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*Article*

# Influence of the Systemic Application of Blue–Green *Spirulina platensis* Algae on the Cutaneous Carotenoids and Elastic Fibers *in Vivo*

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**Abstract:** The objective of the study was to investigate the influence of a food supplement rich in antioxidants on the antioxidant status of the skin. For this reason, the blue-green algae *Spirulina platensis* powder was used for oral application during eight weeks. The effect of oral application of the antioxidant-containing *Spirulina platensis* on characteristic skin aging parameters, e.g., concentration of cutaneous carotenoids and the collagen/elastin index (SAAID), was investigated *in vivo*. A significant average increase from 2.67 ± 0.86 arb. units to 3.25 ± 0.93 arb. units ($p < 0.001$) in the cutaneous carotenoid concentration was detected subsequent to oral application of the carotenoid-containing *Spirulina platensis* powder, showing a significant improvement of the antioxidant status of the skin. A slight but not significant increase ($p = 0.33$) in the dermal SAAID mean values was measured from −0.54 ± 0.11 to −0.51 ± 0.11 subsequent to oral intake of *Spirulina platensis* powder.

**Keywords:** *Stratum corneum*; protection; antioxidants; resonance Raman spectroscopy; two-photon microscopy; collagen; elastin

## 1. Introduction

Skin aging is determined by genetic factors and can be accelerated by individual lifestyle factors and environmental influences [1,2]. As genetic factors cannot be changed, the lifestyle and environmental exposure have to be controlled individually [3]. Excessive exposure to solar irradiation [4–7], pollutant exposure [8,9], alcohol abuse [10,11], high stress exposure [12], and smoking [13] can lead to the generation of an enormous amount of free radicals in the skin, which can result in the development of oxidative stress in the tissue. The manifestation of oxidative stress can give rise to the development of premature skin aging [14,15], inflammation, and even malignant cellular changes [16,17]. The human skin has developed a protection network against the negative action of free radicals in the form of antioxidants [18,19]. The most common cutaneous antioxidants are carotenoids (beta-Carotene, lycopene), vitamins (A, C, D, E), enzymes (superoxide dismutase, catalase, glutathione peroxidase), and other substances (e.g., lipoic acid, uric acid, selenium, coenzyme Q10, *etc.*) [20]. Most antioxidants cannot be synthesized by the human organism itself and thus have to be supplemented by antioxidant-containing nutrition, for instance, fruit and vegetables [21–23]. The antioxidant substances form a balanced network in the skin, thus acting synergistically and protecting each other from direct destruction [24,25]. The concentration of antioxidants in the skin is at an individual and varying level depending on current lifestyle, environmental influences, and antioxidant uptake [26].

Carotenoids are powerful antioxidants acting efficiently against free radicals including singlet oxygen [27,28]. Recent *in vivo* studies using electron spin resonance spectroscopy and resonance Raman spectroscopy show that carotenoids could serve as marker substances for the whole antioxidant status of the human epidermis [29,30].

In order to increase the concentration of carotenoids in the different skin layers, the topical and/or systemic application of carotenoids is recommended [31–33], although it is also critically discussed [34]. Many studies show that both the topical and systemic application of antioxidants lead to an increase of the antioxidant status of the skin [31,35–37]. The use of optical methods is of high importance and is irreplaceable for non-invasive measurements of the antioxidant status of the skin [38].

Several types of algae, for instance, *Spirulina platensis*, are known for their high antioxidant composition including carotenoids and vitamins [39–41]. The systemic administration of algae is recommended as they are rich in antioxidants, easy to acquire, and have obvious health-promoting properties [42], such as anti-viral [43,44], anti-inflammatory [45,46], anti-diabetic [46], anti-allergic [47], anti-atherogenic [48], anti-cancer [49–51], cardioprotective [52], and neuroprotective [53] effects. Only one study shows the inhibitory effects of dietary *Spirulina platensis* on UVB-induced skin inflammatory responses and carcinogenesis, which were attributed to the algae's anti-inflammatory and antioxidant effects [54].

The aim of this study was to investigate the influence of systemic application of blue-green algae *Spirulina platensis* powder on the concentration of carotenoids and collagen/elastin fibers in human skin *in vivo*.

## 2. Experimental Section

### *2.1. Applied Substance*

The blue–green algae *Spirulina platensis* powder test product is a spray-dried loose biomass containing carotenoid antioxidants provided by IGV Institut für Getreideverarbeitung GmbH (Nuthethal, Germany). It was mixed with a fluid peach juice, Granini® (Eckes-Granini Deutschland GmbH, Nieder-Olm, Germany) for oral application. The carotenoid content in the test product was 461 mg/100 g. Most represented were beta-Carotene (214 mg/100 g) and Zeaxanthin (191 mg/100 g). The volunteers were provided the powdered test product, which they ingested at an amount of 0.7 g per dose. For this purpose, the powder was stirred into a glass of peach juice and orally administered twice daily. The dose was measured by means of a measuring spoon delivered with the powder.

