Carrot extract for blue light protection and vivid skin

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Abstract

Blue light-induced skin damage originates from two sources: natural blue light, which is part of sunlight, and artificial blue light, which comes from electronic devices. Topical applications that protect from blue light have reached the cosmetic market. But efficient blue light absorbers are rare.

Nature's answer to blue light are carotenoids, which shield blue light and protect against blue-light induced oxidative stress in plants. Lipoid Kosmetik's active ingredient Carotolino is designed to supply carotenoids to the skin, where they can naturally act as a defensive shield against blue light and reduce oxidative stress. Besides its photo-protective activity, topically applied carotenoids improve skin color. Indeed, our *in vivo* study demonstrates that Carotolino creates a subtle color optimization, shifting pale and dull skin tones to a more lively and healthy appearance.

Carotolino incorporates the best of carrots including stabilized carotenoids. This makes our botanical active a trendy ingredient that synergistically reduces blue light-induced skin damage while at the same time adding a vivid look.

Blue light protection has reached the cosmetic market. Topical application of blue light absorbers protect skin from the direct and indirect effects of blue light radiation. A class of natural blue light absorbers are carotenoids. They effectively capture blue light and reduce light-induced oxidative stress. Interestingly, carotenoids do also change skin color - a key factor for a healthy and attractive appearance of skin. In a recent, scientific study[1,2] researchers concluded that skin attractiveness correlates with carotenoid levels in skin.

Using the unique properties of carotenoids, Lipoid Kosmetik presents a new cosmetic active that combines both concepts: blue light protection and skin color improvement.

Carotenoids – blue light absorbers invented by nature

Carotenoids are fascinating molecules: they specifically filter blue light (hence their orange color) and due to their molecular structure, they effectively neutralize radicals, reducing light induced oxidative stress.

Carotenoids are highly abundant in nature - in fact, there is no green plant without them. In plants, carotenoids are light-harvesting antenna molecules that absorb visible light (VIS) and transfer its energy to the center of photosynthesis. Their most important function, however, is to protect the chlorophyll from triplet oxygen radicals that are generated in the process of photosynthesis under conditions of excess light.

In human skin carotenoids perform similar functions: During exposure to sunlight, or to light from electronic devices, visible blue light generates oxidative stress. In skin, carotenoids filter a certain portion of the incident blue light and neutralize reactive oxygen species formed.

Carotenoids protect skin from blue light

All types of light can damage skin - but each by a different mechanism. For example: UV radiation is directly absorbed by DNA causing DNA lesions. Such lesions may be mutagenic, cause photoaging, or lead to other health problems, such as skin cancer. Humans produce melanin to protect the skin against UV exposure.

By contrast, visible light radiation (VIS), especially blue light, acts through photosensitization. [3] Here, visible light is absorbed by chromophores in the skin (e.g. melanin) that upon excitation generate reactive oxygen species. Reactive oxygen species, in turn, damage DNA and other biomolecules, disturb the epidermal barrier, or induce inflammation. Sufficiently high doses of blue light cause extensive cell death.[3]

It is therefore a persistent misconception that visible light is safe for skin. In fact, after sun exposure, about 50% of free radicals produced in human skin originate from visible and infrared light.[4] Due to its shorter wavelength, particularly blue light generates radicals that accentuate the signs of premature skin aging, including hyperpigmentation, wrinkles, loss of elasticity, or dryness.

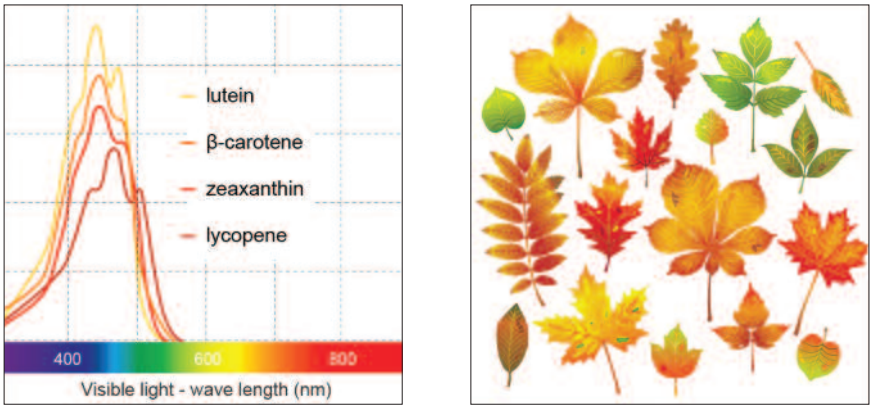

Figure 1: Carotenoids are perfect blue light absorbers. Selective absorption of blue light, and very high extinction, results in blue light protection and brightly orange colors – e.g. as seen in autumn leaves or carrots.

