

Utilizing Microalgae Addition in Novel Food Products which might Improve Health-A Review

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Abstract

With the increasing trends in world population need for increasing demand for meeting food production arises. Only such sources seem to be from Microalgae that might offer a much better source of getting proteins than other raw materials that are being used at present. Also a lot of bioactive compounds get produced from the microalgae. Getting bulk protein from Microalgae, within the food industry still has loopholes, in view of the underdeveloped technologies and processes that are available for Microalgae processing. The gradual improvement of the technological readiness level (TRL) could help in improving the situation at present in case of cultivation and processing. Also lot of applications in Microalgae cultivation and processing and technologies also needs improvement of economy of scale, investment in resources in new facilities and research.

Antioxidative, immunomodulatory, antihypertensive, anticarcinogenic, hepatoprotective and anticoagulant activities have been related to some Microalgae derived compounds like peptides. Still little research exists and evidence regarding potential health benefits are also not that convincing. But in the past decade interest has been revived, though interest focuses on the addition of whole cells, or some compounds that already exist in the market. Thus we reviewed the role of using Microalgae as an ingredient in novel food products having potential health benefits using pubmed search engine using MeSH terms like microalgae derived products; bioactive compounds, marketing, processing, gelling safety profiles. We found a total of 609 articles of which we utilized 98 articles for this review. No metaanalysis was done.

Introduction

There has been increasing demand of food available with populations rising and this is expected to get 2 times what it is right now and one expects the population growth by 2050 to roughly 9.8 billion people [1,2]. That protein demand would increase was reported by Wijnandes *et al* in 2007 [3]. Approximately 1 billion people don't have adequate protein intake, with inadequate conventional sources [4]. Main sources of worldwide protein intake both for food and feed has been plant based. However in the European Union (EU), animal based proteins get used more than plant based proteins [5], still there have been problems regarding health as well as environmental problems along with more concentration regarding saving animals which might add to the usage of plant based products.

There has been concerns regarding environmental effect with food production being responsible for 20-30% of the same [6], as well as 30% of the global greenhouse emissions of gas [7]. In Europe over 80% of animal needs of protein gets imported from non-european countries. Most of these are nonsustainable and environmentally damaging sources [8]. Need is there for technologies that are new, developing fresh products which help in the effect of eating behavior of people on the environment [9]. This could be brought about by changes in dietary patterns [10]. Germany gave an exemplary performance by shifting from an omnivore diet to one of ovo-lacto vegetarian diet [11]. This would decrease food-based greenhouse gas release by one third, and to 50% by shifting to a vegan diet [11]. Based on same principle in USA, switching to an ovo-lacto vegetarian diet might decrease the consumption of energy [12]. As per Rosi *et al* adding any type of diet, the effect on environment is based on the individual food habits [13]. This means which types of food are eaten and how frequently i.e in terms of how many times in a day or week [13]. Besides that how much the country can sustain which all are different in countries having high per capita income in contrast to those with lower income [10,14].

Getting Food from Microalgae

For increasing plant based feed, changing the cropping frequency, expanding the area that is cultivated and boosting yields might help in increasing food demand, but crop production might be approaching a maximal limit for optimizing it.

Using animal based proteins banks on the availability of correct, cost effective plant based protein for feed [16]. An alternative protein source, that might be sustainable protein source is microalgae. Algae may account for 18% of the protein source by the middle of this century, in a market that is more diverse market [17]. Food safety aspects of algae are not known properly in terms of contamination, allergens, or any substances which are harmful that get produced during microalgae processing. Thus the time which is estimated to market microalgae vs other protein sources are different [17].

Spirulina the general trade name of *Nostoc*, *Arthrospira* and *Aphanizomenon* are protein rich microalgae, which have been part of the human diet for over 1000's of years [18]. Spanish reporters watched Aztecs taking a blue green cake that was made of *Arthrospira* [19]. In 1952, a suggestion was given regarding exploiting microalgae regarding food and biochemical applications in the Algae Mass-Culture Symposium, after the earlier progress in early 1940's. Japan developed the facilities for commercial production of *Chlorella* first, while Mexico developed *Arthrospira* cultivation in the 1970's [18]. Total microalgae species in nature ranges from 200,000 to 800,000, although very few get used for applications for food production [20]. Food and Drug Association regulates the status of algae in USA which gives it a generally recognized as safe (GRAS) status [21]. While in Europe, it is the competent authority of a member state which makes assessment of a new product, that gets later authorization from the EU, if there are no objections made by the member states. If such objections are raised then the European Food Safety Authority (EFSA), is responsible for doing the safety assessment of the novel foods [17].

