Manufacturing Microalgae for Skin Care

Patrick Stolz and Barbara Obermayer
Pentapharm LTD, Basel, Switzerland

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ABSTRACT: *Biotechnology methods are described for manufacturing of contaminant-free microalgae with potential skin care benefits, such as collagen synthesis and reduction of intracellular oxidative stress.*

Microalgae from the sea are sources of vitamins, pigments, proteins and other substances providing benefits in skin care compositions. Recently, biotechnology has enabled the manufacture of high-quality micraoalga cultures that are completely free of contaminations. This article describes the manufacturing process and uses in vitro cell tests to illustrate the ability of three manufactured microalga extracts to influence intracellular oxidative stress and collagen I synthesis.

Algae and Microalgae

Algae belong to the oldest vegetable organisms on Earth dating as far back as the Precambrian era (3.8 billion years ago) with the development of prokaryotic cyanophytes.

Algae are characterized by a large diversity of species: their total number is estimated at approximately 280,000, of which approximately 39,000 are described. The algae range in length from 70 m for macroalgae to a few microns for microalgae (such as some protozoans). Microalgae are present in plankton, where they form the so-called phytoplankton.

Due to their composition, algae constitute a valuable source for different organic substances, including proteins, carbohydrates, fibers, vitamins, polyunsaturated fatty acids, inorganic substances, trace elements and pigments. They have been utilized by different industries, which explains why their worldwide production is increasing.

After having been extensively applied in the food and animal food industry, algae have finally penetrated the skin care industry. Among many different species of macroalgae, only few microalga species are established on the skin care market, the main ones being *Spirulina* and *Chlorella*. Here are some examples of commercially available products:

- A protein-rich extract[a] from *Spirulina* repairs the signs of early skin aging, exerts a tightening effect and prevents striae.
- A fraction[b] from *Chlorella* acts against wrinkles and protects skin against harmful environmental aggressions.
- An extract[c] from *Chlorella vulgaris* stimulates collagen synthesis in skin, thereby supporting tissue regeneration and wrinkle reduction.

Manufacturing Extracts of Phototropic Microalgae

In this article, *Nannochloropsis oculata* and *Dunaliella salina*, two microalgae with excellent skin care properties, are presented and compared to *Chlorella vulgaris*.

[a] *Protulines, Exsymol S.A.M., Monaco*
[b] *Caelico, RTC (Relocation Trading Consulting Ltd), Nordsehl, Germany*
[c] *Dermochlorella, Codif, St. Malo, France*

As can be seen in Figure 1a, the microalga *Nannochloropsis oculata* is made of coccoid cells 2-4 µm in length. This microalga is characterized by a high content of polysaccharides and amino acids. It contains valuable essential amino acids that cannot be created by the body itself. Moreover, *Nannochloropsis oculata* has a high content of vitamin B_{12} and vitamin C and contains a well-balanced mixture of substances with antioxidant activity.

Dunaliella salina (Figure 1b) is a protozoic microalga with an oval or elliptical body 11-24 µm long and 6-13 µm wide. It is often used in the pigment industry as a natural source of carotene and carotenoids, but also contains further interesting components such as

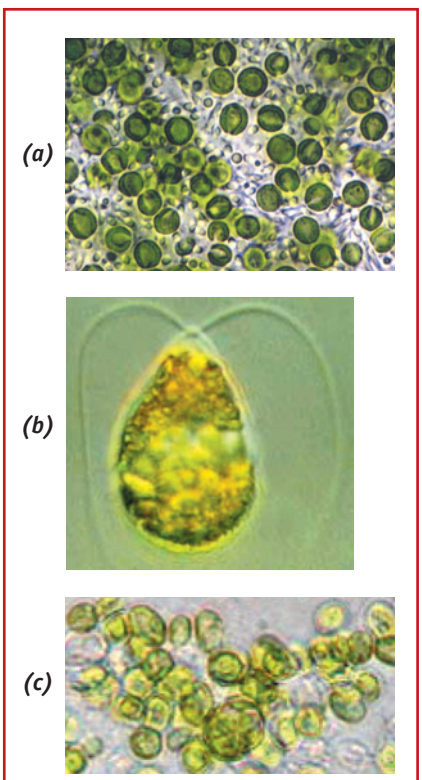

Figure 1. Nannochloropsis oculata (a), Dunaliella salina (b) and Chlorella vulgaris (c)