Carotenoids make skin color more attractive

Human skin color is composed of three pigments: hemoglobin, melanin and carotenoids, each contributing a distinct tone. Interestingly, melanin makes skin darker, whereas carotenoids predominantly impart orange, thereby balancing pale and unhealthy skin tones.[1] People with higher carotenoid levels have skin that looks healthier and more attractive.[2] This is the result of a digital color simulation study, where volunteers were asked to modify the carotenoid and melanin content in skin, until they reached the most attractive and healthy skin tone. The study provided evidence that skin attractiveness correlates with carotenoid levels. Remarkably, skin with insufficient carotenoid levels were perceived as dull, pale or greenish.

Still another study directly compared the contributions of melanin pigment and carotenoid coloration in skin. The researchers concluded that the human eye selectively prefers carotenoid coloration over melanin coloration.[5] In conclusion, attractive skin needs adequate levels of carotenoids.

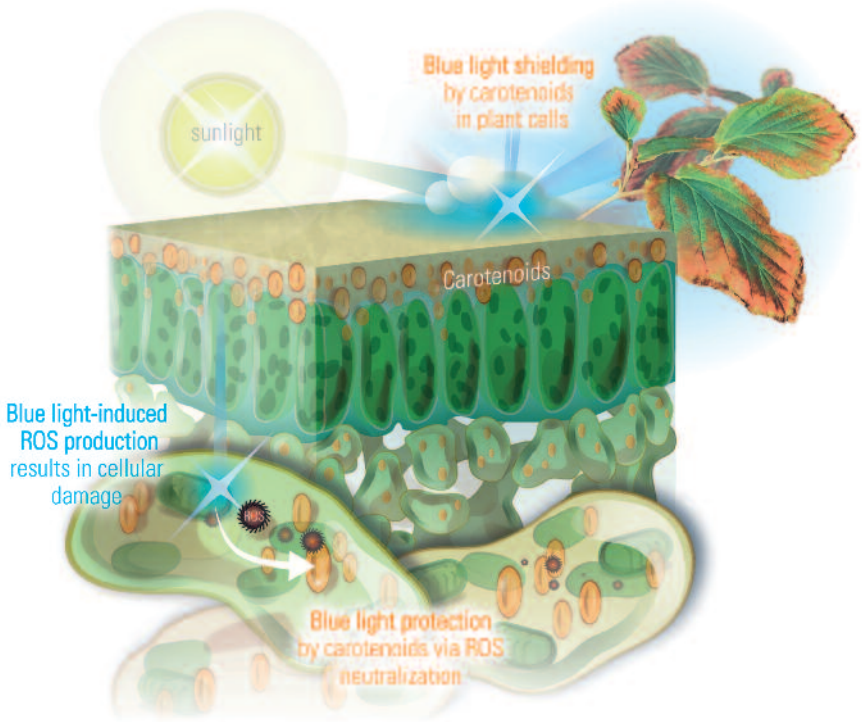

Figure 2: In plants, carotenoids capture blue light and protect plants against light-induced oxidative damage.

Carotenoid supply to skin

The human organism is unable to synthesize carotenoids; we obtain them through diet. Carotenoids are abundant in fruits and vegetables (e.g. broccoli, tomatoes, or carrots), and when consumed, accumulate in all layers of skin. Not surprisingly, people who eat more fruits and vegetables have skin tones that look healthier and more attractive.[2]

After ingestion, carotenoids are non-uniformly distributed throughout the tissues of our body.[6] Remarkably, carotenoid content is particularly high in light-exposed tissues such as eyes and skin. Indeed, before the age of sunscreens and sunglasses, humans depended entirely on biological protection from sunlight - carotenoids being a major class of protecting substances.

The topical application of carotenoids is an opportunity to supply extra carotenoids to skin. Due to their highly lipophilic nature, carotenoids readily integrate into the biomembranes of skin where they protect cellular components (e.g. lipids, enzymes, melanin) against the direct and indirect effects of blue light and improve skin color.