Making bulk proteins utilization is a new thought. Proteins made by this way could lead to meeting the needs of the protein of the population, having many advantages over protein used presently. Low land requirements are there as compared to animal based proteins: 2.5kg/m²/kg of protein [22], as compared to 47-64m² for pork, 42-52m² for chicken, and 144-2582 for beef production [23]. These land usage is also lesser than that of some of plant based protein used for food and feed, like soyabean meal, pea protein meal, and others [24]. The nonarable land getting used for cultivation, minimal consumption of fresh water, possibility of growing in seawater, along with potential replacement of nonsustainable soy imports are the few advantages of algae over other plant based proteins [25]. *Chlorella* and *Arthrospira* accumulates high quality proteins, with both species having well balanced amino acid profiles as per WHO/FAO/UNU recommendations regarding human requirements of essential amino acids [25,26]. These amino acid profiles for both are similar to that of other conventional sources like egg and soybean [25], though some microalgae showed deficiency in some amino acids [27]. Comparison between amino acid profile of different algal products that are available commercially like *Chlorella* pills or *Arthrospira* flakes, proved that some supplements could provide high amounts of essential amino acids (EAAs). Cultivation conditions or sources of the biomass used for production, might lead to differences in the amino acid profiles of various products [27]. But during consumption protein bioavailability is important. Three concepts are important, namely bioaccessibility, bioavailability and bioactivity [28]. Bioaccessibility is examined by *in vitro* tests, which represents the fraction of the compound released from the food matrix that becomes available for absorption. After that the compounds might reach the systemic circulation and get utilized that is known as, bioavailability. Bioavailability is tested using *in vivo* tests. Bioactivity of a compound is the physiological response like antioxidative, antihypertensive or anticarcinogenic activities, that can be tested both *in vivo* and *in vitro*.

Thus a compound might be bioaccessible, although not bioactive. Bioavailability of proteins from whole microalgae cells might be increased by pretreating these cells that =>disruption of cell walls that interferes with degradation [26].

Microalgae act as sources of various other compounds which possess health benefits like polyunsaturated fatty acids, carbohydrates, essential minerals along with vitamins [25, 26, 29] that might increase the nutritive value of food product once incorporated. Both polysaccharides as well as oligosaccharides have received attention in lieu of their prebiotic applications [30-32]. Gibson and Roberfroid defined these prebiotics initially as food ingredients that are nondigestible food ingredients by which host benefits because of stimulating the growth and/or activity of one or a limited number of bacteria in the colon, improving health thereby [31]. *Chlorella*, *arthrospira* and *nannochloropsis* besides supplying proteins, are also good sources of polysaccharides and oligosaccharides, and hence have been thought of as possible prebiotic candidates [29-31]. Also lipids, especially long-chain polyunsaturated omega-3 fatty acids (ω -3 PUFAs) have beneficial effects once added to food products. α -linolenic acid (ALA; 18:3, n-3) eicosapentaenoic acid (EPA; 22:5, n-3) and docosa hexaenoic acid (DHA; 22:6, n-3) are some groups of ω -3 PUFAs which have been proven to add to health in human beings [29]. For preventing cardiovascular disease or renal diseases, EPA and DHA were found to be of use [29]. Since these long chain essential fatty acids can be only produced by plants, it is essential to supplement diet with them [33]. Although EPA and DHA can also get manufactured through ALA the process is not efficient and thereby fish oil remains the main source of these PUFAs that are available commercially [33]. Microalgae also are great sources of ω -3 PUFAs. *Chlorella*, *Arthrospira*, *Dunaliella*, *Haematococcus*, *Schizochytrium*, *Porphyridium cruentum* and *Cryptocodium cohnii* retain the GRAS status [34]. Commercially whatever biomass is there is available as pills and capsules. *Chlorella* and *Arthrospira* are taken commonly as food supplements. *Tetraselmis chuni* is used as a seafood flavouring agent, the diatom *Odontella auria* is taken as a food supplement in view of it being rich in EPA [17]. Other products which get produced from microalgae include β -carotene obtained from *Dunaliella*, DHA from *Cohnii* and phycocyanin obtained from *Arthrospira* that acts as a blue colorant [34,35].