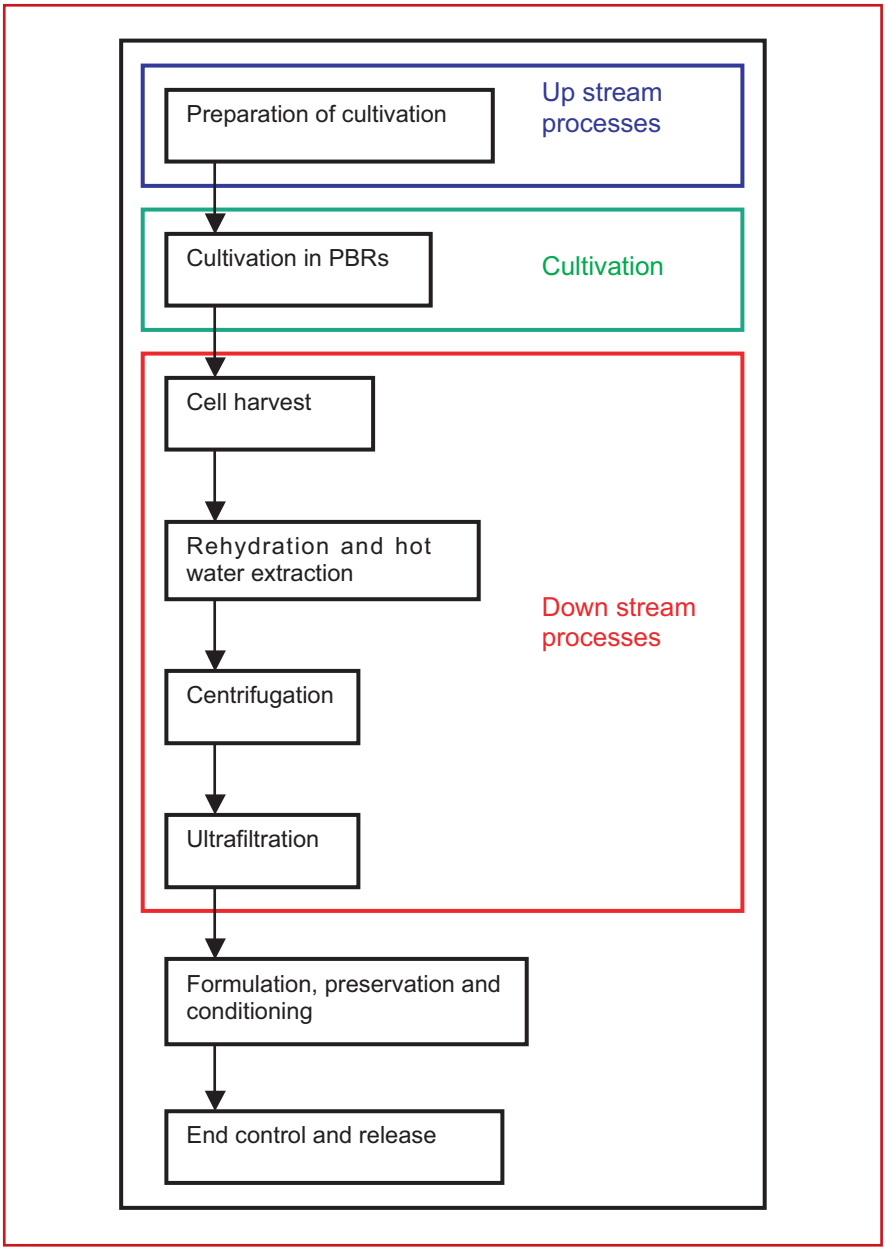

Figure 2. Schematic process of manufacture of microalga extracts

amino acids and polyphenols.

