ash levels influence the digestibility outcomes [117]. Species like *Lemna minor* and *Lemna gibba* typically show higher digestibility compared to *Spirodela polyrhiza*, which in comparison has higher fibre content.

Lignin content is an important component of plant cell walls, and if its content is too high, it can make the cell walls more difficult to break down, which is not conducive to the digestion and absorption of nutrients. Additionally, lignin can form complexes with proteins, which resist breakdown in the stomach environment, thereby reducing the solubility and digestibility of proteins [118]. Research has shown that the lignin content in duckweed is extremely low, which has a minimal impact on the absorption and utilisation of proteins [119]. However, the unique cell wall structure is primarily composed of cellulose embedded in a matrix of other polysaccharides, which can pose challenges in accessing the plant's bioactive compounds and ensuring adequate digestibility. This is naturally recalcitrant to the deconstruction carried out by microbes and enzymes [52].

Duckweeds, being a rapidly growing and highly efficient phytoremediator, can potentially accumulate various contaminants and toxins from the environment, which can lead to the presence of antinutrients. Antinutrients are substances present in plants that can interfere with the absorption or utilisation of nutrients by the human or animal body [24,71].

The presence of other potential antinutritional factors in duckweed can also affect the absorption of nutrients, such as oxalates, phytates, and tannins, which are common in plant-based foods [120]. Soluble oxalates can form insoluble crystals with Ca_{2+} and Mg_{2+}, leading to reduced absorption and utilisation of calcium in the body, and they can easily induce hypocalcaemia and kidney metabolism disorders [121]. About 70% of phosphorus (P) in plants exists in the form of phytate-P, which is difficult to digest and absorb in monogastric animals (such as pigs and chickens) and in humans without sufficient phytase. Therefore, high levels of phytates can lead to lower biological phosphorus utilisation [122]. High concentrations of tannins in food can inhibit the digestion and utilisation of nutrients such as proteins, carbohydrates, vitamins, and minerals by binding with them to form indigestible substances, suppressing digestive enzymes and gastrointestinal bacteria [123]. Hu et al. (2022) conducted an analysis of the contents of oxalates, phytates, and tannins in duckweed, finding that their levels in duckweed are relatively low, with minimal impact on the digestion and utilisation of nutrients [50]. Another potential antinutrient in duckweeds is phytic acid, which can chelate essential minerals such as iron, zinc, and calcium, reducing their absorption [87].

Another challenge arises from the variations in bioactive compounds' contents and distribution within duckweed. Factors such as the source/species, growth conditions, and processing methods can significantly impact the concentration and bioavailability of these compounds [14]. *In vivo* studies have been conducted to assess and mitigate these antinutrient factors. For example, a study incorporated raw and fermented *Lemna polyrhiza* leaf meal into diets for rohu fish (*Labeo rohita*) fingerlings. Fermentation with a specific bacterial strain significantly reduced tannin (from 1.0% to 0.02%) and phytic acid (from 1.23% to 0.09%) levels. Fish fed diets with up to 30% fermented leaf meal exhibited improved growth performance compared to those fed raw leaf meal, indicating that fermentation effectively reduces ANFs and enhances nutritional value [124]. Other researchers have evaluated the impact of different types of duckweed batches (with *Lemna minuta* and *Lemna minor* (A, 17.5% crude protein), *Spirodela polyrhiza* (B, 24.6% crude protein), and *Lemna obscura* (C, 37.0% crude protein)) on broiler chickens, focusing on feed intake, body weight, and the ileal digestibility of amino acids and phosphorus [111]. Studies have shown that there are significant differences in the standardised ileal digestibility of crude protein and amino acids among different batches of duckweed, primarily due to variations in the nutrient composition and levels of antinutritional factors in different species of duckweed [111]. Furthermore, the growth environment of duckweed, such as carbon dioxide concentration and microorganisms, can also affect its nutrient composition [125]. Research has found that duckweed has higher protein content in its leaves under high carbon dioxide concentration and suitable nutrient supply conditions, but this high concentration may have a feedback inhibitory effect on certain nutrients.

Furthermore, the rapid growth rate of duckweed, which can double in biomass in less than 30 hours under optimal conditions, may present unique challenges in terms of maintaining consistent quality and bioactive compound profiles [57].

