Enrichment of Microalgae with Vitamins for Aquaculture and Food Industries Using Nannochloropsis, Tetraselmis and Isochrysis Species (A Review)

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***Abstract*- Microalgae play an important role as a source of nutrient in aquaculture and food industries due to their high content of protein, vitamins, carbohydrate and lipid. They also have therapeutic uses owing to their antitumoral, antiviral, antifungal and antibacterial activities. Tetraselmis species are ellipsoidal, brightly green and spore forming algal massively cultivated on guillard media best known for its cytotoxic properties due to high concentration of omega 3 fatty acid. It is an important alga commonly used as live food in aquaculture. Nannochloropsis species were found to have supplements in lying hen's diet for the production of enriched egg and meat with Omega 3 fatty acid. Isochroysis species cells lost their capacity to divide and became enlarged, with a reduction in the content of photosynthetic pigment and photosynthetic capacity when grown in batch culture for a prolonged period. In order to produce a microalgae enriched with high vitamin content, supply of nutritionally balanced mixture of nutrients must be sufficient, production cost must be reduce, as well as the response to different environmental factors.**

***Key words:*Microalgae, aquaculture, enrichment**

I. INTRODUCTION

Algae are a very large and diverse group of autotrophic organisms which ranges from unicellular to multicellular forms. They are primary producers which are a source of many nutrients and it has high protein content [1]. Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications.

They can be used to enhance the nutritional value of food and animal feed, due to their well-balanced chemical composition. Moreover, they are cultivated as a source of highly valuable molecules such as polyunsaturated fatty acids, pigments, antioxidants, pharmaceuticals and other biologically active compounds. The application of micro algal biomass and/or metabolites is an interesting and innovative approach for the development of healthier food products. Algae are a very large and diverse group of autotrophic organisms which ranges from unicellular to multicellular forms. They are primary producers which are a source of many nutrients and it has high protein content. The most complex marine forms are called seaweeds. Blue green algae, red algae, green algae assigned higher contents of dietary fibres. Certain beverages are prepared from sea algae. Mainly marine algae have been used as food and medicine for many centuries. They are not only used as food but also used as extracts in food, dairy, cosmetics, and industrial uses [4]. Microalgal biotechnology is similar to conventional agriculture, but has received quite a lot of attention over the last decades, because they can reach substantially higher productivities than traditional crops and can be extended into areas and climates unsuitable for agricultural purposes. Microalgae production is an important natural mechanism to reduce the excess of atmospheric CO_2 by bio fixation and recycling of fixed Carbon in products, ensuring a lower greenhouse effect, reducing the global environmental heating and climate changes [2].

II. LITERATURE REVIEW

The implications of diet on health sustainability have assumed a major importance, supported by considerable epidemiological evidences, and is well recognized by the scientific community and general public, on developed countries. Microalgae are able to enhance the nutritional content of conventional food and feed preparation and hence to positively affect humans and animal health due to their original chemical composition, namely high protein content, with balanced amino acids pattern, carotenoids, fatty acids, vitamins, polysaccharides, sterols, phycobilins and other biologically active compounds, more efficiently than traditional crops [2].A review by [3] provided a background on the usage of microalgae in aquaculture, focusing on their nutritional value and transfer of nutrients through food chains.

The current status of knowledge was summarized and potential areas of research and industry development are

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identified. The review was divided into six sections which included general attributes of microalgal species used in aquaculture, nutritional properties, production systems, alternatives to fresh algae, use of algae to enrich zooplankton and directions for future research.

According to [5],the cells of *Isochrysis*aff.*galbana* (*Isochrysis* Tahitian strain T. Iso) lost their capacity to divide and became enlarged, with a reduction in the content of photosynthetic pigment and photosynthetic capacity when grown in batch culture for a prolonged period (up to 11 months). Upon returning to fresh medium, the cells of a 11-month-old culture regained their capacity to divide within 3 days under light conditions. The resulting cells possessed similar size, photosynthetic pigment and photosynthetic capacity as those of logarithmic growth phase. The results suggest that the aged cultures of *Isochrysis* can be used as starter cultures for the production of live food for aquaculture animals.*Tetraselmis* is an important alga commonly used as live food in aquaculture. A research by [6] reported the laboratory culture of its strains collected from the coasts of China, covering Guangdong, Zhejiang and Shandong Provinces. The effectiveness of various physical and chemical methods for the removal of contaminants from the microalgae, *Tetraselmissuecica*, and culture was investigated. The information obtained was used as the basis for the development of improved protocol for the preparation of axenic culture to be adapted to heterotrophic cultivation.