Chlorella vulgaris (Figure 1c) is a protozoic green alga a few µm in length. Its natural habitat is fresh water. *Chlorella vulgaris* has a high content of proteins and inorganic substances.

The use of these microalgae in the cosmetic industry implies strict requirements in the cultivation and breeding conditions. Due to the small size of the organisms, biotechnology offers highly modern cultivation methods that have proved suitable for the cultivation of microalgae.

During their manufacture, the tested microalga extracts pass through three classical, biotechnological steps (Figure 2):

1. Upstream processes
2. Cultivation
3. Downstream processes

Upstream Processes in Manufacture of Microalgae

The upstream of microalga cultivation comprises the preparation and sterilization of the breeding equipment and the preparation of the culture media. The photosynthetic production of biomass is generally represented with Reaction 1, where *nhv* corresponds to the number of energy (light) quanta needed to produce 1 mole of O_2. This equation is not stoichiometric as, besides photosynthesis, further energy-consuming metabolic reactions are also participating in the biomass production. Because the photosynthesis reaction on the educt side only requires CO_2, H_2O, NH_3 and inorganic substances besides energy quanta, media with simple composition can be used for the cultivation of phototrophic microalgae.

$$\alpha CO_2 + \beta H_2O + \gamma NH_3 + \text{inorganic substances} + nhv \longrightarrow \alpha\{\text{CHON inorganic substances}\} + O_2$$

Reaction 1. The photosynthetic production of biomass

Cultivation of Phototrophic Microalgae

Phototrophic microorganisms are limited above all by high cell densities in their ability to use visible light quanta as energy sources for their metabolism. Whereas with heterotrophic microorganisms, such as yeasts and bacteria, simple mixing ensures an even substrate distribution in the culture, phototrophic microorganisms are supplied with photons only near the surface. Therefore, facilities for the cultivation of microalgae have to be designed in such a way that sufficient light reaches the inside of the system, requiring that the light path is sufficiently thin. Today, microalgae are cultivated in both closed and open systems (Table 1).

Open cultivation systems: Most commercial processes for the production of microalga biomass take place in open cultivation systems. Due to the high contamination risk, however, processes in open systems are difficult to reproduce. For this reason, they are less suitable for the production of high-quality microalga products for cosmetic and pharmaceutical applications.

Open cultivation systems can be classified as either natural or artificial. Natural systems include lagoons, lakes and ponds. Among the artificial systems are artificial ponds, raceway ponds and so-called inclined surface systems. These culture systems have in common that they require large surfaces as well as a sunny and moderate climate.

Table 1. Comparison between open and closed microalgal cultivation systems

Parameter	Open systems*	Closed PBR systems
Contamination risk	Extremely high	Low
Required space	Large	Small
CO_2 loss	High	Insignificant
Variability of cultivatable species	Not given, only restricted to a few algal species	High, nearly all microalgal species can be cultivated
Flexibility of production	Changing from one microalga species to another is nearly impossible	Change of microalga species is possible without any problems after cleaning of the facilities
Reproducibility of process parameters	Not given; dependent on exterior conditions	Possible within certain tolerances
Standardization	Hardly possible	Possible
Weather dependence	Absolute; production impossible during rain	Insignificant, because closed configurations allow production even during bad weather
Biomass concentration during production	Low, approx. 0.1 - 0.2 g/l	High, approx. 2 - 8 g/l