Composition and stability of carotenoids in cosmetic formulations

Carotenoids are unstable compounds - they are highly sensitive to oxygen and light. This is a big disadvantage and makes formulating with carotenoids a challenge. Carotolino, the lipophilic active ingredient used throughout the presented studies, is a highly stable form of carotenoids. It combines the best of carrots: carrot seed oil, lipophilic carrot root extract, and β-carotene all united in a matrix of canola oil and stabilized with tocopherol acetate.

Objective: To analyze β-carotene stability in Carotolino (now referred to as 'the carotenoid active') under different storage conditions and to compare its stability to other cosmetic preparations.

Figure 3: The relationship between a carotenoid-rich diet and skin color.

Technique: Measuring long-term stability of β-carotene to light and oxygen: β-carotene preparations were soaked on an inert, white support and stored for 60 days under 4 different conditions: Condition 1 - daylight and oxygen atmosphere. Condition 2 - dark and oxygen atmosphere. Condition 3 - daylight and inert atmosphere (nitrogen). Condition 4 - dark and inert atmosphere (nitrogen). Carotenoid degradation was monitored at regular time intervals by measuring the discoloration of the support using a colorimeter. Heat stability: An emulsion with 2% carotenoid active was heated to 80°C and β-carotene levels were measured after 2 and 4 hours.

Results: The carotenoid active has excellent long-term stability, superior to other cosmetic preparations. Almost no carotenoid degradation (discoloration) was observed, even in the combined presence of atmospheric oxygen and daylight. By contrast, rapid degradation and discoloration was evident for reference preparations in sunflower oil and in isopropyl myristate (IPM). Further, the carotenoid active is stable at elevated temperatures: 80°C for up to 4h (data not shown).

In conclusion, the active ingredient is the most stable form of carotenoids. It secures long-time storage stability and provides a lasting cosmetic activity on the skin. Further, as a lipophilic active ingredient, the carotenoid active can be used in hot process manufacturing.

Selectively shielding blue light

Objective: To show that the carotenoid active selectively absorbs blue light, as an active ingredient, but also in a cosmetic formulation.

Techniques: The absorbance spectrum of the carotenoid active was recorded with a photospectrometer between 350 - 800 nm (Fig 6 A). In an LED light experiment, green, blue and red light was passed

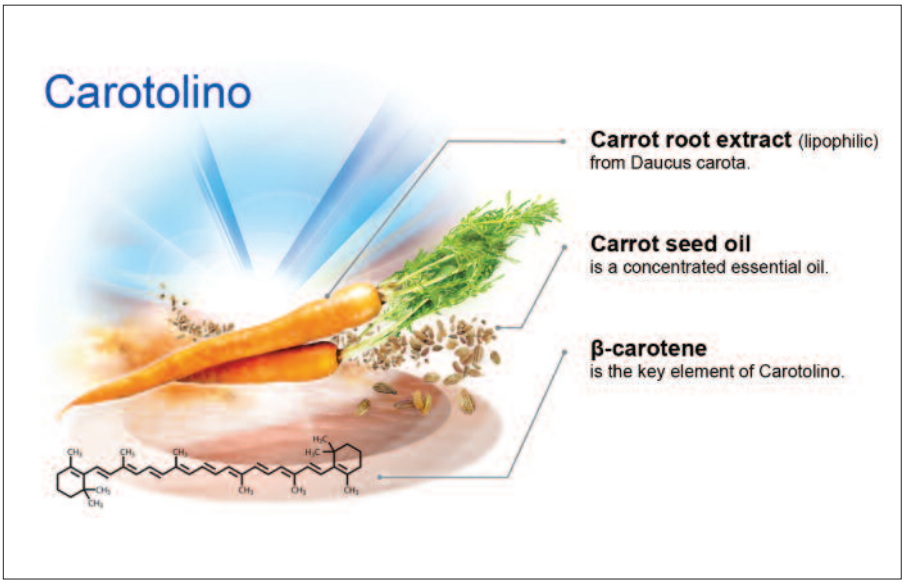

Figure 4: Carotolino - the best of carrots: It contains, a lipophilic carrot root extract from *Daucus carota* - an excellent source of carotenoids (provitamin A); and carrot seed oil - a concentrated essential oil produced through vapor steam distillation of carrot seeds. Key element though, is β-carotene an extremely efficient blue light absorber, that neutralizes reactive oxygen species and that has a characteristic lively color.

