ALGAE AS PRODUCTION SYSTEMS OF BIOACTIVE COMPOUNDS

Dr. Izabela Michalak
PROJECTS CONCERNING ALGAE

**New generation of biological feed supplements with microelements – elaboration of method of production**

- **MNiSW**
  - R05 014 01

- **Start Date:** 13.09.2006
- **End Date:** 12.09.2009

**Technology of feed additives from seaweeds enriched with microelements via biosorption and bioaccumulation method**

- **MNiSW**
  - N N209 146136

- **Start Date:** 20.04.2009
- **End Date:** 26.02.2010

**Biologically active compounds in extracts from Baltic seaweeds**

- **NCN**
  - 2012/05/D/ST5/03379

- **Start Date:** 04.02.2013
- **End Date:** 03.02.2016

**Innovative technology of seaweed extracts – components of fertilizers, feed and cosmetics**

- **NCBiR**
  - PBS/1/A1/2/2012

- **Start Date:** 11.11.2012
- **End Date:** 31.05.2016

**The effect of bioactive algae enriched by biosorption in the certain minerals such as Cr(III), Mg(II) and Mn(II) on the status of glucose in the course of metabolic syndrome horses. Evaluation in vitro and in vivo**

- **NCN**
  - 2015/18/E/NZ9/00607

- **Start Date:** 01.04.2016
- **End Date:** 31.03.2019
Engineering in Life Sciences

Izabela Michalak
Katarzyna Chojnacka

Department of Advanced Material Technologies, Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland

Review

Algae as production systems of bioactive compounds

Algal extracts are gaining increasing interest due to their unique composition and possibilities of wide industrial applications. Various extraction techniques are used for conversion of algal biomass into extracts. Recently, attention of scientists has been paid to novel methods, such as enzyme-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, and ultrasound-assisted extraction, which enable the extraction of biologically active compounds without their degradation. In this review, the properties of biologically active compounds extracted from the biomass of algae reported in the literature are presented in a structured way. Algal extracts contain compounds such as carbohydrates, proteins, minerals, oil, fats, polyunsaturated fatty acids as well as bioactive compounds such as antioxidants (polyphenols, tocopherols [vitamin E], vitamin C, mycosporine-like amino acids), and pigments, such as carotenoids (carotene xanthophyll), chlorophylls, and phycobilins (phycocyanin, phycoerythrin), which possess antibacterial, antiviral, antifungal, antioxidative, anti-inflammatory, and antitumor properties. Finally, we assemble a list of applications of algal extracts in different developing branches of agriculture (biostimulants, bioregulators, feed additives) and in pharmaceutical industry.
ALGAE AS A SOURCE OF BIOLOGICAL COMPOUNDS

1. Minerals
2. Polysaccharides (agar, ulvan, alginians)
3. Carotenoids (carotene, xanthophyll)
4. Vitamins, antioxidants (vit. C)
5. Proteins (essential amino acids)
6. Fatty acids (omega-3, omega-6)

* Phe, Val, Thr, Trp, Met, Leu, Ile, Lys, His
In many papers, compounds that are responsible for these properties are not determined.
Therefore, they should be isolated and fully characterized.
**PROPERTIES OF ALGAL ACTIVE COMPOUNDS**

**Target:** Staphylococcus aureus, Proteus vulgaris, Escherichia coli, E. faecalis, Bacillus subtilis, Pseudomonas aeroginosa, Salmonella typhi, Shigella flexineri, Klebsiella pneumonia

**Target:**
- Candida albicans
- Aspergillus niger
- Aspergillus flavus
- Colletotrichum lagenariu

**Target:**
- Hepatitis A (HAV, Hep A)
- Human immunodeficiency virus (HIV)
- Herpes simplex virus type 1 and 2 (HSV)

**Target:**
- Total antioxidant activity of the extract
- Reducing power of the extract: 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay

**Target:**
- ear derma (tests in mice)

**Target:**
- Human promyelocytic eukemia cell line
- Inhibition of the proliferation of human breast, lung cancer cells
- Human hepatocellular carcinoma cell lines
Components of algal cell wall