### *2.2. Volunteers*

For the study, 10 healthy Caucasian volunteers (eight females and two males) of skin type II and III according to the Fitzpatrick classification [55], aged between 25 and 54 years, who had not consumed any food supplements, were recruited at the Charité-Universitätsmedizin Berlin. Prior to the beginning of the investigations, the study had been submitted to and approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin. The investigations were carried out in accordance with the ethical guidelines of the Declaration of Helsinki. All volunteers gave their informed written consent.

### *2.3. Study Design*

The volunteers were instructed to apply the test products twice daily for a period of eight weeks. The volunteers were instructed not to apply their usual skin care products on the palms and forearms at least for 48 hours and not to take a bath or shower at least four hours before the beginning of the experiments.

Prior to the start of the *Spirulina platensis* application period and one day after its termination, the cutaneous antioxidant status of the volunteers was determined by measuring the carotenoid marker substances using resonance Raman spectroscopy. The measurements were performed non-invasively and *in vivo* on the thenar eminence of the volunteers. In addition, the collagen/elastin index (second-harmonic generation to autofluorescence aging index of dermis (SAAID)) was determined in all volunteers by multiphoton tomography at time points $T = 0$ and $T = 8$ weeks on the inner side of the forearm at a distance of 30 cm from the distal end of the middle finger. The carotenoids were measured on five adjacent areas, while the SAAID was measured on two areas located closely together, and the obtained mean values were used for further analysis. All measurements were conducted in the winter months to avoid any seasonal influences on the cutaneous carotenoid concentration [26].

### *2.4. Determination of the SAAID*

For the investigation of the elastic fibers, a multiphoton tomograph (JenLab GmbH, Jena, Germany) was used. This system operates using a femtosecond titanium sapphire laser (Mai Tai XF, Spectra Physics, Santa Clara, CA, USA) emitting a wavelength of 760 nm and generation pulses of 100 fs at a repetition rate of 80 MHz as a source of excitation. The utilized multiphoton tomograph was described

in detail in previous studies [56,57]. For the analysis of the skin structure at different depths, sensitive photo multipliers were employed for detection of autofluorescence (AF) and second harmonic generation (SHG) signals. The dermal signal was averaged over an area of 127 × 127 μm$^2$. In order to determine the SAAID, which is defined as (SHG − AF)/(SHG + AF), the signals of the collagen and elastin in the dermis had to be detected.

The AF of the dermis is predominantly determined by the elastin concentration. Consequently, the SAAID value decreases with photoaging, reaching approximately −1 when the collagen has been completely replaced by elastin [58,59]. The mean SAAID value was calculated by averaging the values obtained at dermal depths from 80 to 120 μm where collagen and elastin are strongly presented.

### *2.5. Determination of the Carotenoid Concentration*

The carotenoids were measured non-invasively in the volunteers' palmar skin area using resonance Raman spectroscopy [60]. Based on the absorption properties of the carotenoids, the wavelength of an argon continuous wave laser at 488 nm was chosen as a resonant excitation wavelength. The intensity of the carbon-carbon double-bond stretch vibration of the conjugated backbone of carotenoid molecules (C=C) measured at 1525 cm$^{-1}$ was analyzed. The C=C chemical bonds of the carotenoid molecules are responsible for their antioxidant properties in the reaction of neutralization of free radicals [61]. The utilized Raman device was previously described in detail by our group [62]. Resonance Raman spectroscopy was chosen due to its high sensitivity and advantages compared to other non-invasive techniques.

### *2.6. Statistical Analysis*

The statistical analysis was conducted using IBM® SPSS® Statistics 22 for Windows (IBM Corporation, Armonk, NY, USA). The Kolmogorov-Smirnov test was applied to test for normal distribution of continuous data. All described data were analyzed using nonparametric tests. The Wilcoxon signed-rank test was used to analyze differences between groups, while $p$-values < 0.05 were considered to indicate any statistical significance.

## 3. Results

### *3.1. Carotenoid Concentration in the Skin*

After the *Spirulina platensis* algae powder had been orally administered for a period of eight weeks, a highly significant increase in the carotenoid concentration of the skin was observed ($p < 0.001$). The mean value of the carotenoid concentration increased from 2.67 ± 0.86 arb. units at $T = 0$ to 3.25 ± 0.93 arb. units at $T = 8$ weeks. Figure 1 represents the subsequent box plots. The obtained results are summarized in Table 1.