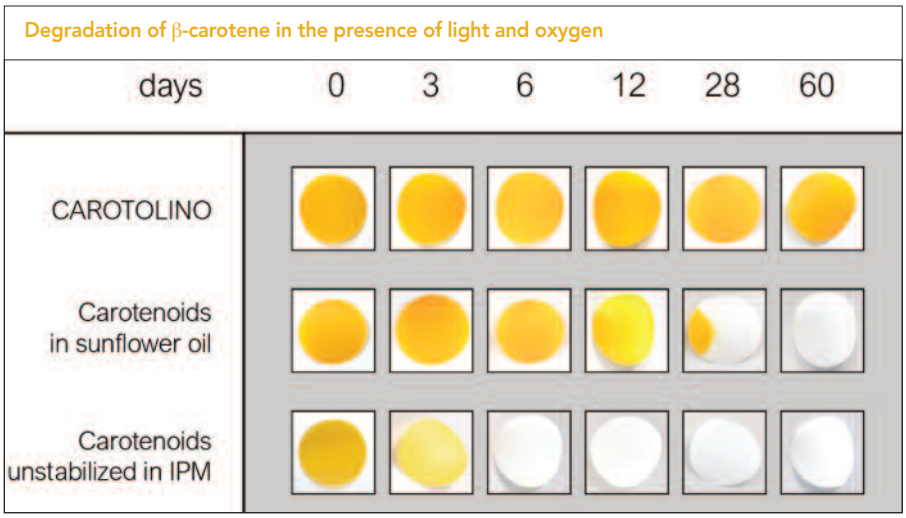

Figure 5: Carotolino is long-term stable in the presence of light and oxygen. β-carotene preparations were soaked on a white support and stored for 60 days under day light and ambient oxygen atmosphere. Carotenoid degradation was monitored at regular time intervals by measuring the discoloration of the support using a colorimeter.

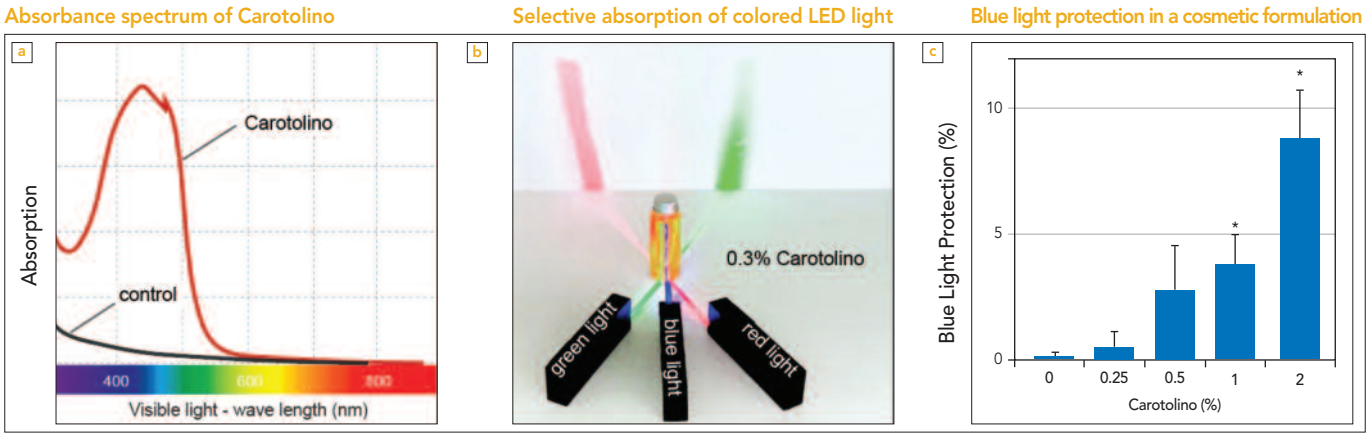

Figure 6: Carotolino is an extremely selective and efficient blue light shield. (A) Maximal absorbance of Carotolino ranges in blue light between 400 - 500 nm. (B) Carotolino shields blue light selectively. Our LED light demonstration shows that Carotolino specifically shields blue light. (C) Light, which has been passed through Carotolino-containing creams, showed significantly lower blue light intensities. N=6; Mean +/- SEM. Student's unpaired t-test; * = $p<0.05$.

