The Identification of Vitamins from Marine Algae of Black Sea by Spectrodcopic Methods

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Abstract

Modern research from the last years show that some vitamins have changed in their role of maintaining a healthy life. The paper presents information about the concept of vitamins and possible sources of vitamins from marine algae found in the Black Sea. The existence of vitamins in green algae from Enteromorpha and Cladophora species were identified through spectral analyzes in IR.

Keywords: vitamin, marine algae, Enteromorpha, Cladophora, Black Sea

1. Introduction

Modern research from the last years show that some vitamins have changed in their role of maintaining a healthy life. That's why, in recent studies, the importance of vitamins has been highlighted in different diseases such as: (atherosclerosis, diabetes, Alzheimer disease, degenerative diseases etc). There were also researches on the antioxidant capacity of some vitamins (A, C, E). In addition there are numerous studies about the vitamin doses needed to maintain the homeostatic balance in the human body, that's why vitaminology is a dynamic field in a continuous change [1].

The importance of maintaining a healthy diet has been acknowledged before the identification of vitamins. The ancient Egyptians knew that feeding a patient with liver, will help him with eyesight diseases, now this is known to be caused by vitamin A deficiency. In 1747 the Scottish surgeon James Lind discovered that citrus prevents scurvy, a lethal disease, which manifests in teeth bleedings and acute pain. In 1753 Lind published "A treaty on Scurvy". Nevertheless his discovery hasn't been accepted unanimously. For example in Royal Marine expeditions, it's been thought that scurvy could be prevented by a good hygiene on the ship, by regular exercises and by maintaining a high morale of the crew instead of a fresh diet based on citric. As a result these expeditions continued to be compromised by scurvy. In that period when

Robert Falcon Scott made his two expeditions in Antartica, in the early 20's century, the accepted theory was that scurvy was caused by contaminated can food [2].

In 1881, the Russian surgeon Nikolai Lunin fed rats with an artificial combination of all the constituents in milk which were known at that moment in time: proteins, fats, carbohydrates and salts. These rats died while the rats fed with natural milk grew up normally. He reached the conclusion: "a natural aliment like milk must contain, alongside the main ingredients small unknown substances which are essential to life". His conclusion was rejected by the other researchers who couldn't replicate the results of his experiments [2]. A main difference was that he used normal sugar (sucrose), while the other researchers used the sugar from milk (lactose) which contained small quantities of vitamin B. In 1905, William Fletcher discovered that by eating undecorticated rice instead of the decorticated one people could prevent the beri-beri disease. In the following year, Frederick Hopkins claimed that aliments contain "accessory factors" alongside proteins, carbohydrates, fats etc which are necessary to the human body. For this observation and for other various contributions in the year 1929 he received the Nobel prize for Physiology and Medicine [2]. The existence and the important role of vitamins have been highlighted indirectly by studying diseases which are caused by vitamin deficiencies. Different diseases like scurvy, pellagra, rickets or beri-beri were observed in the Extreme Orient since ancient times and were caused by faulty alimentation in different factors. When Casimir Funk isolated the water-soluble complex of micronutrients identified previously by Fletcher, he proposed the name "Vitamin" to be associated with it. The name rapidly became equivalent with Hopkins "accessory factors" and remained so until it was proved that not all vitamins are amines [2]. In the early XX's century researchers were capable to isolate and identify a larger number of vitamins. The clinical era of vitamins market at the start of the XX's century by the discovery of vitamin B1 or thiamine was followed by the metabolical era of vitamins marked by the studies of O. Warburg on their structural cells. Studies on the structure, the physico-chemical properties, the physiological action, the role of vitamins in alimentation, etc led to the creation of an important branch of biochemistry named vitaminology [2].

Vitamins are organic compounds of either endogenous or exogenous origin with various structure's, necessary in different quantities indispensable to all factors of life (plants, animals, microorganisms and of course man). Vitamins are synthesized in important quantities by plants and microorganisms and only in small proportions by animals and humans. People are gaining their essential vitamins from food (in free from and in the form of inactive compounds, provitamins which will transform afterwards inside the organism in vitamins.

The IUPAC commission (International Union of Pure and Applied Chemistry) in agreement with IUB (International Unions of Biochemistry) proposed in 1966 a nomenclature based on the chemical structure of vitamins. This systematic

nomenclature it's being used alongside the outdated one especially for papers about nutrition and physiology. Often the naming of vitamins were related with their main actions for example vitamin C was named antiscorbutic, vitamin D antirachitic, vitamin B1 anti beri-beri vitamin B3 pellagra preventive etc. [1], [2].

Some of these vitamins are known by their name from the older papers. For example vitamin B2 it's also named vitamin G, vitamin B7 or the biotin it's known as "vitamin H". Vitamin B3 it's also called "vitamin PP" a name derived from the old "preventive factor of pellagra". At the moment, there are 13 vitamins recognized. There are split into 2 groups , 4 which are soluble in fats (A, D, E, K) and the other 9 which are soluble in water (the 8 vitamin B's and vitamin C). The lack of vitamins in alimentation leads first to a state named predeficiency, when the symptoms are not yet visible but the organism is weakened for future aggression.