Application: medicine, food (stuff), pharmaceutical industry (cosmeceuticals, nutraceuticals, as functional ingredients, such as stabilizers), agriculture
**Application:** natural colorants that can be applied in many products, e.g., as **food pigments** (in dairy products, beverages, etc.), as **feed additives** (e.g., aquaculture), as components of **cosmetics** and **pharmaceuticals**

**Phycobilins**
- phycocyanin
  - cyanobacteria (Blue-green algae)
- phycoerythrin
  - Red algae (Rhodopyta)

**Carotenoids**
- carotene:
  - $\alpha$-carotene
  - $\beta$-carotene
- lycopene
- xanthophyll:
  - astaxanthin
  - fucoxanthin
  - zeaxanthin
  - lutein

**Chlorophylls**
- chlorophyll a
- chlorophyll b
- chlorophyll c
**COMPOUNDS WITH ANTI-OXIDANT ACTIVITY**

| Glutathione (GSH) | \(\text{ascorbate (vitamin C)}\) | \(\alpha-, \gamma-, \delta- \text{ tocopherol}\) |
| - vitamins | \(\text{tocopherol (vitamin E)}\) | \(\alpha-\text{carotene and } \beta-\text{carotene}\) |
| - Carotenoids | \(\text{fucoxanthin and astaxanthin}\) | \(\text{fucol}\) |
| - Polyphenols | | \(\text{phlorotannin - brown algal polyphenol}\) |

- Prevention of damage to cellular components caused by ROS, eg. free radicals.
- Protection from UV radiation and oxidative damage.

**Integral structural components of cell walls**

- **Phlorotannin**
- **Polyphenols**
  - Catechin
  - Epicatechin/Epicatechin gallate
  - Epigallocatechin/Epigallocatechin gallate
  - Anthocyanins
  - Flavonols
  - Flavanols
  - Flavanones
  - Flavones
  - Isoflavones
- **Mycosporine-like amino acids**
  - Mycosporine-glycine
- **Cytokinins** – influence cell division and shoot formation.

- **Auxins** – influence cell growth (cell enlargement), bud formation and root initiation.

- **Gibberellins** – regulate growth and influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf and fruit senescence.

- **Abscisic acid** – functions in many plant developmental processes, including bud dormancy. Is also produced in the roots in response to decreased soil water potential and other situations in which the plant may be under stress.

- **Ethylene** – stimulates or regulates the ripening of fruit, the opening of flowers and the abscission of leaves.
### Betaines
Organic osmolytes, substances synthesized or taken up from the environment by cells for protection against **osmotic stress**, drought, high salinity or high temperature. Intracellular accumulation of betaines permits **water retention in cells**, thus protecting from the effects of **dehydration**.

### Polyamine
Plays multiple roles in cell growth, survival and cell proliferation, are important chelating agents.

#### PLANT GROWTH-PROMOTING SUBSTANCES/HORMONES

<table>
<thead>
<tr>
<th>Betaines</th>
<th>Polyamines</th>
<th>Sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>fucosterol</td>
<td>Brown algae (Phaeophyta)</td>
<td>Ergosterol</td>
</tr>
<tr>
<td>cholesterol</td>
<td>Red algae (Rhodophyta)</td>
<td>24-methylenecholesterol</td>
</tr>
<tr>
<td>cholesterol</td>
<td></td>
<td>Cholesterol</td>
</tr>
<tr>
<td>ergosterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-methylenecholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTHER COMPOUNDS</td>
<td>vitamins</td>
<td>minerals</td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>vitamins</td>
<td>$B_{12}$, K, C, E, A, D</td>
<td></td>
</tr>
<tr>
<td>minerals</td>
<td>K, Ca, Mg, Na, Zn, Cu, Co, I, B</td>
<td></td>
</tr>
<tr>
<td>peptides and proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lectins</td>
<td></td>
<td>polyunsaturated fatty acids (PUFAs)</td>
</tr>
<tr>
<td>lipids</td>
<td>fatty acids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sterols</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diterpenes</td>
<td></td>
<td>extended sesquiterpenoids</td>
</tr>
</tbody>
</table>
THE NUMBER OF SCIENTIFIC PAPERS FOCUSING ON THE EXTRACTION OF ALGAE IN THE LAST 36 YEARS

Algal biomass/lipids are treated as a source of bioenergy.

