

# EFFICACY STUDIES

## PRO-RESOLVING SKIN INFLAMMAGING

Our pack of efficacy studies are focused on showing the benefits of Bicoalgae  $\omega$ 3 in improving three typical situations related with skin inflammaging:

### 1. PRESENCE OF PRO-INFLAMMATORY CYTOKINES IN SKIN TISSUE (*skin explants*)

The decrease of age-related inflammatory cytokine IL-1 $\beta$  and IL-8 in inflamed skin explants were evaluate post treatment with Bicoalgae $\omega$ 3.

### 2. SKIN OXIDATIVE STRESS FROM EXPOSURE TO ELECTRONIC DEVICES and UV-VIS radiation (*skin explants*)

Bicoalgae $\omega$ 3 capacity to decrease oxidative inflammatory states related to exposure to Blue light from electronic devices and UV-Vis radiation in different skin layers.

### 3. CHRONIC INFLAMMATORY DISORDER (*in vivo*).

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae $\omega$ 3 at 3% twice a day, morning and evening, during 30 days. The measurements were carried out at 0 and 30 days under Dermatological control.

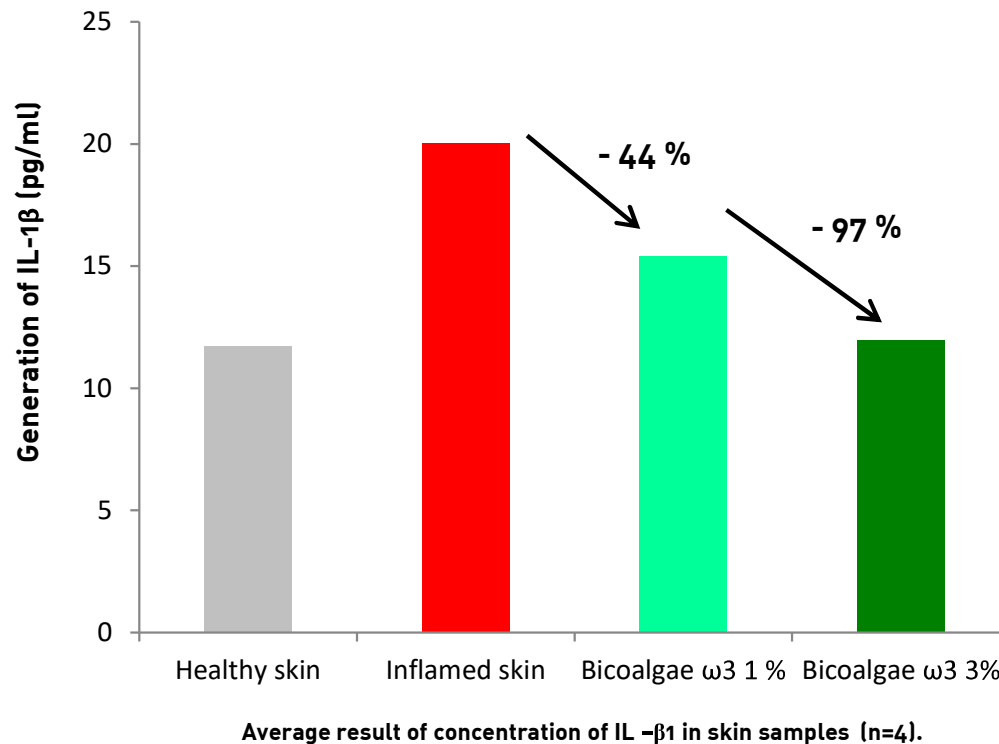


bicoalgae $\omega$ 3  
by bicosome $\text{\textsuperscript{®}}$

# DECREASE OF PRO INFLAMMATORY CYTOKINES

*in vitro*  
Inflamed skin explants

The constant release of ROS and pro-inflammatory cytokines characterise skin chronic inflammation. The decrease of pro-inflammatory cytokines in inflamed tissues indicate improvement of inflammaging.



**Bicoalgae® ω3** decreases the level of the age-related pro-inflammatory cytokine IL-1β in inflamed skin

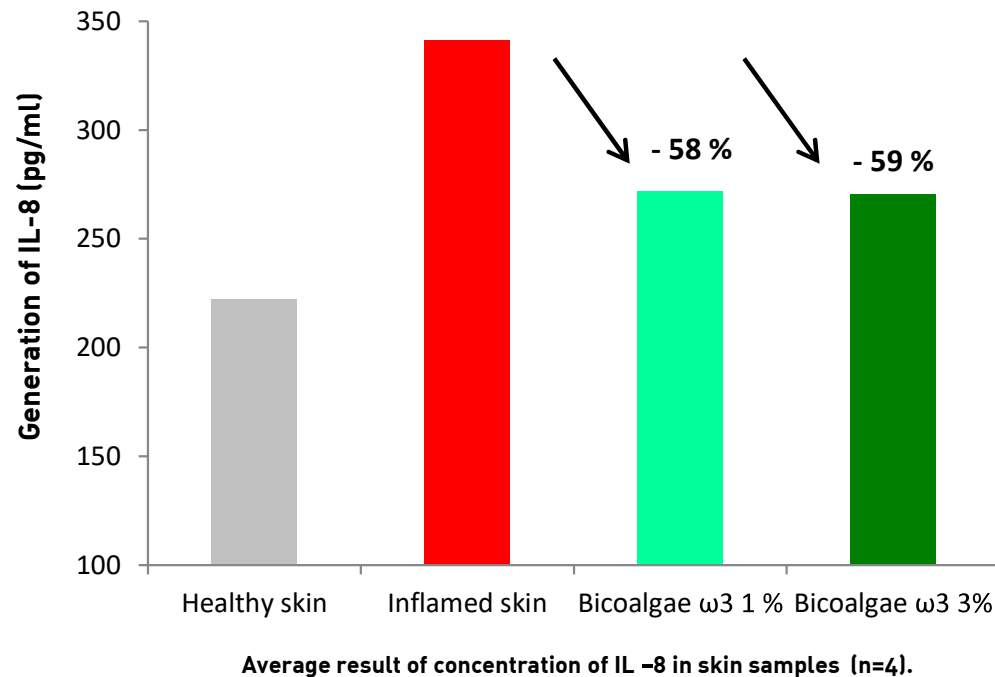
## Experiment procedure:

