Innovative Algae Processing for Nutraceuticals in Food and Feed (iAlgaePro)

(CORNET)

Coordination: Forschungskreis der Ernährungsindustrie e. V. (FEI), Bonn

National Agencies:
- AiF - German Federation of Industrial Research Associations, Germany
- IWT - Institute for the promotion of Innovation by Science and Technology, Flanders/Belgium

Research Associations:
- FISCH - Flanders Innovation Hub for sustainable Chemistry, Brussels/Belgium
- BB – Boerenbond Projecten vzw, Leuven/Belgium

Research Institutes:
- DIL – German Institute of Food Technologies, Quakenbrück
  Dr. Volker Heinz/Dr. Bastian Dörrbecker
- ILU - Institute for Food and Environmental Research, Nuthetal
  Prof. Dr. Sascha Rohn/Dr. Michael Sandmann
- VITO NV - Vlaamse Instelling voor technologisch Onderzoek, Mol/Belgium
  Dirk Fransaer/Dr. Kathy Elst

Industrial Branch: Confectionery Industry

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Initial Situation:

Microalgae have many special properties that allow a sustainable production of food and feed. High biomass productivity, nearly 100 % fertilizer utilization efficiency, the possibility of using infertile areas, salt water and secondary streams as sources of nutrients, as well as the use of CO_2 enable a sustainable production of numerous valuable ingredients. These include proteins (up to 70 % of the dry substance), functional groups, unsaturated fatty acids, pigments and other bioactive ingredients. Algae ingredients can be used in the food and feed industry as natural dyes, nutraceuticals, hydrolyzed proteins and as a general source of protein in animal breeding and aquaculture industries.

The aim of the interdisciplinary research project was the development of an innovative algae processing based on the "mesh ultra-thin-layer" technology, membrane-based harvesting methods, high-voltage pulse technology as well as novel separation and extraction techniques. Three phototrophic model strains, including extremophiles such as *Spirulina* and higher algae, such as *Chlorella* and *Scenedesmus*, were to be used. This approach opens up an improved source of microalgae as an alternative source of functional proteins for human as well as for animal nutrition and provides innovative technological innovations, such as efficient cultivation and better drainage concepts, but also gentle extraction of sensitive functional ones ingredients. The interdisciplinary approach in iAlgaePro by combining innovative methods was intended to demonstrate a concept for use in food and feed as well as in biotechnological and biochemical applications on a pre-competitive basis.

Research Results:

In this project the cultivation of *Spirulina platensis* and *Scendedesmus dimorophus* in a Mesh Ultra-Thin Layer (MUTL) prototype was successfully established and optimized. The coupling of a 5 kW-construction for pulsed electric fields (PEF) to a photobioreactor for the continuous stimulation of the cultures was successfully established and

characterized. In addition to the continuous stimulation, batch experiments for short-time stimulation of the algae were performed. Therefore, a screening with different energy levels and their effect on the physiology of the tested algae was possible. It was shown that low energy levels influence the development of cell cultures, resulting in a different nutraceutical potential. The antioxidant capacity and the level of polyphenols were increased and the composition of fatty acids was changed significantly. Apart from that, biomass was produced in adequate amounts and was available for the partners as "Slurry" or dry weight and fresh biomass respectively. Regarding the different cultivation techniques, i.e. the tubular reactor and the MUTL, differences in the efficiency and several physiological parameters were determined. For the green alga *Scendedesmus* the MUTL technology showed a consistently higher efficiency in biomass production than the reference. Due to the mechanical stress for the cyanobacteria *Spirulina* the results were not reproducible. For the biomasses produced in the MUTL, antioxidant capacities were strongly increased, as well as the amounts of phenol. In addition, the composition of the fatty acids was significantly changed. The PEF-stimulated cells as well as the cells cultivated in the MUTL showed an increased nutraceutical potential in contrast to the reference material. Hence, they can be applied to innovative products in the future.

Furthermore, a screening with different harvesting technologies was performed. With respect to energy saving aspects, the most promising technology is the „centrifugal-brush-technology", developed by the company USEGY. In comparison to the reference, this technology could save about 70 % of the energy. In order to analyze the efficiency of ingredient extraction and inactivation of the contaminating flora, the PEF technology was applied. Different approaches in lab scale and pilot scale were established in this context, and the contamination was successfully reduced. In order to purify ingredients, different technologies were tested. Via size-exclusion chromatography, green fractions of chlorophyll and a blue fraction containing phycocyanin were isolated. Further chromatographic techniques like hydrophobic interaction, anion-exchange or cation-exchange chromatography were tested. Best results were achieved with size-exclusion chromatography and the hydrophobic interaction chromatography concerning purity and yield, respectively. The composition of isolated proteins was analyzed via SDS-PAGE. It could be shown that physical procedures do not negatively affect the composition. Furthermore, a lipoxygenase activity was detected in supernatants of *Scenedesmus* treated with PEF during cultivation. For the analysis of antioxidant and antimicrobial properties algae hydrolysates were used. Therefore, different hydrolyzation techniques like acid, alkaline and pepsin hydrolysis were established. In conclusion, several hydrolysates possessed antioxidant and antimicrobial activity.