In-depth understanding of the bioaccessibility and bioavailability of duckweed's bioactive compounds, as well as the optimisation of cultivation and processing methods, can unlock the full potential of this aquatic plant as a functional food source. Factors influencing the digestion rate of nutrients and the bioavailability of bioactive compounds in duckweed are crucial for the conversion of duckweed into food resources for human consumption and raw materials for animal feed. Understanding the factors that affect the digestion rate of nutrients and the bioavailability of bioactive compounds is helpful in developing more effective food processing methods and nutritional supplementation strategies, providing a theoretical basis for optimising feed/food formulations and improving feed/food processing techniques in animal feeding and human consumption [14,114].

7. Possible Technological Solutions for Improving Protein Digestibility and Bioaccessibility of Bioactive Compounds in Duckweed

Duckweed, a small floating aquatic plant, has garnered significant attention as a potential source of protein, vitamins, and other valuable compounds [125]. However, the efficient extraction and utilisation of these compounds from duckweed can be challenging due to the plant's cellular structure and composition [126], along with antinutritional factors.

Improving protein extraction yields from comparatively sustainable aquatic biomass (microalgae, seaweeds, aquatic plants) is the present focus of the food industry. Kumar et al. (2022) mentioned that protein extraction from microalgae is quite challenging due to the presence of pigments and polysaccharides in the protein due to cell disruption, hence requiring a proper purification process. Similarly, during the extraction of protein from seaweed, polysaccharides and phenolics are present as co-extracted compounds and interact with seaweed proteins [127]. The article does not specifically mention the extraction of duckweed proteins, but it does mention steps of methods for obtaining proteins from aquatic plants, such as tissue disruption (mechanical pressing), protein solubilisation, and/or fractionation (alkaline pH, ammonia treatment), followed by protein purification (precipitation by heat or acid) and protein concentration (centrifugation, drying). This information suggests that, as in the case of duckweed, protein release must be a multi-step process.

To overcome these challenges, researchers have explored various chemical and physical approaches to improve the efficiency of nutrient extraction from duckweed. Enhancing the bioaccessibility of bioactive compounds in duckweed is essential to fully realise its potential as a sustainable source of nutrients and bioactive compounds for human and animal consumption.

The main objectives are to overcome structural barriers, improve nutritional value, eliminate antinutritional factors, and enhance bioavailability for human and animals. Enhanced bioaccessibility translates to improved bioavailability—the degree to which bioactive compounds are absorbed and utilised in the body. Employing these techniques ensures maximum utilisation of duckweed's bioactive compounds, reducing waste and making it a more efficient alternative to traditional crops. Some techniques are energy-efficient and environmentally friendly, aligning with sustainability goals. Enhancing bioaccessibility makes it possible to incorporate duckweed into a wider range of functional foods and supplements, thus unlocking its full potential as a superfood.

7.1. Chemical Methods

One such chemical method involves the use of specific enzymatic degradation to disrupt the cell walls and facilitate the release of nutrients, such as proteins. Strategies to address the cell wall barrier have been explored in the context of other algal and plant-based sources. A study on *Nile tilapia* showed that the addition of exogenous enzymes and probiotics to the diet can alleviate the negative effects of plant-derived ingredients, such as increased levels of non-starch polysaccharides and phytates, on nutrient digestibility and gut health [121]. Further research is needed to identify the specific enzymes that can target and break down the antinutritional factors present in duckweed, thereby improving its nutritional profile and suitability for use in animal feed and other applications [128,129]. Dwinanti et al., (2023) determined the effects of NSP multi-enzymes (containing xylanase, beta-glucanase, cellulanase, and mananase) on the growth and feed digestion efficiency of fish fed with duckweed-based feed [130]. Feeding was carried out under four different conditions: feed without enzymes and without duckweed (T0), feed without duckweed but with added enzymes (T1), feed without enzymes but with 5% duckweed (T2), and feed with enzymes and 5% duckweed (T3). The results showed that T3 had the best feeding effect, with an absolute weight gain of 0.91 g, absolute length increase of 0.63 cm, and specific growth rate of 1.35%, compared to a specific growth rate of 0.41%, a survival rate of 100%, and a feed digestion rate of 28.59% for the control group.