through a solution of 0.3% carotenoid active and transmitted light projected onto a white screen (Fig 6 B). To test the carotenoid active in cosmetic formulations, blue light protection was determined in o/w emulsions with different concentrations of the carotenoid active (0% (control), 0.25%, 0.5%, 1% or 2%), which were spread over a transparent, roughened slide (4 mg/cm^2). Incident visible light was passed through the emulsions and transmitted light was analyzed by a photomultiplier. A selective reduction in blue light intensity is expressed as blue light protection. Blue light protection was calculated as reduction of blue light intensity in visible light.

Results: The carotenoid active, as a raw material, efficiently absorbed blue light between 400 - 500 nm (Fig 6 A). Further, the carotenoid active selectively shields blue light as shown in the LED light demonstration experiment: red light and green light pass through; only blue light is filtered (Fig 6 B). Finally, in a cosmetic formulation, the carotenoid active selectively absorbs blue light, as light transmitted through a carotenoid active cream contained less blue light than incident light (Fig 6 C). The effect was dose dependent.

Reducing oxidative stress induced by visible blue light

Objective: To show that topical application of the active ingredient reduces VIS blue light-induced oxidative stress in biological tissues.

Technique: Test substances were applied onto the surface of a 3D tissue model (*Oryzias latipes*) and allowed to penetrate for 5 min. Thereafter, test substances were completely removed, and the tissues were irradiated with visible blue light for 60 min. Finally, free oxygen radicals were visualized by a fluorescent ROS sensor and quantified fluorometrically.

Results: The carotenoid active reduces blue light induced oxidative stress (ROS emission) in biological tissues. (Fig 7: untreated control, ROS=100%). Previous treatment with the carotenoid active reduces ROS by -29% (Fig 7: Carotolino, ROS=71%).

Carotolino reduces blue light induced oxidative stress.

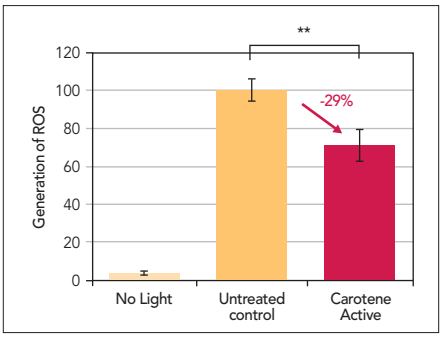

Figure 7: Carotolino reduces blue light induced oxidative stress. ROS emissions diminished by 29%. N=7; Mean + SEM; Student's unpaired t-test; ** = p<0.01.

Carotenoids protect melanin from high intensity blue light

Melanin, the skin's dark pigment, protects skin from visible and UV light. Although melanin is a stable chromophore, it can be degraded by high intensity visible light in a process called photo-beaching.[7] In this process, incident light is absorbed by melanin, which produces oxygen radicals (by photosensitization) that finally auto-degrade the pigment (photobleaching). To test the efficacy of the carotenoid active, the photobleaching experiment was performed either in its presence or absence.

Objective: To show that the active ingredient protects melanin from photobleaching through high intensity visible light (VIS) exposure. (Experiment adapted from reference[7].)

Technique: Melanin pigment is adsorbed to an inert surface either supplemented with the carotenoid active or not (control). The pigment was exposed to high intensity visible light (LED) for 3h (inner circle in Figure 8). The outer area was not exposed (control). Melanin bleaching was quantified using a colorimeter (L* value of CIE Lab color system).

How to create a vivid skin color - a consumer self-assessment test

Objective: To show that a carotenoid active formulation perceptively changes skin color by adding an expression of liveliness and vividness and by compensating pale and sallow skin tones, without being perceived as too orange. Further, to show that the application of a carotenoid active cream changes the general appearance of skin, including its overall beauty, healthiness and attractiveness. And finally, to show that the carotenoid active adds value to a cosmetic formulation that convinces consumers to recommend and buy a cosmetic product.

Technique: In a placebo-controlled consumer test, two groups of 20 female volunteers each applied a test cream with the carotenoid active (group A) or a placebo cream (group B) for 7 days, twice a day. The volunteers rated skin parameters according to a questionnaire before the first application and after 7 days of application.