* Raceway ponds

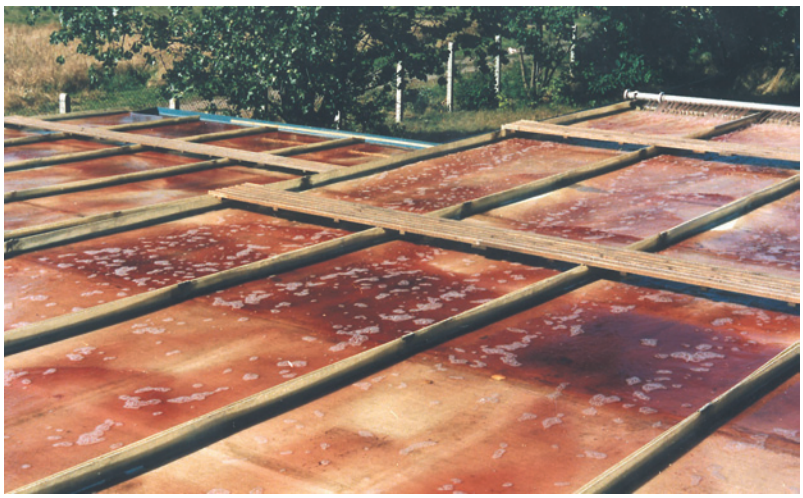

Figure 3. Open cultivation system

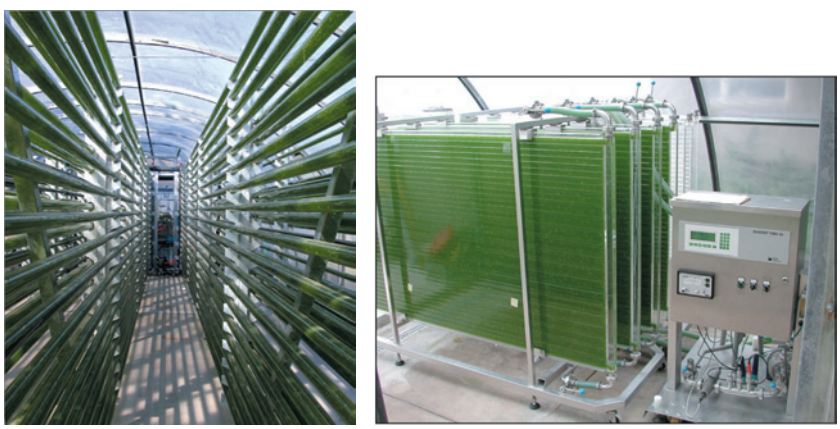

Figure 4. Tubular PBR (left) and plate-type PBR (right) in a greenhouse

Closed cultivation systems: Compared to the natural environment of microalgae, the environment in artificial culture systems is different in terms of lighting conditions and many further growth-determining factors. To be able to optimize the growth of microalgae and better adapt the culture conditions to the different species-specific needs, nearly all important biological parameters have to be regulated in photobioreactors. To meet these requirements, various technical solutions and systems for the closed cultivation of microalgae have been developed.

The technical construction of the first generation of photobioreactors, to which tanks and plastic bags belong, was based on closed containers. During up-scaling trials, however, it could be rapidly observed that this design is suitable only for volumes up to approximately 50 liters. With larger volumes, the microalgae are no longer supplied with sufficient light quantities. On the other hand, tubular and plate-type photobioreactors (PBRs) (Figure 4) can be up-scaled without limits. For this reason, they have been generally accepted on the production scale and have also been selected for the cultivation of the microalgae described in this article.