Hypovitaminosis (relative deficiency) is determined by the lack of a certain vitamin from alimentation and leads to symptoms of a smaller intensity. Avitaminosis (absolute deficiency) is the result of the complete lack of a vitamin from alimentation and determines the appearance of a grave state, with the feature of each vitamin deficiency. If this state lasts for a long time, there will be irreversible changes in the organism and could lead to death.

The absence of more than 1 vitamin at the same time, which happens ofter leads to poliavitaminosis. The appearance of deficiency problems can be delayed by the fact that in the large intestine of mammals there is a coliform flora which synthesizes vitamins beneficial to the organism. The reports published by the Organization for Alimentation and Agriculture and by the World Health Organization (FAO/WHO Handbook on human nutritional requirments, 2002-2006) shows that there are certain refuges, victims of the war or stranded because of natural disasters who suffer from malnutrition and avitaminosis because most of the cereals and other aliments sent for their aid are not supplemented with micronutrients [2].

The main enemies of vitamins are: oxygen, heat, ultraviolet radiation, industrial treatments, acidity and antivitamins, chemical substances which oppose their assimilation. To prevent the loss of vitamins during the processing state, countries with high life expectancy use vitaminization or revitamization of end products so the aliment has supplemented percentage of vitamin B. For example fruit juices are supplemented with vitamin C etc .

A viable source of vitamins are marine algaes. Marine algaes are knows and used since a long time ago in alimentation, in medicine and in soil fertilization. Today researchers from around the world are trying to explore and exploit this precious resource. Studies about the chemistry and biology of algae, represent the importance of these in different branches of activity. The first data about the uses of algae dates from the year 2500 bc, and also in Chinese literature from VIII-IX centuries bc. In japan the product Kombu prepared from different species of *Laminaria* (*angusta, japonica, religiosa*) and *Alaria* is consumed, but only since 1670 it's being cultivated on a large

scale [3]. The ancient greeks used algae's as remedies for curing intestinal worms. Countries in the west are not yet accepting algae's in alimentation maybe because of their appearance and their assimilation. In certain places of Europe algae's were used for the alimentation of poor people. For example, In Ireland, Dulza (*Palmarian palmata)* was used as anthelmintic and sexual arouser, like an aphrodisiac. In the ancient Tomis the locals salad was a well knows algae named *Ulva Lactuca* who was found on the Black Sea shores [3]. Along the time algae's have suffered progresses and regresses altogether because of the various factors more or less favorable. Algae's are part of the inferior plants (*Thallophyta*). They are plants with a simple structure, without flowers or seeds. They have a lot of pigments, the most important ones are: phycobiline, *Chlorophyll*, xanthophyll and carotenoids. Depending on the pigments that is in majority, algae's suffer a simple classification: blue algae (*Cyanophyta*), green (*Chlorophyta*). There is a great diversity of algae which can vary from a few microns to hundreds of meters. There are also: green, red, golden, blue algae who multiply through simple division or complex transformations [4]. Algae are set on the taxonomic groups according to the International Congress of Botanists after many discussions. The most frequent species belong to *Enteromorpha* and *Ceramium* but also *Cladophora, Porphyra Ag*. and *Callithamnion Lyngh.*

Green algae *Enteromorpha intestinalis* and *E. Linza* have conquered little by little more and more space creating a prime line of vegetation. *E. flexuosa* (Wulf) J. Ag. and *E. prolifera* are sometimes added to the previously mentioned ones especially in the warm season. An interesting phenomenon has been proved recently where *Enteromorpha* species were discovered 6 meters deep, a depth thought inaccessible for them [4].

Usually *Enteromorpha* species blend with different species of *Cladophora,* especially *Cladophora Sericea* (Huds.) Kutz. and sometimes *Cladophora albida* (Huds.) Kutz. and *Cladophora laetevirens* (Dillw.) Kutz [5].. From a quantitative point of view, the stocks of the main macrophytes have suffered a permanent decrease all along the Black Sea shores. Recent data on macrophytes biomasses have shown that in place of the old vegetation which was stable and rich, appears a different flora with a different qualitative composition, physiognomy and productivity, grown in the new conditions of shallower water [5]. The main characteristics of this flora is the small number of species.

2. Research Methods

The identification methods used were spectrophotometric studies in IR of marine algae powder in comparison with standard substances [6].

The IR spectral scope is larger than the UV-VIS one and it stretches from 780 nm to 300 nm, but analytic determinations use a narrower scope between 2,5 um and 1,5 um. There are 3 zones in IR: near, mid and far-infrared.

For structural analyses including detection only the medium IR scope is being used, which offers the most information. The close IR scope offers less data and that's why it's being used only in research [7], [8]. IR spectrophotometry is mostly used for organic substances identification including medicine without destroying the integrity of the molecules [8], [9].