A consumer acceptance study was conducted, before several innovative products like algae flips, algae pasta, algae chocolate on the basis of white chocolate, drops based on standard reference material and different algae cookies were prepared. The determination of the nutritional value revealed a distinct benefit in comparison to products without the addition of algae.

Economic Value:

In 2010, a total of 5,000 tons of *Spirulina* biomass were produced worldwide. Only 3 % of the biomass was added to extracts, e.g. the dye phycocyanin (*Spirulina* blue). One of the most important functional proteins from microalgae in the food application is phycocyanin. This dye is the only natural blue dye for food applications, with which a "clean label" with a list of ingredients without E numbers can be realized. Phycocyanin has been validated by FDA, the Food and Drug Administration, 2013 and its GRAS ("Recognized as Safe") status has been confirmed. Artificial dyes such as Brilliant Blue (E 133) are suspected of causing hyperactivity or other health risks. After replacing this artificial dye with *Spirulina* blue, Nestle Rowntree recorded a 9 % increase in sales in the confectionery sector in 2008. Other applications of algae proteins in the fields of enzymes, bioactive substances and ingredients can also be developed for food and feed.

A purity of 20 % for phycocyanin currently achieves on the world market a price of 200 - 3,800 €/kg, depending on the quality. The yearly growth rate (Compounded Annual Growth Rate CAGR) of Spirulina biomass is indicated at 14 % and for pycocyanin at 10 % (market data 2012). Due to the recent FDA approval of the GRAS status of phycocyanin in the US market, the demand is expected to increase significantly. For further applications of algae proteins, there are currently no market analyzes as many data are treated confidentially.

The largest share of algae biomass is produced in Asia. The biomass is harvested, dried and shipped to Europe, where it is later resuspended and made

available for extraction. This approach is extremely energy and cost intensive. Ultimately, the volatility of the raw material price over the last few years has created additional challenges, especially for small and medium-sized enterprises (SMEs), in order to maintain their competitiveness in domestic and foreign European countries.

New cost-effective and sustainable developments for improved nutritional profiles in food and feed are necessary to significantly strengthen SMEs. Algae value creation chains can contribute here in Europe, but only with the development and optimization of highly efficient process solutions. The main obstacles to European microalgae production are operational limitations with inefficiencies in productivity as well as personnel costs during harvesting, dewatering, extraction and purification. This leads to economic problems during production, in particular for food and feedstuffs. The key technologies for the production of microalgae not only include the identification of optimized cultivation conditions as well as the development of efficient and economical cultivation systems but also the combined harvesting, separation and extraction of the biomass.

Publications (Choice):

1. FEI-Final Report 2017.
2. Sandmann, M., Lippold, M., Saalfrank, F., Odika, C.P. and Rohn, S.: Multi-dimensional single-cell analysis based on fluorescence microscopy and automated image analysis. Anal. Bioanal. Chem. 409 (16), 4009-4019 (2017).
3. Sandmann, M., Lippold, M., Seffelaar, H. and Rohn, S.: Anwendung der neuen „Zentrifugen-Bürsten-Technologie“ für die Separation von Zellen der Grünalge *Scenedesmus obliquus.* Chem. Ing. Tech. 88 (9),1383-1383 (2016).
4. Sandmann, M. and Rohn, S.: Mikroalgen: unerschöpfliches Potenzial für Gesundheit und Ernährung, Nutrition-Press. 9, ISSN 2196-1271 (2016).

Further Informations:

German Institute of Food Technologies (DIL)
Dr. Volker Heinz/Dr. Bastian Dörrbecker
Prof.-von-Klitzing-Str. 7, D-49610 Quakenbrueck
Phone: +49 5431 183-140
Fax: +49 5431 183-450
E-Mail: info@dil-ev.de

Institute for Food and Environmental Research (ILU)
Prof. Dr. Sascha Rohn/Dr. Michael Sandmann
Arthur-Scheunert-Allee 40/41, D-14588 Nuthetal
Phone: +49 33200 518-815
Fax: +49 33200 518-820
E-Mail: office@ilu-ev.de

Research Association of the German Food Industry
GFPi/FEI EU Office
Dr. Jan Jacobi
47-51, Rue du Luxembourg
B-1050 Brussels/Belgium
Phone: +32 2 28208-40
Fax: +32 2 28208-41
E-Mail: gfpi-fei@bdp-online.de

Vlaamse Instelling voor technologisch Onderzoek (VITO)
Dr. Kathy Elst
200, Boeretang, B-2400 Mol/Belgium
Phone: +32 14 33 56 17
Fax: +32 14 32 11 86
E-Mail: Kathy.Elst@vito.be

BB – Boerenbond Projecten vzw
Dr. Ilse Geyskens
40, Diestsevest, B-3000 Leuven/Belgium
Phone: + 32 16 28 61-33
Fax: + 32 16 28 61-29
E-Mail: ilse.geyskens@innovatiesteunpunt.be

Flanders Innovation Hub for Sustainable Chemistry (FISCH)
Dr. Tine Schaerlaekens
80, Boulevard Auguste Reyerslaan
B-1030 Brussels/Belgium
Phone: +32 22 389 764
Fax: +32 25 230 7118
E-Mail: tschaerlaekens@fi-sch.be

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