To the best of our knowledge, enzymatic hydrolysis of duckweed proteins has only been performed three times on duckweed protein powder. Tran et al. (2021) performed enzymatic hydrolyses of proteins from defatted *Lemna minor* using Flavourzyme and alcalase to evaluate the antioxidant properties of the hydrolysates [131]. Duangjarus et al. (2022) used alcalase to hydrolyse duckweed (*Wolffia globosa*) proteins and investigated the antimicrobial and functional properties of the fractions obtained [132]. Bernier et al. (2024) conducted the first investigation on enzymatic hydrolysis (by pepsin, chymotrypsin, papain, and trypsin) of duckweed proteins to produce bioactive peptides with therapeutic applications [133]. They found antihypertensive peptides that inhibit angiotensin-converting enzyme (ACE), effectively reducing blood pressure levels. ACE plays a significant role in the regulation of blood pressure, converting angiotensin I into the potent vasoconstrictor angiotensin II while also breaking down the vasodilator bradykinin, leading to an elevation in blood pressure [134,135].

In case of proteins, the selection of appropriate extraction buffers and the optimisation of parameters, such as pH and temperature, can play a crucial role in ensuring the efficient extraction and preservation of the duckweed proteome [16,130]. The degree of protein hydrolysis can be increased with an additional step of chemical extraction of the proteins to produce a protein isolate on which enzymatic hydrolysis can be performed. This step allows the proteins to be concentrated up to higher percentage (~80%) in the product and removes unwanted compounds, such as insoluble proteins and fibres, but several compounds with valorisation potential can also be lost [133].

The most commonly used treatment, fermentation, might also help to decrease the antinutritional factors and crude fibre content, thereby increasing the nutritional value and potential for incorporation into animal and human diets [136]. The fermentation process, carried out by microorganisms or their secreted enzymes, breaks down proteins into smaller peptides and amino acids, thereby increasing the digestibility and bioavailability of protein. Furthermore, fermentation can effectively reduce antinutritional factors in plant-based materials, such as tannins and phytic acid, thus reducing restrictions on the digestion and absorption of protein [124]. Currently, fermented plant protein has been used as an excellent substitute for fish meal in aquafeed to increase and sustain the production of sustainable aquaculture [137].

Fermentation (the process by which complex substances are broken down into simpler substances by microorganisms) is an ancient, simple, and inexpensive method to process plant raw materials (among others), reduce antinutritional components, and increase digestibility. In the literature, duckweed has been reported to contain antimicrobial active substances that inhibit the growth of microorganisms [138]. However, Mahoney and colleagues (2021) found that both *Lemna minor* and *Spirodela polyrhiza* duckweeds are suitable substrates for the propagation of *B. subtilis* KATMIRA1933 and *B. amyloliquefaciens* B-1895. Both bacilli grew on duckweed and reached the 9.48–11.31 Log CFU/g cell concentration after 48 h of growth. However, the variety and origin of the duckweed are also important, as it was observed that the best growth was found with *Lemna minor*, followed by *Spirodela polyrhiza*, while the commercial duckweed product was a less favourable substrate [139]. The fermentation process can also cause significant changes in certain cases and components. When fermentation was carried out with five different lactic acid bacteria, significantly lower moisture contents and lower crude fibre contents were measured in the fermented duckweeds compared to the non-fermented one, but with no significant differences in protein content. There were also no significant differences in free amino acid contents. However, a much lower (61%) phytic acid content was detected in fermented than in unfermented duckweed [140]. Meanwhile, Bairagi and co-workers (2002) [124] reported that the fibre content of leaf meal was reduced from 11.0% to 7.5%, and the antinutritional factors tannin and phytic acid were reduced from 1.0% to 0.02% and from 1.23% to 0.09%, respectively, after fermentation, when *Lemna polyrhiza* was fermented by a cellulose-degrading fish-gut *Bacillus* sp. bacterium. Similarly to the findings of Flores-Miranda and co-workers (2014), fermented duckweed showed a decrease in crude fibre, but in this case, a decrease in crude protein content and a significant increase in free amino acid contents were also observed [140]. Cruz and colleagues (2011) fermented various aquatic macrophytes with a mixed culture containing *Lactobacillus plantarum* strains DSM 8862 and DSM 8866 and found that, while fibre and ash were slightly reduced in the fermented product, the protein content increased slightly [141]. However, there was a significant decrease in antinutritional components (trypsin inhibitors, phytates, tannins) compared to the unfermented original sample.