Lipid extraction from algae has the highest potential for upscaling and commercialization.

The most promising area for the utilization of algal lipids is biodiesel production.


e.g., „extraction of microalgae” in the topic
ALGAE AS A SOURCE OF BIOLOGICALLY ACTIVE COMPOUNDS

Basic Search

extraction of polysaccharides from algae

Topic

Search

TIMESPAN

All years

From 1926 to 2016

According to the Web of Knowledge (http://apps.webofknowledge.com), 27.10.2016
According to the Web of Knowledge (http://apps.webofknowledge.com), 27.10.2016

![Bar chart showing the number of papers for various substances.]

- **L**: lipids
- **P**: polysaccharides
- **P-X**: pigments – xanthophyll
- **P-C**: pigments – carotenoids

The chart displays the number of papers for different substances:

- **Lips**: 1227 papers
- **Fatty acids**: 642 papers
- **Polysaccharides**: 628 papers
- **Antioxidants**: 394 papers
- **Carotenoids**: 319 papers
- **Alginate**: 157 papers
- **Carotene**: 150 papers
- **Fucoidan**: 124 papers
- **Astatanthin**: 106 papers
- **Xanthophyll**: 89 papers
- **Polyphenols**: 74 papers
- **Unsaturated fatty acids**: 61 papers
- **Fucoxanthin**: 31 papers
- **Plant hormones**: 20 papers
- **Laminarin**: L (lipids)

The chart indicates the number of papers for each category, with lipids having the highest number at 1227 papers.
The opportunity to commercialize algal biologically active compounds is to find appropriate method of their extraction.

- The choice of the method should depend on the nature of the extracted compound.

- The optimal process conditions (solvent, pH, temperature, pressure) might have a significant influence on the outcome of the extraction process.

Cell wall matrix with organic molecules

Bioactive compound

AIM OF WORK

Extract obtained by SFE is a concentrate of biologically active compounds in a solvent-free environment and is safe to plants, animals and humans.
Review

Algal extracts: Technology and advances

A number of studies on the development of a variety of extraction techniques to produce algal extracts have been reported. At present, attention is paid to the production of algal extracts without resorting to toxic organic solvents or aggressive extraction conditions that could deteriorate biologically active compounds found in algal cells. In this review, we present a critical overview of novel techniques to extract biologically active compounds from algae without their degradation. We compare the advantages and disadvantages of classic and novel extraction techniques such as supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, enzyme-assisted extraction, pressurized liquid extraction. Additionally, we review the different biomass pretreatment methods. Products derived from algae are safe to plants, animals, and human. Therefore, they can be used in modern agriculture (biostimulants, bioregulators, feed additives) and in food, cosmetic, and pharmaceutical industries.
One of the most promising future techniques for the extraction of biologically active compounds is **Supercritical Fluid Extraction**.

In a **supercritical region** (temperature $T_c=31^\circ C$ and pressure $p_c=73$ atm), CO$_2$ has properties of both liquid and gas. Above the critical point, it becomes a supercritical fluid.

CO$_2$ is considered as a “green” solvent which is:
- non-toxic
- completely inflammable
- inexpensive
- easily separated from the extract (reduction of pressure)
OPPORTUNITIES TO COMMERCIALISE ALGAL EXTRACTS

Disadvantages of Supercritical Fluid Extraction.

1. High investment cost
2. Low polarity of supercritical CO₂ (nonpolar nature)
3. SC-CO₂ will not extract any polar substance (the addition of a polar solvent such as methanol or water to supercritical CO₂ allows it to extract polar compounds)
4. Labor-intensive step of sample processing
Preparation of formulations containing supercritical algal extract for a given application.
APPLICATION OF ALGAL EXTRACTS IN AGRICULTURE

- Supercritical algal extract as a feed additive to the drinking water of laying hens