Products Obtained from Microalgae-Marketing

Problems are associated with trying to commercialize microalgae based products on a large scale. Main microalgae based products in bulk availability at present are obtained from seaweed or algae, that get harvested from natural habitats. Currently large scale facilities which are present are reserved for aquaculture or producing high value compounds [23]. It is very difficult to obtain a market demand along with market value for microalgae based products can't be ensured mainly regarding food product. Investors look for opportunities having in mind long term market demand before settling for investments and more research on microalgae cultivation is important for developing sustained production that is required by competitive markets. Comparing production of microalgae to other crop based protein sources which have been grown for years and thus both cultivation and processing get maximized. Raising the production facility from 1 to 100 hectares, cultivation, along with biorefining costs/kg of biomass could be decreased 10 times as per Ruiz *et al* [36]. While it is not easy to implement microalgae production, Brennan L *et al* and Chacon Lee *et al* reviewed the production process in detail [37,38].

Biomass production costs get influenced by harvesting and dewatering steps, and one needs to take into account the process of harvesting and dewatering, the cultivation systems as well as the size of facility [39]. Improvement of the technology readiness level (TRL), might help in obtaining more maturity in development of technology regarding microalgae cultivation and processing [40], helping in the improvement of economy of scale.

In biorefining cost comes to 20-40% of the total production costs, while when processing common biomass though it hiked to 50-60% for microalgae because of underdeveloped technologies and the processes that are available at present [41]. Disruption of cells, extraction along with fractionation, besides other processes could cut down on the cost on optimization. Lot of research has been done aiming for reduction of biomass cost for biofuels production, that should be below 1€/kg for competitive fuels [42], and these results have helped in optimizing technologies and processes available at present for microalgae cultivation and processing. Also other than cost one can improve other aspects which are related to optimize the sustainability of the whole value chain for microalgae based proteins can be improved, on processing for meat substitutes, a high moisture which gets extruded *Chlorella* (green heterotrophically) resulted in a more environmentally sustainable product than pork and beef [23], with the current TRL end economy of scale for microalgae production.

Various products like β -carotene, ataxanthin and phycocyanin turn out to be very costly requiring hundreds to thousands of euros/kg, based on their purity [37,43] and since they seek high value in the market, it increases their attraction for business. While the whole microalgae cells used as food supplements are there in the market at a cost below 40€/kg [24,35,37] seek high value in the market, increases their attraction for business. Thus microalgae based products having biofunctional compounds have higher selling prices as compared to common food supplements might better their economic value, with the better selling prices might help in recovering the higher costs which is needed for newer cultivation and processing technologies. Hence microalgae product, be it as a final product or as biomass for microalgae based products is gradually expanding, and thereby larger enterprises or companies have started to show increased interest in them globally. Importantly companies are showing interest in the markets of microalgae and microalgae based products has been noted by Sharma and Sharma *et al* [44].

Besides that better economic feasibility might also better in development of sustainable biorefinery models for recovering multiple types of products having food applications. In the biorefinery approach, approach, changing the residual biomass into biofuel markedly adds to reduction in the biomass production costs. Like anaerobic digestion process is an important alternative that develops biogas from microalgae residues [45]. This biogas which gets produced might be converted to biomethane, that can be utilized for vehicles and at the same time, the aqueous stream which gets generated and is rich in nitrogen, might be used as a fertilizer for microalgae cultivation [46,47]. The CO₂ thus obtained from biogas upgrading can be utilized as a source for cultivating microalgae [47].

Food Supplement Value of Bioactive Compounds

In addition to giving nutrients along with energy to maintain, cause growth along with physical activity, foods can be utilized for giving bioactive compounds that have health benefits.