Not only bacteria but also fungi can grow on duckweed and cause significant changes in its composition. Fermentation using *Trichoderma harzianum* and *Saccharomyces cerevisiae* has significant effects ($p < 0.05$) on the dry matter, ash, and crude fat in duckweed. The fermentation process increased the contents of minerals, and the content of crude fat in fermented *Lemna* meal increased by up to 5.76% after fermentation. However, the crude fibre content in the fermented duckweed reduced by 15.27% [141].

In case of a mixed fermentation, *Lemna minor* fermentation was performed by the probiotic microorganisms *Lactobacillus casei* and *Saccharomyces cerevisiae*. It was found that the proportion of protein increased significantly after fermentation, while crude fibre and ash decreased slightly. The results showed that the fermentation process could improve the protein quality of the duckweed meal; however, the bacteria could be an important source of high-quality protein [124].

It is considered that duckweed is a highly nutritious plant due to its high protein content compared to other aquatic weeds; however, the presence of antinutritional factors limits the direct use of the weed as a dietary ingredient. Several reports have indicated that the unfermented form of duckweed is a suitable alternative protein source for animal feeding, but the fermented duckweed can be used in a higher percentage in feed [139]. Therefore, fermentation is considered to be a potential way to solve the limited use of duckweed [142]. However, the variety, origin, and cultivation of the duckweed have a significant impact on its protein content and nutritional availability [124].

7.2. Physical Methods

Promising approaches include the use of traditional pretreatment processing methods such as drying, crushing, or pressing to enhance the digestibility and bioavailability of compounds in duckweed.

The use of hydrothermal liquefaction, a thermochemical conversion process, has been explored to enhance the extraction of valuable compounds from duckweed biomass [37].

The use of ultrasound-assisted extraction has been shown to facilitate the release of important biomolecules from microalgae, such as *Dunaliella salina* [143]. While these strategies have shown promise in other systems, their applicability and effectiveness in overcoming the cell wall barrier of duckweed remain to be thoroughly investigated. Ultrasound technology has emerged as a promising solution to address these challenges, offering the potential to enhance the digestibility, bioavailability, and bioaccessibility of duckweed-derived compounds. Ultrasound-assisted extraction has been investigated as a sustainable and environmentally friendly approach to extracting proteins and other bioactive compounds from various plant sources, including duckweed [144]. The use of ultrasound can disrupt the plant cell walls, facilitating the release of intracellular compounds and improving mass transfer, thereby enhancing the extraction yield and functionality of the extracted compounds. The propagation of pressure oscillations in the liquid medium during ultrasound-assisted extraction can result in the formation, growth, and collapse of microbubbles, allowing for cell disruption and improved mass transfer. This could contribute to the more effective utilisation of duckweed as a valuable source of nutrients, pharmaceuticals, and biofuel feedstock [126,144,145].

Mirón-Mérida et al. (2024) optimised the alkaline extraction of protein from duckweed (*Lemna minor*) with ultrasound by a Box–Behnken experimental design [146]. In addition, an exploration of the effects of ultrasound on the morphological, structural, and functional properties of the extracted protein was also carried out. They concluded that the cellular disruption produced by ultrasound was beneficial for the extraction of more protein. Furthermore, the application of ultrasound during the extraction of protein generated changes in the colour and structure of the duckweed protein, which resulted in improvements in its properties. These results are promising when considering the utilisation of duckweed in diverse food products.

Nitiwuttithorn et al. (2024) investigated the effects of duckweed forms (fresh and oven-dried), duckweed-to-aqueous solution ratio (DSR), alkaline extraction (AE), ultrasound-assisted alkaline extraction (UAAE), and ultrasound-assisted water extraction (UAWE) on protein yield and, furthermore, the physicochemical and techno-functional properties of duckweed protein extracts (from *Wolffia arrhiza*) [147]. Duckweed in fresh form resulted in a 2.5-fold higher protein recovery than oven-dried duckweed. UAAE significantly enhanced the extraction yield and protein recovery. The optimal extraction process was a DSR of 1:6 using UAAE at pH 8.5, resulting in a 16% yield and 34% protein recovery. Furthermore, the study found that UAAE facilitated the extraction of non-polar/hydrophobic amino acids, while AE proved to be efficient in extracting sulphur-containing amino acids (in addition to enhancing dietary fibre extraction, which helped stabilise the emulsion). This study, for the first time, revealed the role of UAAE and AE in promoting the extraction of different profiles of amino acids. The duckweed protein extract produced using UAAE contained 65% protein (with an excellent essential amino acid content), meeting the WHO/FAO/UNU recommended protein intake for preschool-aged children. Furthermore, the duckweed protein extract exhibited excellent emulsifying properties and oil-holding capacity comparable to commercial soy protein isolates. Overall, UAAE was identified as a promising approach for producing techno-functional and nutritious protein ingredients from *Wolffia arrhiza*. It proved to show great potential for functional plant-based food and feed applications. Nitiwuttithorn et al. (2024) declared that they plan to investigate the gastrointestinal digestibility of duckweed protein extract and the potential bioactivities of the digested peptides in the future. Moreover, their goal is to improve the greenish appearance and grassy flavour of duckweed protein extract and develop zero-discharge production processes.