Repeated centrifugation and rinsing effectively removed the free bacterial contaminants from the microalgae culture while sonication helped to loosen up the tightly attached bacterial contaminants on the microalgae cells. Removal of bacterial spores was accomplished using a mixture of two antibiotics, 5 mg/mL vancomycine and 10 mg/mL neomycine. Walne medium formulation with natural seawater was preferred for the enhancement growth of *T. suecica*.

Adaptation of growth from photoautotrophic to heterotrophic conditions was achieved by the repeated cultivation of photoautotrophic culture with sequential reduction in illumination time, and finally the culture was inoculated into the medium containing 10 g/L glucose, incubated in total darkness to obtain heterotrophic cells.

Changes in the morphology and composition of *T. suecica* cells during the adaptation from photoautotrophic to heterotrophic condition, as examined under Transmission Electron Microscope, were also reported by [7]. The total protein, carbohydrate, lipid and ash compositions, and fatty acid contents of two species of marine microalgae, the eustigmatophyte *Nannochloropsisoculata* (formerly '*Chlorella* sp., Japan') and the chrysophyte *Isochrysis* sp. (Tahitian) used in tropical Australian mariculture, were studied. The microalgae were grown under a range of culture conditions (41 and 601 laboratory culture, 3001 bag culture, and 80001 outdoor culture) and four light regimes (100 to 107 μ E m^{-2} s^{-1}, 240 to 390 μ E m^{-2} s^{-1}, 340 to 620 μ E m^{-2} s^{-1}, and 1100 to 1200 μE m^{-2} s^{-1} respectively) to determine the effect of light intensity on the chemical composition of large scale outdoor cultures. Laboratory and bag cultures were axenic and cultured in Walne medium while outdoor cultures were grown in a commercial medium designed for optimum nutrition in tropical outdoor aquaculture operations. Change in growth medium and photon flux density produced only small changes in the proximate biochemical composition of both algae. *N. oculata* and *Isochrysis* sp. both showed a trend towards slightly lower carbohydrate and higher chlorophyll *a* in shaded outdoor culture. *Isochrysis* sp. showed significant concentrations of the essential polyunsaturated fatty acid 22:6(n−3) (docosahexaenoic acid) from 5.3 to 10.3% of total fatty acid, and 20:5(n−3) (eicosapentaenoic acid) ranged from 0.6 to 4.1%. In contrast, *N. oculata* had high concentrations of 20:5(n−3) (17.8 to 39.9%) and only traces of 22:6(n−3). The fatty acid composition of *Isochrysis* sp. grown at high photon flux density (1100–1200 μE m^{-2} s^{-1}) under outdoor culture showed a decrease in the percentage of several highly unsaturated fatty acids, including 20:5(n−3), and an increase in 22:6(n−3). *N. oculata* showed a similar decrease in the percentage of 20:5(n−3). High light intensity caused a decrease in the ratio of total C_{16} unsaturated fatty acids to saturated 16:0 in *N. oculata*, and a decrease in the ratio of total C_{18} unsaturated fatty acids to saturated 18:0 together with a decrease in the ratio of total unsaturated fatty acids to total saturated fatty acids in both microalgae.Twelve algal strains representing the classes *Cyanophyceae*, *Prymnesiophyceae, Bacillariophyceae, Rhodophyceae, Cryptophyceae, Chlorophyceae, Xantophyceae*and *Eustigmatophyceae* were selected mainly from the culture collection of the Norwegian Institute for Water Research (NIVA). The algae were grown as continuous cultures in a 1.8 l. reactor, internally illuminated with an 11 W fluorescent tube. The retention time was adjusted in the range 2–4 days to fit the growth rate of the algae. The growth responses and fatty acid composition were analysed. The maximum production rate was obtained with *Pseudokirchneriellasubcapitata* (0.63 g l^{-1} day^{-1}) and the lowest with *Porphyridiumcruentum* 0.13 g l^{-1} day^{-1}.

Arachidonic acid (AA) and eicosapentaenoic acid (EPA) were the dominating polyunsaturated fatty acids (PUFAs) in *P. cruentum*, while only EPA accumulated in *Phaeodactylumtricornutum*. Docosahexaenoic acid (DHA) was the major PUFA in *Isochrysisgalbana*, while *Pavlova* sp. had both EPA and DHA. This is the first report on the fatty acid profiles of *Nannochloropsisoceanica, Chroococcus* sp., *Synechococcus* sp. and *Tribonema* sp. [11]. According to [12], the biotechnology of microalgae has gained considerable importance in recent decades. Applications range from simple biomass production for food and feed to valuable products for ecological applications. For most of these applications, the

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market is still developing and the biotechnological use of microalgae will extend into new areas. Considering the enormous biodiversity of microalgae and recent developments in genetic engineering, this group of organisms represents one of the most promising sources for new products and applications. With the development of sophisticated culture and screening techniques, microalgal biotechnology can already meet the high demands of both the food and pharmaceutical industries.