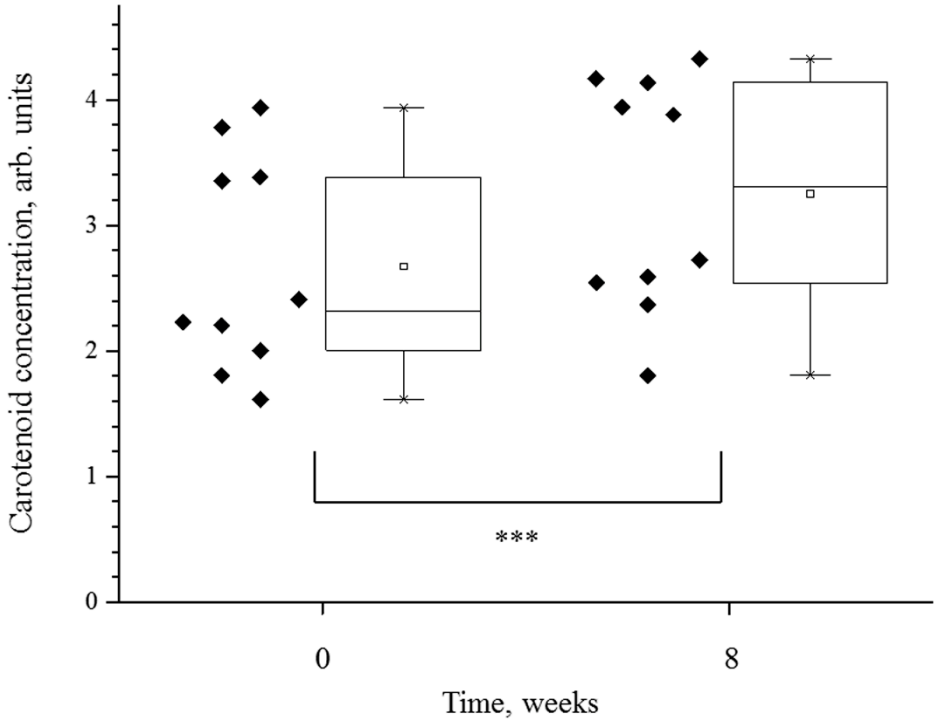


**Figure 1.** Boxplot of the changes in the carotenoid concentration prior to and subsequent to eight weeks of *Spirulina platensis* powder systemic application, *** $p < 0.001$. The box represents the middle 50% of carotenoid values including mean value and median. The "whiskers" above and below the box show the locations of the minimum and maximum.

**Table 1.** Cutaneous carotenoids and SAAID before and after oral intake of *Spirulina platensis*.

| Volunteer's | Carotenoids (arb. units) | | SAAID = (SHG − AF)/(SHG + AF) | |
|---|---|---|---|---|
| Number | Before Application | After Application | Before Application | After Application |
| 1 | 3.3850 | 4.1358 | −0.48228 | −0.35563 |
| 2 | 3.9346 | 3.8812 | −0.41115 | −0.44907 |
| 3 | 2.0042 | 1.8042 | −0.38178 | −0.38967 |
| 4 | 3.3532 | 3.9430 | −0.46049 | −0.39780 |
| 5 | 2.2032 | 4.1676 | −0.62912 | −0.62319 |
| 6 | 1.6136 | 2.5906 | −0.46187 | −0.51366 |
| 7 | 2.4084 | 2.5426 | −0.66280 | −0.56581 |
| 8 | 3.7788 | 4.3250 | −0.70285 | −0.66643 |
| 9 | 2.2284 | 2.7234 | −0.62638 | −0.60336 |
| 10 | 1.8060 | 2.3704 | −0.55371 | −0.57417 |
| Mean ± SD | 2.67154 ± 0.85594 | 3.24838 ± 0.92721 | −0.53724 ± 0.1128 | −0.51388 ± 0.10939 |
| *p*-value | 0.00000011911 | | 0.33 | |

### *3.2. Collagen/Elastin Index (SAAID)*

The results of the collagen/elastin index (SAAID) measurements averaged from the depths of 80 to 120 μm are presented in Figure 2 and are summarized in Table 1.

The obtained results showed that the eight weeks of systemic application of the antioxidant-containing *Spirulina platensis* powder resulted in a slight and insignificant increase in the mean values of the SAAID in the dermis from −0.54 ± 0.11 at $T = 0$ to −0.51 ± 0.11 at $T = 8$ weeks ($p = 0.33$).