7.3. Novel Methods

Duckweed contains bioactive compounds such as phenolics, flavonoids, and proteins that are often encapsulated within rigid cell walls or bound to complex molecules like fibre or lignin. The following novel techniques can overcome these structural barriers by disrupting cell walls, releasing these compounds and improving their bioaccessibility. Furthermore, they can enhance cell permeability, allowing for more efficient extraction of bioactive compounds.

While the techniques mentioned in the previous section have rarely but nevertheless been applied to duckweeds, to the best of our knowledge the techniques listed in this chapter have not been applied to duckweed yet. The applicability of these techniques to duckweed remains to be explored.

The **pulsed electric field technique (PEFT)** involves the application of short, high-intensity electric pulses to a substance, which can induce the formation of pores in cell membranes. This effect has been extensively studied for its potential applications in food preservation, as well as for enzyme inactivation and the enhancement of secondary metabolite production in plant cells [146,148]. While most of the research on pulsed electric field technology has focused on liquid food products and plant cell cultures, there is limited information on its potential for processing duckweed. The high nitrogen and phosphorus contents of duckweed, particularly when grown in wastewater, could make it a promising candidate for the application of this technique [37].

High hydrostatic pressure technology (HHPT) is a non-thermal processing technique that has shown promise in enhancing the bioavailability and bioaccessibility of bioactive compounds in various food systems [37,96]. The impacts of high hydrostatic pressure on the bioactive properties of duckweed, however, have not been extensively explored.

HHPT is a preservation process in which liquid or solid foods are subjected to pressures between 100 and 800 MPa. The advantage of the treatment is that the hydrostatic pressure is applied instantaneously and uniformly throughout the mass of the food in a submerged, preferably airtight and flexible package according to the Pascal principle. This ensures that the effect of the treatment is not dependent on the size and shape of the product [149]. The efficacy of the treatment is contingent upon a number of factors, including the level of pressure applied, the duration of the treatment, the temperature of the treatment, the rate of pressure rise and release, the initial temperature of the product, the temperature distribution within the equipment, and the pH, composition, and water activity of the product [150]. High hydrostatic pressure is increasingly recognised as an effective method for extracting proteins and bioactive compounds from various food sources. In the vast majority of cases, the application of high-pressure processing results in a modification of the texture of cell-structured food systems. The primary cause is the damaging effect of HHPT on cell membranes and cell walls; this phenomenon is intensified in products with a high proportion of air-filled pores, due to the differences in the compressibility of the tissue materials and the entrapped air [151]. The other factor that can enhance the yield of extraction is that the cell membrane of the sample is destroyed during extraction, so the solvent enters the cell and many components are easily eluted out of the cell [152]. Dehnad and co-workers (2024) investigated the impact of HHPT on legume proteins. Their findings revealed that 300/400 MPa pressure resulted in the most significant enhancement in the solubility of legume proteins. Additionally, HHPT at 400 MPa exhibited the highest emulsion activity index of legume proteins [153].

The "**instant controlled pressure-drop technology", known by its French acronym DIC** (Détente Instantanée Contrôlée), was created in 1988 as a solution to the drying shrinkage/collapse issues, in order to obtain a better quality of the dried foods or plants in terms of texture, colour, aroma, etc.