Detection is based on the absortion of radiation and less on reflection. IR absortions are so distinctive for some functional groups somuch that the IR scope can be considered like fingerprints to studied molecules fact which eases their recognition. Each substance owns its own scope different to the others and helps its recognition. By measuring the intensity of a band the concentration of the substance can be determined [6], [10].

By comparing scopes of a great number of substances It's been decided that each type of connection manifests through one or more bands which are very little influenced by other atoms or molecule connections [9], [10].

Tab. 1 Marine algae gathered from the Black Sea

Nr. crt.	*Algae name*
1	*Cladophora*
2	*Enteromorpha intestinalis*

IR specters can help by recognizing the presence of some atoms and the nature of its connections. The studied types of algae are showed in tab 1 and standards used are carotene Fluka products and vitamins presented in Tab 2

Tab. 2 Standards used

Nr. crt.	*Standard name*
1	*B carotene*
2	*Cholecalcipherol - vitamin D3*
3	*Ergocalcipherol - vitamin D2*
4	*Retinol Vitamin A*

Equimpent – IR 4200 Jasco Spectrometer [6]

The equipment used for creating the specters is JASCO FT-IR 4200. The characteristics of the machinery are the following : Fourier Transform Infrared Spectrometer FTInfrared Spectrometer FT/IR 4200 Jasco; - wavelength 7800-350cm^{-1} accuracy of wavelength ±0,01 cm^{-1}; - maximum resolution 0,5 cm^{-1}; - the signal-noise balance 30000:1; system with one beam ; high radiation ceramic source ; - interferometer Michelson, mirror covered with Al; - radiation divider Ge/KBr ; speed 1,2,3,4 mm/sec; detector DLATGS (standard); Gain switching AUTO; - correction ATR (Attenuated Total Reflection).

3. Results and Discussions

The identification of vitamins in green algae powder Specters were made in IR for each algae (*Cladophora* and *Enteromorpha intestinalis*) (see figure 1 – *Cladophora* and figure 6. *Enteromorpha intestinalis*) and each standard mentioned (see Figure : 2, 3, 4, 5, 7, 9, 10).

After that there were overlaps of each alga specter with the standards to follow the eventual overlaps or structural modification of the same wavelength.

In *Cladophora* case we find similarities between the alga colecalcipherol and ergocalcipherol as we see in figure 3 and figure 4.

In figure 6-10 we find similar results for *Enteromorpha intestinalis*.

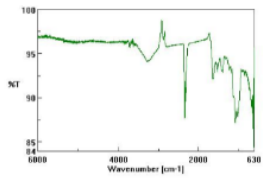

Fig.1 *Cladophora* Spectre IR

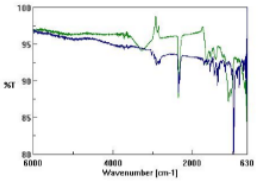

Fig.2 *Cladophora and β Caroten* Spectre IR

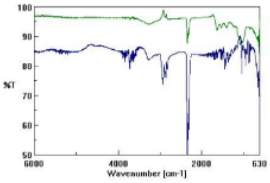

Fig.3 *Cladophora and calecalcipherol* Spectre IR

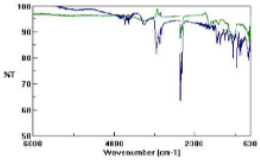

Fig.4 *Cladophora and ergocalcipherol* Spectre IR

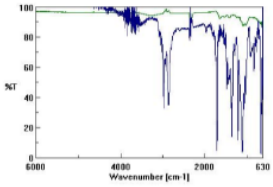

Fig.5 *Cladophora and retinol* Spectre IR

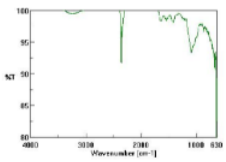

Fig. 6
Enteromorpha intestinalis
Spectre IR

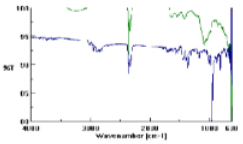

Fig. 7 *Enteromorpha intestinalis and β Caroten* Spectre IR

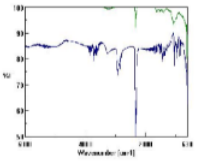

Fig. 8 *Enteromorpha intestinalis and colecalcipherol S*pectre IR

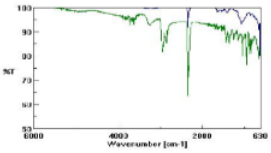

Fig. 9 *Enteromorpha intestinalis and ergocalcipherol S*pectre IR

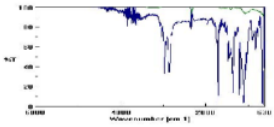

Fig. 10
Enteromorpha intestinalis and retirol Spectre IR

4. Conclusions

Marine algae can be a natural source of vitamins. From the spectral analyses in IR we can conclude that vitamin D2 and D3 certainly exist in analyzed green algae powder (*Cladophor*a and *Enteromorpha intestinalis*).

Modern research from recent years have brought to attention some of the vitamins and changed their role into healthcare.

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