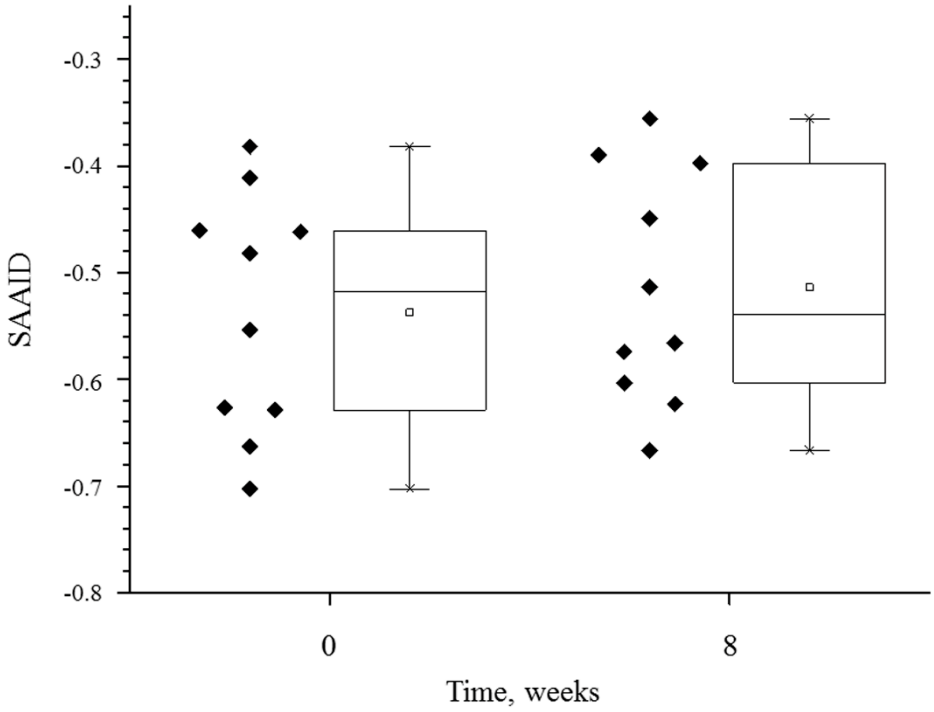


**Figure 2.** Boxplot of the SAAID (mean from the depth between 80 µm and 120 µm) prior to and subsequent to eight weeks of *Spirulina platensis* powder systemic application. The box represents the middle 50% of SAAID values including mean value and median. The "whiskers" above and below the box show the locations of the minimum and maximum.

## 4. Discussion

The results of the study show that the oral administration of the powdered algae *Spirulina platensis* improves the antioxidant status of the skin as demonstrated by the highly significant increase of the carotenoid concentration from 2.67 ± 0.86 arb. units at $T = 0$ to 3.25 ± 0.93 arb. units at $T = 8$ weeks on average. The obtained highly significant increase was found to be 22% on average, which is comparable to results obtained in other studies utilizing other carotenoid-rich test products (increases of 20% [36] and 40% [33]). The observed variety could be due to differences in the initial carotenoid concentration in the test products, application doses, seasonal influences, and the volunteers' individual lifestyles [26]. Taking into consideration that carotenoids could serve as marker substances for the complete antioxidant status of the epidermis [29,30], the obtained increase can be directly correlated with the increased ability of the skin to counteract generated free radicals.

Another parameter that has been evaluated prior to and subsequent to the systemic administration of the *Spirulina platensis* is the SAAID (collagen/elastin index). It was expected that additional antioxidant protection of the skin will result in a decrease of the amount of free radicals interacting with the dermal elastic fibers, *i.e.*, collagen and elastin. Thus, an increase of the SAAID parameter was expected. The results showed that the systemically administered *Spirulina platensis* slightly but insignificantly

improved the dermal collagen concentration and, thus, the SAAID index. It can be assumed that the investigation of a larger number of volunteers or over a longer period of time might permit a significant statement. Nevertheless, it could be expected that due to the additional antioxidant protection of the skin, collagen and elastin fibers are more resistant to external stress conditions than lower-protected skin, which was confirmed in a previous study [59].

## 5. Conclusions

The oral intake of the antioxidant-containing *Spirulina platensis* powder for 8 weeks was shown to increase the concentration of the cutaneous carotenoid antioxidants significantly, showing a strong improvement of the antioxidant status of the human skin. The collagen/elastin index of dermis (SAAID) increased slightly and insignificantly.

Optical methods, such as resonance Raman spectroscopy of carotenoids and two-photon tomography of the cutaneous structure are well suited non-invasive techniques for analyzing the human skin *in vivo*.

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## Author Contributions

Maxim E. Darvin, Martina C. Meinke, Gisela Thiede, Juergen Lademann and Elke Kurth designed the research; Maxim E. Darvin, Sora Jung, Sora Jung and Heike Richter performed research and analyzed data; Maxim E. Darvin, Sora Jung, Martina C. Meinke, Gisela Thiede and Juergen Lademann wrote the paper. All authors have revised the paper critically and approved submission.

## Conflicts of Interest

The authors declare no conflict of interest.

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