As far as proteins are concerned the composition of peptides is 20-30 amino acids/molecule, that remain inactive within the primary structure of the proteins, till they get released into the GIT, once food gets digested or during processing of food like ripening, fermentation, cooking and storage [48]. Body functions can be influenced by these protein fragments. Initially in 1950's increased bone calcification was increased in rachitic infants [49]. Also some microalgae products possess antioxidative, antihypertensive, immunomodulatory, anticarcinogenic, anticoagulant and hepatoprotective properties [50-54]. Though not much knowledge on humans regarding potential benefits for health have been published regarding these peptides. Still use of macroalga along with microalgae peptides for novel functional food products has created interest recently [18,29-32,42,54-59].

Regarding microalgae products, those possessing antioxidant qualities are the most lucrative for industrial applications. Importantly both proteins and lipids present in foods in the process of industrial processing/storage are prone to oxidation and thus essential nutrients get destroyed, besides that toxic compounds get generated. These low molecular weight off flavour compounds that get generated following oxidation are not accepted by the consumers. Furthermore these potentially toxic products might damage their health by initiating chronic diseases like cancer, diabetes, arteriosclerosis, coronary heart disease along with neurological disorders [54]. Methods used in food processing for preventing food oxidation are i) decreasing the prooxidant content meaning substances which generate reactive oxygen species or those that inhibit antioxidant systems like free fatty acids, metals and oxidized compounds ii) preventing exposure of food to light iii) removing air or adding oxygen scavengers and iv) putting antioxidants [59]. The commonly used antioxidants in the food industry from 1970's are butylated hydroxytoluene (BHT), EDTA and others [54]. Though law regulates and controls the adverse effects on health of these synthetic compounds have been reported [60]. Since consumers correlate synthetic as being unhealthy [61], they prefer to consume more natural products [60].

Thus for meeting consumer requirements food industries have tried to develop technologies by which usage of these synthetic additives are decreased. In EU, the very popular clean label movement that focuses on more natural and healthy foods which are free from additives, labeling foods that guarantee the absence of synthetic additives are some of the methods to attract the users [61]. Hence natural additives, that claim to possibly have natural antioxidant properties is a field for research. Thus peptides that have antioxidant or preservative properties might prolong food shelf life by delaying or preventing oxidation [53,54]. However no research has been reported regarding usage of microalgae derived compounds in food.

Adding Microalgae in Foods Having Health Benefits

Bioactive compounds can be added to most of foods that have been accepted/regularly consumed. Although peptides addition to foods has not been documented till now, other microalgae derived compounds as well as whole cells have got used as food ingredients for various purposes. Positive effects on the techno functional along with antioxidizing properties of food emulsions was seen by Raymondo *et al* [62] and Gouviela [63], on incorporation of some microalgae species. It was suggested that gels be used as a vehicle for providing important microalgae based compounds [64-71]. To provide antioxidants and some ω -PUFAs, different microalgae were added in gels by Batista *et al* [64]. Gouverira *et al* carried out studies having similarities by adding other microalgae species [65].

One can also incorporate microalgae in dairy products, for providing bioactive compounds [68]. That *Arthrospira* spp, might stimulate growth of desired probiotic bacteria in yoghurts along with fermented milk for improving the viability of prebiotics has been proposed by different authors [69]. Presence of trace elements, vitamins and other bioactive compounds in microalgae powders helps the development of the bacteria needed [69,70]. Asynergy exists between microalgae and bacteria, with the former releasing exopolysaccharides into the medium and thus stimulating bacterial growth [71]. Likewise *Chlorella* has become a part of yoghurts [72] and cheeses [73].