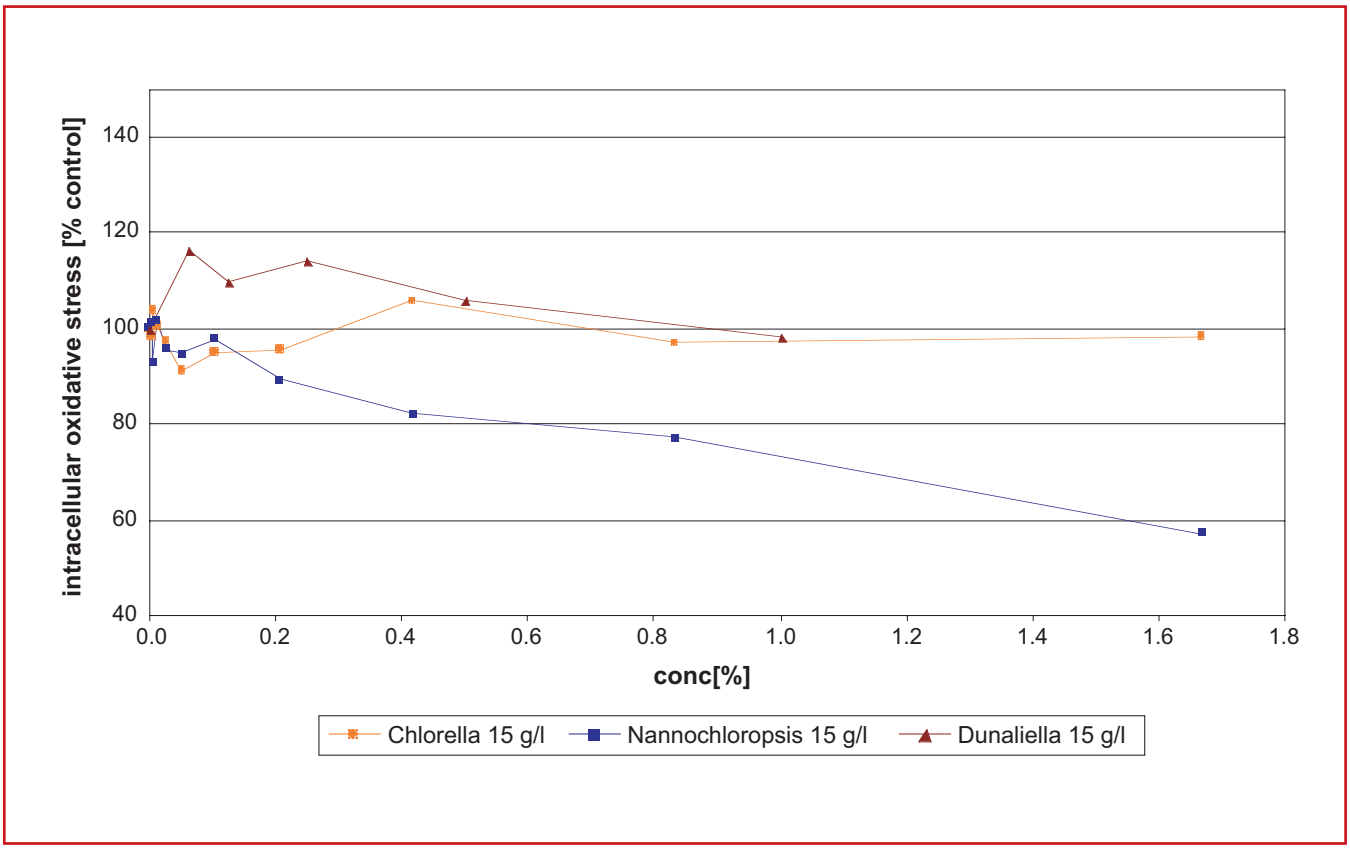

Figure 5. Influence of selected microalga extracts on intracellular oxidative stress