A research on a high-performance liquid chromatography (HPLC) method for simultaneous determination of thiamine and riboflavin and the results of its application to a number of edible seaweeds that are sampled in dried form (Himanthaliaelongata, Laminariaochroleuca, Undariapinnatifida, Palmaria sp., and Porphyra sp.) or as canned food (H. elongata and Saccorhizapolyschides).

Samples are prepared by acid and enzymatic hydrolysis. Optimized conditions for reversed-phase HPLC with fluorescence detection are as follow: column, Kromasil 100 C18; column temperature, 35°C; mobile phase, a 72:28 (v/v) mixture of 0.005M ammonium acetate (pH 6.7)–methanol; and flow rate, 1.35 mL/min. With these conditions, recovery is 95.52% for thiamine and 90.08% for riboflavin, and the method precision (relative standard deviation) is 2.66% for thiamine and 2.21% for riboflavin. On a dry weight basis, thiamine contents range from 0.14 µg/g in dried H. elongata to 2.02 µg/g in dried Porphyra and riboflavin contents from 0.31 µg/g in canned H. elongata to 6.15 µg/g in dried Porphyra [8].An easy and sensitive method for measuring thiamine and its phosphate esters in small biological samples of microalgae.

The method consists of extraction of thiamine and its derivatives in acid solution, followed by liquid chromatography with fluorescence detection. The detection limit is as low as 15 fmol of thiamine. For comparison to microalgae, the method has been applied to evaluate thiamine levels in the crustacean Artemiasalina Leach and is suitable for nutritional studies of the food web of the Baltic salmon, which suffers from thiamine deficiency. This method of HPLC analysis can be readily utilized to follow uptake and interconversion of thiamine and its phosphate esters in many micro and macroalgae[9].

III. DISCUSSION

Algae were used as one of important medical source due to its antioxidant, anticancer, antiviral properties. Therapeutic properties of algae is used for promotion of health edible algae is recognized as complete foods which provides correct balance of proteins, carbohydrates ,vitamins ,and minerals [1].

The need for nutritional sources safer than traditional animal products has renewed interest generally in plants and particularly in microalgae. Microalgae have diverse uses in aquaculture; their applications are mainly to provide nutrition and to enhance the colour of the flesh of salmonids. The larvae of molluscs, echinoderms and crustaceans as well as some fish larvae feed on microalgae. Several studies have confirmed that a live multi-specific, low bacterial and microalgal biomass remains essential for shellfish hatcheries. Major advances are expected from new production system, designs and operations from batch run open tanks to more sophisticated continuously-run and closed loop reactors. Currently, studies are underway to examine the cost-effectiveness of the on- and off-site microalgal production systems which can only be achieved by substantial scaling-up and improved quality control. In order to attain sustainability in the usage of microalgae, a systems-based approach is required which integrates different fields such as biotechnology, bioprocess and management procedures as suggested by [10]. In order to industrialize massive production of microalgae with biotechnologically important traits, it is essential to reduce production costs through the design and evaluation of different culture media, which are reflected in the value of the final product. It is important to know and understand the dynamics of growth and behavior of the strain of interest, as well as the response to different environmental factors, in order to obtain an adequate and permanent production of biomass.

*Tetraselmis*specie is an ellipsoidal mobile, compressed alga with four flagella and the acute rear end with the anterior end of four lobes. It is green bright, 7 µm in diameter and 10-16µm in length. It is a euryhaline species with ability to form spores, whose limits of tolerance to physical variables are very broad. It contains 49.22% protein, 10.60% lipid and 16.38% from carbohydrates. The microalgae Tetraselmis specie was massively cultivated with Guillard media, and their biomass was harvested every 72hrs, in which a biomass average of 1.35 g/L/d were obtained [4].The implications of diet on health sustainability have assumed a major importance, supported by considerable epidemiological evidences, and is well recognized by the scientific community and general public, on developed countries [2].

IV. CONCLUSION

Microalgae are a diverse group of microscopic plants with the wide range of physiological and biochemical characteristics and contain up to 50-70% protein, 30% lipids, over 40% glycerol, up to 8-14% carotene and a fairly high concentration of vitamins B1, B2, B3, B6, B12, E, K, D, etc., compared with other plants or animals (Avagyan, 2008). Moreover, microalgae are meant to be an important raw material for vitamins and productions of other pharmaceuticals. The cultivation of microalgae is known to be the most profitable business in the biotechnological industry. It is a wasteless, ecologically pure, energy and resource saving process.

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