Results: Consumer ratings confirmed improvements in skin color (Fig 9). Consumers were more satisfied with their skin color and they perceived their skin as healthier and more attractive. Further, pale and sallow skin tones were reduced, and skin color was more vibrant. Nevertheless, color changes were not perceived as too orange.

The observed color changes also influenced the general appearance of skin. Volunteers judged their skin as livelier, healthier, younger and more attractive (Fig 10).

As part of the same study, volunteers were asked to estimate the purchase price of the test product. Remarkably, consumers believed that a cream containing the

Photobleaching of melanin by high intensity visible light

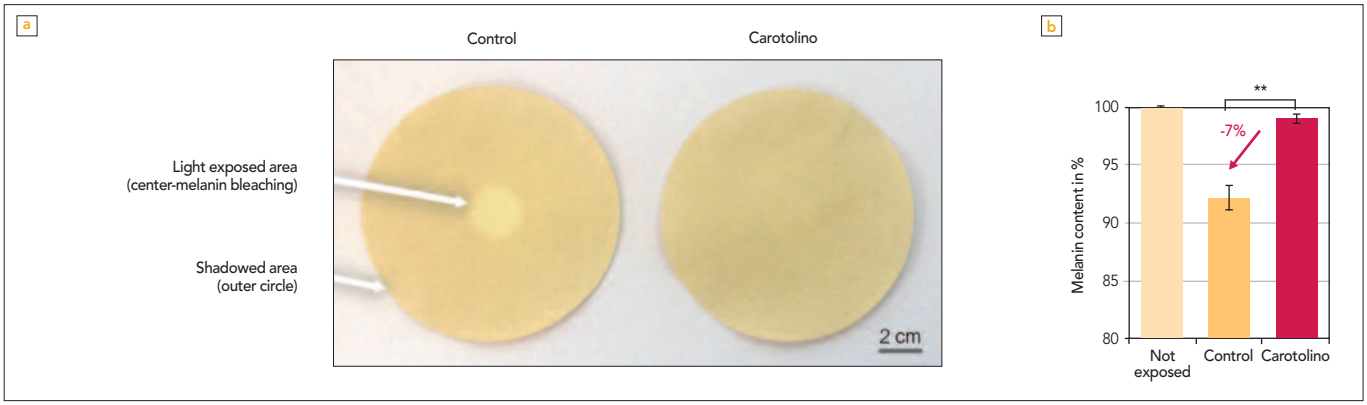

Figure 8: Carotolino protects melanin from photodegradation by visible light. The inner circles of both supports were exposed to high intensity visible light. The outer circles were covered and not exposed to light. The control (left) was photo-beached, reducing melanin content by -8%. Carotolino protected melanin (right); almost no photobleaching was detected (-1%). N = 3; Mean +/- SEM; Student's paired t-test; ** = p< 0.01.

Optimized skin color - consumer self-assessment test

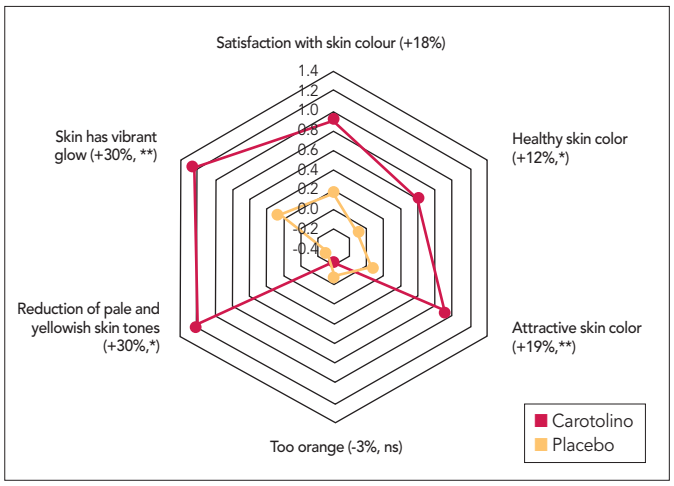

Figure 9: Carotolino improves skin color. Consumers applied a Carotolino formulation or a placebo product for 7 days, twice a day. They were asked to rate individual skin parameters on a 10-degree-scale (0 = lowest, 10 highest evaluation), before the first application (day 0) and after the last application (day 7). The data points show changes in absolute numbers between day 0 and day 7. Changes in % between day 0 and day 7 are indicated in brackets. Statistical significance refers to verum vs. baseline. N=20; Mean; Student's t-test, * = $p < 0.05$, ** = $p < 0.01$.