DIC is a hydro-thermo-mechanical treatment that is mainly used with biological materials. The process is characterised by the fact that operating parameters such as temperature and pressure can be tightly controlled. DIC consists in placing the moistened product in a processing vessel and exposing it to a high saturated steam pressure (0.08–1 MPa) at high temperatures (up to 170 °C), over a relatively short duration (from a few seconds to one minute). The treatment duration is determined by a drop, generally instantaneous (<0.1 s), to a low absolute pressure of about 5 kPa. The vaporisation of water from the product produced by the drop in pressure generates micro-mechanical constraints that lead to a modification of the product's texture and result in a porous structure. The short duration of the treatment and the immediate drop in temperature following the fall in pressure prevent further thermal deterioration and ensure that the quality of the end product is maintained [154,155].

Since then, DIC has led to remarkable developments in several aspects.

DIC has shown its potential to remedy the mass transfer resistance linked to cell collapse in drying operations. Thanks to the newly generated porous structure, an increase in the starting and the effective diffusivity of food products was observed [156]. This intensification directly impacts the drying operation, reducing the total drying time (in some cases to 1 h instead of 6 h), and improves the integral quality of dried products [157,158].

In extraction operations, DIC performs excellently as a pretreatment and as an extraction operation itself. First, in the case of DIC as a pretreatment, studies have concluded that, thanks to the new microstructure, the plant/food materials were better at releasing the desired (biologically active compounds, e.g., antioxidants, essential oils, vegetable oils) or undesirable (oligosaccharides) molecules [159].

DIC provides a new way to eliminate vegetative microorganisms and spores, thereby ensuring microbial decontamination for solid and powder foods [155].

In addition, DIC has been used to reduce non-nutritional compounds (e.g., from legumes, nuts), which could directly impact the consumption of plant proteins in human food and feed [160–162]. In this respect, new functional food products could now be developed from dried legumes or with proteins extracted by DIC.

Finally, DIC can also improve other food unit operations, such as oil deodorisation, desolventation, dehydrofreezing, blanching, coffee drying, and roasting, among many others [163]. The operating parameters can be optimised to meet the exact needs of different industrial applications. Thus, it could be said that DIC is a disruptive technology with a high potential to redefine several food processing methods, even in the case of duckweed.

According to the selected operative parameters, optimising DIC as a pretreatment for conventional (e.g., hydrodistillation) and emerging extraction technologies (e.g., ultrasound and microwaves) has been possible. Finally, no study on the application of the DIC process on duckweed has been carried out yet. Therefore, the impact of DIC on duckweed has yet to be explored.

8. Conclusions and Future Perspectives

Duckweed is a typical floating aquatic plant with wide distribution in ponds, lakes, and slow-flowing water. It is a common wild herbaceous plant in nature, and it is edible and nutritious. This is due to its protein content and the wide range of bioactive compounds (such as flavonoids, phenolic acids, sterols, and carotenoids) that have been detected in these plants. Due to its rich composition, duckweed exhibits antioxidant, antibacterial, antifungal, anticancer, immunomodulatory, and anti-adipogenic potential [36]. Duckweed powder from *Lemna minor* appeared to be a rather good source of antioxidants in comparison to other protein-rich nutraceuticals (e.g., *Spirulina* and *Chlorella* powders); nevertheless, it exhibited a much lower antioxidant capacity compared to certain fruit powders [48]. It should also be highlighted that plant-based foods like duckweed are associated with lower cardiometabolic risks in comparison to red meat [36]. The bioactive compounds of duckweed have the potential to be used in the development of pharmaceutical and dietary supplements, but the bioavailability and efficacy of these compounds in the human body remain largely unexplored.

Additionally, duckweed's rapid growth and ability to thrive without competing with agricultural land make it an attractive and sustainable option for food and animal feed production [13]. Despite these potential benefits, the regulation of duckweed as a food ingredient is not yet well established. While some studies have explored the amino acid composition and protein extraction from various aquatic plants, including duckweed, more comprehensive research is needed to fully understand its nutritional profile and potential safety concerns [164]. For these purposes, in vitro and *in vivo* digestion studies—as these topics are less published—are needed to obtain complete information on the bioaccessibility and bioavailability of the good nutrient components, as well as on their remaining or possible lost bioactivity after digestion. It is also important to analyse the food matrix's effect on digestibility, bioaccessibility, bioavailability, and bioactivity. As duckweed is a nutritional novelty, very little attention has been paid to these types of studies yet. Some duckweed species may contain antinutritional factors, such as oxalates and phytates, which could influence nutrient absorption. Research into the presence, concentrations, and effects of these compounds, along with methods for reducing their impact (e.g., soaking, cooking), is essential.