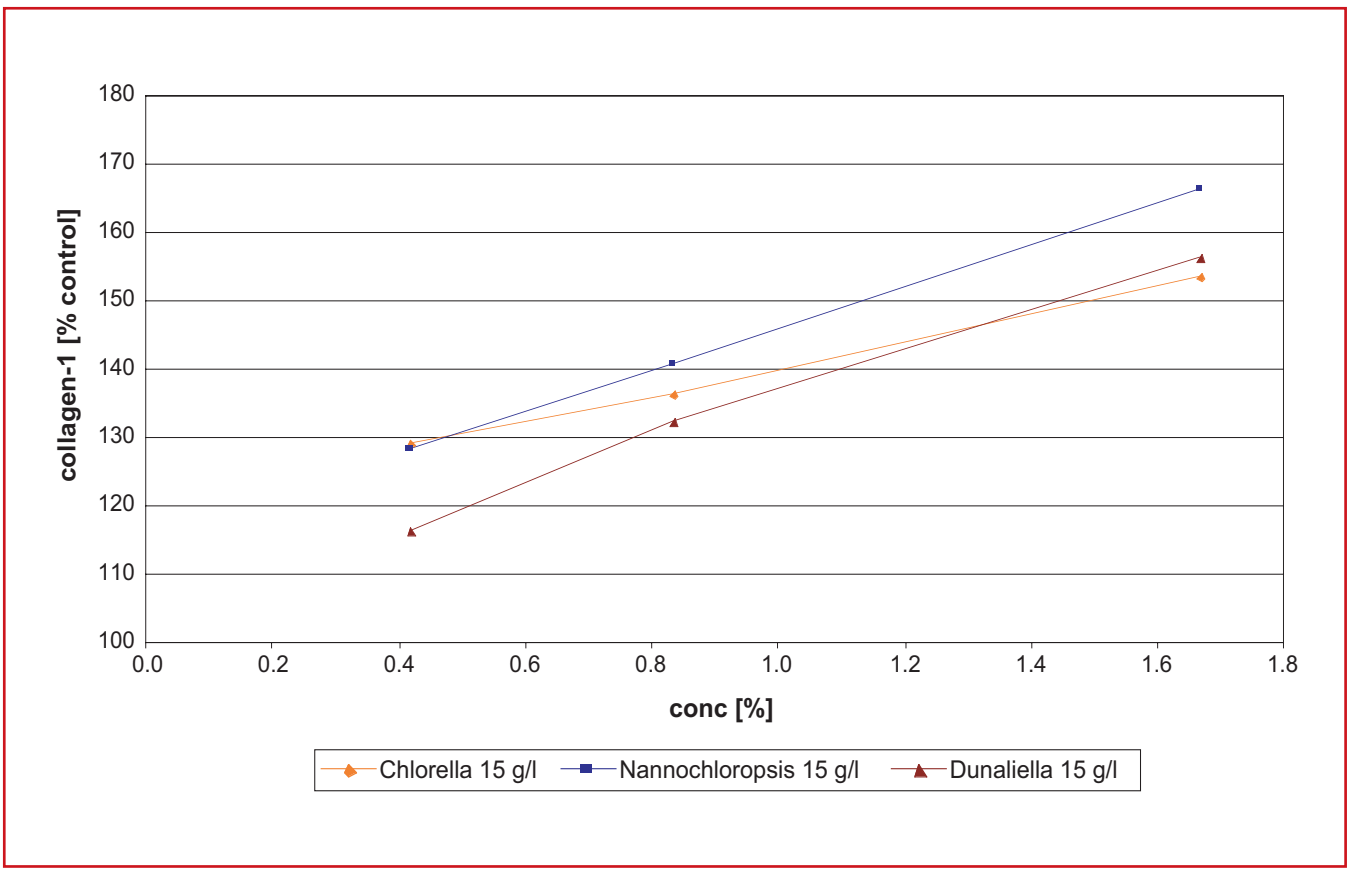

Figure 6. Influence of selected microalga extracts on the synthesis of collagen I

In order to ensure a flow as constant as possible and an even absorption with diffuse light, the horizontal flow-through elements of tube PBRs are vertically bundled. Algal suspension is re-circulated either by pumps or according to the air-lift principle. To be able to use sunlight efficiently, tube PBRs are usually accommodated in greenhouses.

The structure of the plate PBRs with horizontal, meandering channels and a vertical plate is very similar to that of tube PBRs. Therefore, plate PBRs also have a very good surface/volume ratio. Due to their very compact design, plate PBRs need even less surface than tube PBRs. Because production costs for single plates are very high in comparison to those of tubes, tube PBRs are generally used for larger facilities.

Downstream Processes in Manufacture of Microalgae

During cultivation, the microalga biomass is harvested continuously. After rehydration of the biomass, the extract is manufactured by hot water extraction.