Improved general appearance of skin

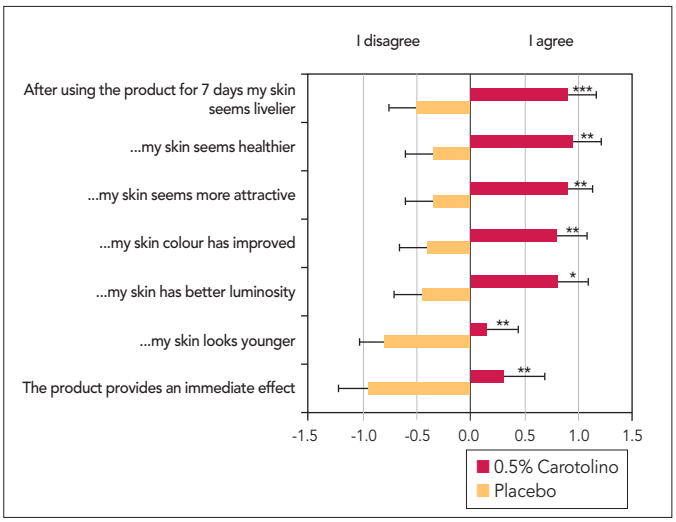

Figure 10: Carotolino improves the general appearance of skin. Volunteers applied a placebo cream or a Carotolino cream for 7 days, twice a day. At the end of the study, they were asked to rate questions on a scale ranging from "I fully disagree" (-3) to "I fully agree" (+3). The graph shows their averaged opinions for each question. N=20; Mean + SEM; Paired Student's t-test versus placebo, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

carotenoid active has a 16% higher price compared to the corresponding placebo cream (20.60 EUR vs. 17.80 EUR, respectively). The added value is remarkable: while the active ingredient increases production costs by only 0.02 EUR, product value increases by 2.80 EUR (Fig 11). What is more, consumers were more inclined to buy the carotenoid active product (+30%) and were more confident to recommend it to others (+45%) as compared to placebo, respectively (Fig 12).

Conclusion

In conclusion, the active ingredient Carotolino produces tangible results, results that are felt, experienced and enjoyed by customers. When specifically focusing on color related parameters, the Carotolino

Increased product value

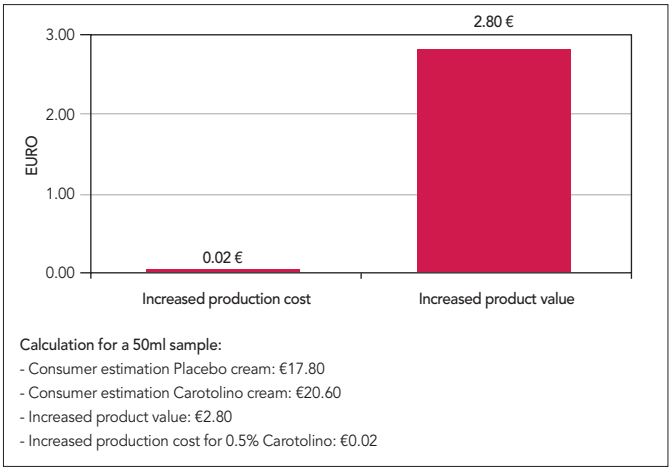

Figure 11: Carotolino adds value to a formulation. Using 0.5% Carotolino in a final formulation increases the bulk costs by 2 cents while increasing the product value by 2.8 Euro (basis 50 ml dispenser). Consumers used a "Placebo test product" or a "Carotolino test product" for 7 days, twice a day. After 7 days of use they estimated the purchase price for each product. Increased product value was calculated as the difference between the estimated price of the "Placebo test product" and the "Carotolino test product". N=20; Mean.