Microalgae derived products may be delivered through cookies or biscuits, in view of taste acceptability, versatility, convenience of consumption because they are easy to conserve and transport, have an acceptable texture and appearance. *Chlorella vulgaris* has been added to cookies, for their potential antioxidant and nutritional supplement properties [74]. *Isochrysis galbana* was incorporated to give ω -PUFAs, providing benefits to human health [75]. Phycocyanin extracts along with whole *A. Platensis* were added to cookies, to get health benefits [76], along with increasing protein and fibre content [77]. Nutritional along with health benefit of cookies was increased by Batista *et al* [78] by adding *A. platensis*, *C. vulgaris*, *Tetraselmis suecica* and *Phaeodactylum tricornutum*. Putting *Haematococcus pluvialis* in cookies increased their antioxidant properties along with lowering the glycaemic response [79]. Bread also gets used in large amounts like cookies. Several yrs earlier it was proposed that microalgae be added to bread for increasing their nutritional value [80,81]. *Dunaliella* was proposed to be a protein supplement for whole wheat bread [80]. Similarly *Arthrospira* [81-83] and a decolorized extract which is obtained from this spp [84] were also added to bread for increasing its protein content [81-84]. Besides other microalgae spp was also used in bread [85]. Recently *Arthrospira* added to gluten free bread [86], markedly increased the protein content, thus improving bread quality in the presence of some essential amino acids as compared to bread that was not supplemented. Same benefits were got by addition of *Arthrospira* in some snacks [87]. Pasta is also a widely liked product. *Arthrospira maxima* and *C. vulgaris*, both raised the nutritional content of fresh spaghetti, and these products were recognized by a sensory panel [88]. Also ω -3 PUFA's could be delivered via pasta by incorporating *L. galbana* and *Diacronema vlkianum* [89] and antioxidants [90]. Though protein content got raised by *Arthrospira*, digestibility of protein got reduced on increasing the microalgae content [90]. By adding *Dunalella salina* powder to pasta, it was tried to improve its nutritive value, but in view of low proportion of microalgae in the ingredients (<3%) not much increase in minerals was seen [91]. Since very little amount of microalgae and microalgae derived products in foods has been reported thus far, these additions did not increase the macromolecular composition of foods like protein content. Despite being rich in antioxidant content of *Chlorella* and *Arthrospira*, the changes in flavor of foods are considered undesirable by consumers [68,73,92]. Their having green colour, prevents daily usage of microalgae, it affects application in daily used products in view of it affecting taste and quality badly [27]. In products like pasta, that is marketed in different colours, consumers are not affected by these colour changes, though a slight fish flavor was not well accepted in certain products on addition of microalgae.

Which additives have to be added to food products vary with their techno functional properties. Emulsifying, foaming, gelation, water and fat absorption capacities are possessed by some microalgae proteins and hydrolysates but many of these remain unknown [28,59,88,93]. Adding microalgae into emulsions caused decrease in the percentage of oil, preserving its structure on the basis that microalgae act like a fat mimetic

as shown by Gouveia, but only some vegetable proteins could be substituted without affecting the emulsions stability. Emulsions resistance to oxidation got increased. On adding to vegetarian desserts (protein polysaccharides mixed gels) as colouring agents, the cell structure of microalgae protected pigments from heat degradation during processing [65]. Rheological and structural properties of gels were also improved, although these properties had species dependency, determined by lipids and microalgae proteins [64]. Further work showed that are also related gel formation and to changes in pH and composition that is derived from the salt content in microalgae [66,67]. Meltability and cohesiveness of processed cheese was reduced by Chlorella biomass, but increased its hardness and springiness [73]. Adding into baked products like cookies and bread lead to positive increase in firmness [74-77]. Microalgae are complex structures possessing proteins, carbohydrates and lipids among other compounds, once added to dough, changes the internal structure of the dough because of changes in water absorption or the incorporation of lipids [78]. Adding microalgae did not change the textural properties of pasta on cooking [88], although mechanical strength of raw pasta was reduced by addition of *Arthrospira*, that became more susceptible to breakage while handling [90]. Also reduced gluten protein content, on replacement of wheat flour partially caused increase in firmness, cohesiveness and chewiness after cooking the pasta. Increased microalgae concentration increases the stickiness of pasta although the elasticity was unaffected [94-98].

Conclusions

Microalgae might be the answer for tiding the need of total populations food demands. Thus one can focus on developing and building on algae based food industry from which novel functional food products might be developed. One is protein content having amino acid profiles that are balanced, besides that adding microalgae into food may help in improving health, in view of the bioactive compounds that are present in some of the microalgae species. Antioxidative, immunomodulatory, antihypertensive, anticarcinogenic hepatotoxic and anticoagulant activities are some of the properties which are possible means by which these microalgae benefit. Still use of microalgae or products derived from microalgae as food substitutes is not competitive because of the low TRL and economy of scale for microalgae cultivation and processing is not there. After these problems are overcome adding microalgae as food ingredients besides giving health benefits will also help in overcoming issues that are related to sustainability, considering the population growth along with our present diet patterns, habits and health.

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