We used an in-house test series to determine the most gentle cell digestion method. For this purpose, hot water extracts, lysozyme, ball grinding and ultrasound models were prepared from the microalga biomass and their biological effect was compared using in vitro skin models. Because hot water extracts showed the highest biological activity in vitro, hot water extraction was the method of choice for production scale manufacturing.

The cell debris formed during hot water extraction is separated by centrifugation. Before the purified hot water extract can be formulated and conditioned, the active agent content of the extract has to be adjusted by ultrafiltration to the desired standard.

In Vitro Effects of Microalga Extracts

The cytotoxicity of the microalgae *Nannochloropsis oculata*, *Dunaliella salina* and *Chlorella vulgaris* was evaluated by means of a cell stimulation/MTT-Test. Later, the extracts were submitted to a large cell culture screening to examine the different influences on the cell metabolism. Here we present the results of two different cell tests, namely the influence of the microalgae on both the intracellular oxidative stress and the synthesis of collagen I.

Determination of the intracellular oxidative stress: The DCF assay allows us to quantify the intracellular oxidative stress in cell cultures. This test evaluates the behavior of the microalga extracts towards hydrogen peroxide-induced oxidative stress on human fibroblasts. If the intracellular oxidative stress is decreased, it can be concluded that the test substance has an antioxidant or even a protective effect against oxidative stress.

Unlike cell-free systems, with which the antioxidant capacity of a test substance is measured in vitro, the DCF method also refers to biological aspects.

As shown in Figure 5, the *Dunaliella salina* extract initially produces a slight,

dose-dependent increase in the intracellular oxidative stress. After reaching a maximum, the stress potential decreases. In the concentrations tested, the *Chlorella vulgaris* extract has no activity towards oxidative stress.

By adding *Nannochloropsis* extract to the cell culture medium, a strong, dose-dependent decrease in the intracellular oxidative stress can be observed; thus, a very good protective effect against oxidative aggressions could be demonstrated.

Collagen I synthesis: Collagen I is an important constituent of the dermal connective tissue. An ELISA test was used to evaluate the influence of microalga extracts on the collagen I content in human fibroblasts. If the collagen I synthesis is highly increased, it can be concluded that the test substance has a connective tissue-strengthening and skin-firming activity. The collagen I content was determined by means of ELISA.

Figure 6 shows that all three microalga extracts exert a dose-dependent, positive influence on collagen I synthesis. The highest efficacy was observed with the *Nannochloropsis oculata* extract.

Conclusion

Due to the increasing industrial use of microalgae, biotechnology has entered the market, ensuring the establishment of highly modern technologies to grow and harvest microalgae. Cultivation in photobioreactors has shown particularly good results, because this equipment provides high-quality alga cultures, completely free of contaminations.

Even the parameters color and odor, that up to now have been a thorn for cosmetic chemists, no longer have a bad influence on the formulation when the microalgae are produced by using the cultivation and extraction technologies described here.

The in vitro screening demonstrated that the tested microalgae seem to possess completely different properties in terms of their activity on the skin.

In the tests described here as well as in further screening tests, *Chlorella vulgaris* did not show the expected efficacy. Therefore, the development of this microalga as a cosmetic active ingredient was not further investigated.

Nannochloropsis oculata not only acts as an optimal protection shield against oxidative stress, but also positively influences collagen synthesis. It has been developed to a cosmetic active ingredient[d] with excellent skin-tightening properties (combination of short-term lifting effect and long-term tightening effect) and is already available on the market.

Dunaliella salina did not show any antioxidant effect, but it increased collagen synthesis. In further assays, this microalga showed ability to massively stimulate cell proliferation and turnover, and to positively influence the energy metabolism of skin. A cosmetic active ingredient[e] made from an aqueous *Dunaliella salina* extract will be launched in April 2005.

[d] *Pepha-Tight, Pentapharm LTD, Basel, Switzerland*
[e] *Pepha-Ctive, Pentapharm LTD, Basel, Switzerland*

Send e-mail to Patrick Stolz at CT_Author@allured.com ■