Increased Buying intention

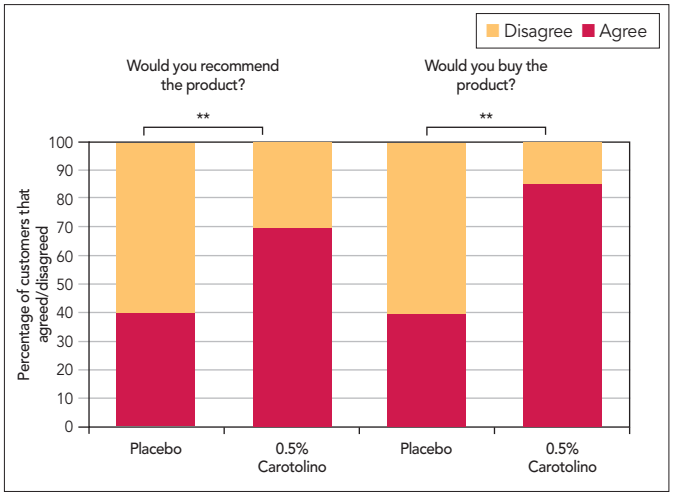

Figure 12: Carotolino convinces consumers to purchase and recommend the formulation. Consumers applied a placebo cream or a Carotolino cream for 7 days, twice a day. After 7 days, they rated their individual opinion on a scale ranging from "I agree completely" to "I disagree completely". Bars reflect percentage of consumers that agreed or disagreed. N=20; Mean; Student's t-test, ** = $p < 0.01$.

formulation improves overall skin color, including luminosity and glow, it reduces pale and sallow skin tones, without being perceived as too intense by consumers. Therefore, the Carotolino formulation improves the general appearance of skin, including its lifelines, visible health and attractiveness. What is more, just 0.5% of Carotolino adds value to a cosmetic formulation, it convinces customers to buy the product and it raises the final product's price expectation. In addition, Carotolino makes consumers confident to recommend the product to others.

In summary, the accumulation of carotenoids in skin after application of Carotolino provides:

- Direct blue light protection: by absorbing and therefore shielding blue light.
- Indirect blue light protection: by neutralizing blue light-induced oxidative stress.
- Improved skin color: by providing a healthy and vivid skin tone.

The combination of blue-light protection and skin color effects make Carotolino a unique active ingredient for skin care applications, suited for anti-age, face and body care, as well as after-sun, lip balms or hair care. PC

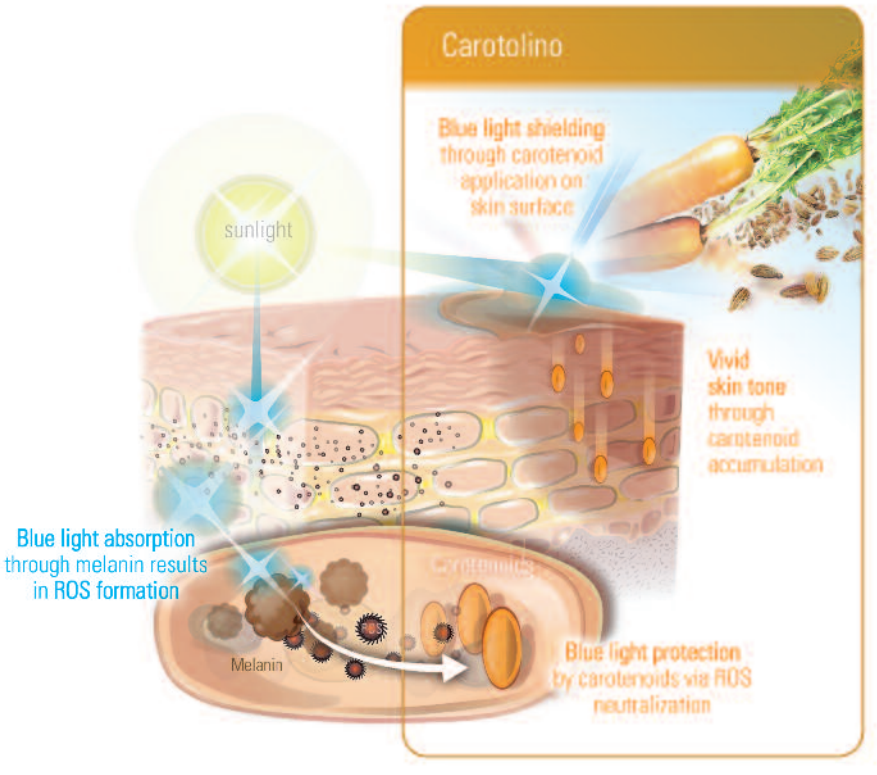

Figure 13: Carotolino's mode of action.

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