This article has been written to give a general overview on the nutritional benefits and bioactive properties of duckweeds, as well as the factors that can affect their bioaccessibility and bioavailability. It was also mentioned in this article what technological treatments have been used so far to improve digestibility and access to bioactive substances. By optimising and coordinating these factors, it would be helpful to develop products with high nutritional utilisation value. In our future work, our priority aim is to select—considering their advantages and limitations—the most effective traditional and novel technologies for the extraction of bioactive components (Table 1). With technological treatments, the aim is also to improve product quality (preservation, elimination of antinutritional components, removal of microbial contamination). So far, the impact of modern techniques (HHPT, DIC, PEFT) on duckweed nutrients have not really been applied, so it would be worth looking at their effects on duckweed samples comparing with traditional techniques. With well-chosen technological processing, the range of nutritious foodstuffs and animal feed could be extended.

There is very little literature on the use of duckweed in food product development, and there have been few nutritional studies of products made from it. It is high time to expand the range of nutrient-rich foods based on duckweed materials. Furthermore, the inherent characteristics of duckweed, such as its fast, clonal growth and simple growth habits, make it a promising platform for biomanufacturing, including the production of proteins, polymers, and small molecules. However, the genetic and biochemical mechanisms that underlie these traits are not fully understood, limiting the ability to fully leverage duckweed's potential in this area.

In conclusion, while the research on duckweed has made significant strides in recent years, there are still several gaps that need to be addressed. These include a better understanding of the factors influencing the nutritional composition and bioavailability of duckweed, as well as the genetic and biochemical mechanisms that govern its growth and metabolic pathways.

Table 1. Advantages and limitations of the traditional and novel technological treatments.

Methods		Advantages	Limitations
Chemical methods	Enzymatic degradation	- Enzymes are highly specific, targeting specific bonds - The process typically occurs under mild temperatures (30–50 °C) and near-neutral pH, requiring less energy compared to thermochemical methods	- As enzymes are highly specific, thus leaving other compounds relatively intact, not all bioactive compounds will be released - Enzymes can lose activity over time or under certain processing conditions, necessitating careful control of the environment (e.g., pH, temperature)
	Fermentation	- Increased nutritional value (reduced antinutritional factors, increased protein or free amino acid content, higher-quality proteins)	- Fermentation conditions, microorganisms, and the raw material have a significant impact on the fermentation process and, thus, on the final product
Physical methods	Traditional pretreatment processing methods such as drying, crushing, or pressing	- Simple - Well known - Require minimal tools	- Less effective alone; can be used in combination with other techniques
	Ultrasound-assisted extraction	- US process can increase the bioaccessibility of bioactive compounds by disrupting the cell walls of the product matrix - The method reduces the extraction time	- The absence of generalizable data has a significant impact on the optimisation of ultrasound applications across a range of products - Sonication has been demonstrated to result in the degradation of protein matrices with molecular weights greater than 20–25 kDa
Novel methods	PEFT	- It requires reduced treatment time and temperature - The process has the potential to result in the disruption of cell membranes, a phenomenon that has been demonstrated to facilitate the extraction of bioactive compounds from plant materials	- The presence of air bubbles in foodstuffs has been demonstrated to result in the generation of sparks within the treatment chamber - The utilisation of this process is incompatible with foods exhibiting elevated or variable electrical conductivity - The efficacy of the process is subject to considerable variation, contingent upon the characteristics of the medium under consideration; this has the potential to engender difficulties in attaining consistent outcomes across a range of products

Table 1. *Cont.*

	Methods	Advantages	Limitations
Novel methods	HHPT	- This process is independent of the dimensions and geometry of the product - The phenomenon of high pressure is not time or mass-dependent; it acts instantaneously, thereby reducing the required processing time - It does not break covalent bonds; therefore, it maintains the original properties of the products - It can be applied at room temperature - It can enhance the antioxidant activity and reduce allergenicity	- The residual enzyme activity and dissolved oxygen levels result in the degradation of certain food components by means of both enzymatic and oxidative processes - Products should have approximately 40% free water
	DIC	- Improving the functional properties of the biological materials - Improving the extraction of bioactive molecules - Decontamination of solid and powder food - Reducing antinutritional factors - Reducing allergens - Maintaining high quality of the end product - An environmentally friendly technology	- The raw material to be treated must be solid or semi-solid

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