**CHALLENGES**
Product registration according to the EFSA requirements

---

**Manuscript Details**

**Manuscript number**
ALGAL_2016_134

**Title**
Supercritical algal extracts as the components of pro-health preparations for animals

**Article type**
Full Length Article

**Abstract**
The aim of the present study was to evaluate the effect of microalga *Spirulina platensis* formulation of algal extract, post-extraction residue and formulation without algal extract on the content of fatty acids in the eggs of laying hens. Microalgae are known as a rich source of unsaturated fatty acids, for example, n-3, n-6 and n-9. These bioactive compounds were extracted from the biomass using Supercritical Fluid Extraction. Microalgae and residues were added to the basal feed, whereas formulation with extract and without constituted a component of drinking water. The content of fatty acids in the lyophilized eggs was examined using Gas Chromatography after 30, 60, 90 and 120 days of feeding experiment. The main differences concerned the content of fatty acids in the groups fed with formulation containing algal extract and algal biomass. After 120 days, the eggs from the group fed with *Spirulina platensis* extract contained 11% more gamma-linolenic acid (GLA, C18:3 n-6) when compared to the control group, 12% – eicosadienoic acid (20:2 n-6), 13% – eicosatrienoic acid (ETE, 20:3 n-3) and 11% – docosapentaenoic acid (DPA 22:5, n-3). In the group fed with algae, the content of eicosapentaenoic acid (EPA 20:5, n-3) increased by 12% and DPA by 20% when compared with the control group. However, the differences between the examined groups were not statistically significant (p<0.05). It can be concluded that the highest impact on the fatty acids profile in the eggs had the supercritical algal extract. This kind of eggs can constitute a designer food fortified with health promoting ingredients – polyunsaturated fatty acids.

**Keywords**
*Spirulina platensis*; supercritical fluid extraction; extract; laying hens; eggs; fatty acids
Biostimulants are materials, other than fertilisers, that promote plant growth when applied in low quantities.
APPLICATION OF ALGAL EXTRACTS IN AGRICULTURE

Characteristics of supercritical algal extracts and analysis of their properties in the germination tests.

- Inorganic (macro- and microelements) and organic (plant hormones: auxins – phenylacetic acid and cytokinins – 6-benzylaminopurine; polyphenols) composition of SC algal extract
- Chlorophyll and carotenoid content in the cultivated plants (garden cress and wheat)
- Method (foliar, maceration) of algal extract application on plant growth
APPLICATION OF ALGAL EXTRACTS IN AGRICULTURE

Evaluation of Supercritical Extracts of Algae as Biostimulants of Plant Growth in Field Trials

Izabela Michalak1*, Katarzyna Chojnacka1, Agnieszka Dmytryk1, Radoslaw Wilk1, Mateusz Gramza2 and Edward Rój2

1 Department of Advanced Material Technologies, Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, Poland; 2 AGRECO Ltd, Wroclaw, Poland; 3 Supercritical Extraction Department, New Chemical Synthese Institute, Pila, Poland

The aim of the field trials was to determine the influence of supercritical algal extracts on the growth and development of winter wheat (variety Akteur). As a raw material for the supercritical fluid extraction, the biomass of microalgae Spirulina plantensis, brown seaweed – Ascophyllum nodosum and Baltic green macroalgae was used. Firthial and Asahi SL constituted the reference products. It was found that the tested biostimulants did not influence statistically significantly the plant height, length of ear, and shank length. The ear number per m² was the highest in the group where the Baltic macroalgae extract was applied in the dose 1.0 L/ha (statistically significant differences). Number of grains in ear (statistically significant differences) and shank length was the highest in the group treated with Spirulina at the dose 1.5 L/ha. In the group with Ascophyllum at the dose 1.0 L/ha, the highest length of ear was observed. The yield was comparable in all

CHALLENGES

Product registration.

There are no specific regulations in many countries to control the use of algal extracts as products for plants – biostimulants of plant growth

This sector of biostimulants is still emerging and is still being defined.

3-year field tests on winter wheat
CHALLENGES IN COMMERCIALISATION OF ALGAL PRODUCTS

- The choice of the appropriate **source of algal biomass** for the extraction

- Selection of the **best method of extraction** of biologically active compounds from algae

- **Detailed characteristics** (the content of biologically active compounds) of the obtained algal extracts

- Increase the **scale of the production**

- The obligatory **law** – introduction of specific regulations to control the use of algal extracts as components of products
THANK YOU FOR THE ATTENTION

Dr. Izabela Michalak
Department of Advanced Material Technologies
Faculty of Chemistry

izabela.michalak@pwr.edu.pl
+48 71 3202434; +48 600197245