To evaluate the effect of Bicoalgae-ω3 in reducing pro-inflammatory cytokines, levels of IL-1β were measured in inflamed organotypic cultures of human skin explants treated and non-treated with Bicoalgae-ω3 (1% and 3%). Human skin explants were treated with the compounds in the presence of bacterial Lipopolysaccharides (LPS), simulating a generic skin inflammation. The cytokines IL-1β were calculated after 24 hours of incubation.

# DECREASE OF PRO INFLAMMATORY CYTOKINES

*in vitro*  
Inflamed skin explants

The possibility that circulating pro-inflammatory cytokines in inflammaging are being originated in the skin is now being explored by several research groups around the world.<sup>6</sup> Evidence of this would change forever the way we treat our skin.



**Bicoalgae®** ω3 decreases the level of age-related pro-inflammatory cytokine IL-8 in inflamed skin

## Experiment procedure:

To evaluate the effect of Bicoalgae-ω3 in reducing pro-inflammatory cytokines, levels of IL-8 were measured in inflamed organotypic cultures of human skin explants treated and non-treated with Bicoalgae-ω3 (1% and 3%). Human skin explants were treated with the compounds in the presence of bacterial Lipopolysaccharides (LPS), simulating a generic skin inflammation. The cytokines IL-8 were calculated after 24 hours of incubation.

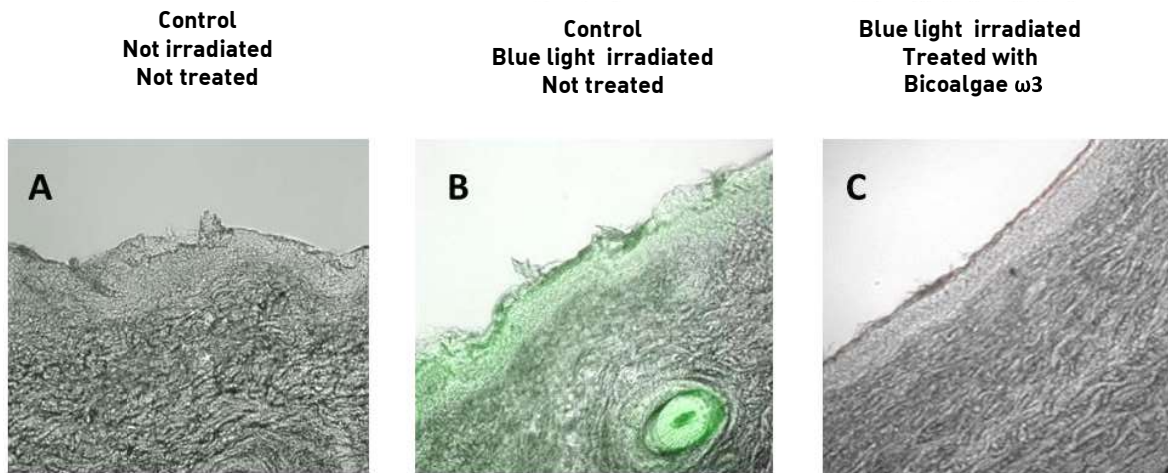
bicosome®

6. Ye et al. (2019). Topical applications of an emollient reduce circulating pro-inflammatory cytokine levels in chronically aged humans: a pilot clinical study.

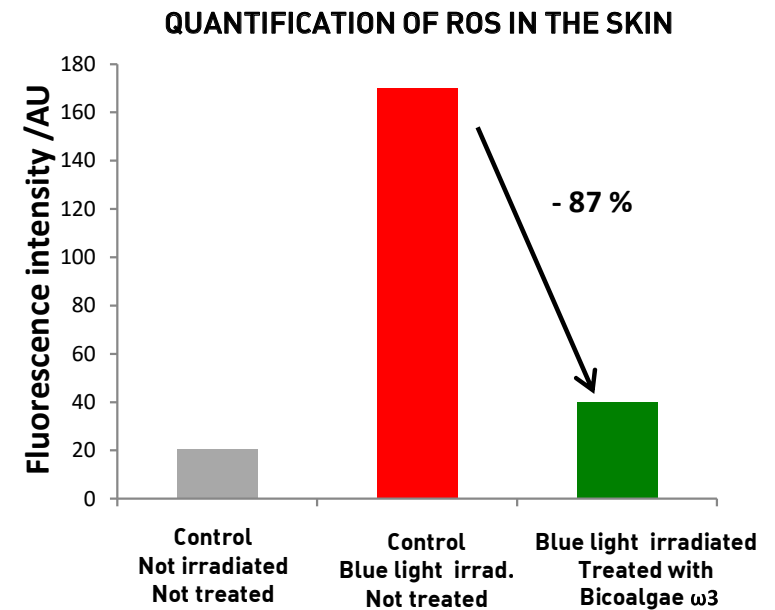
*in vitro*  
skin explants

# BLUE LIGHT PROTECTION

Exposure to blue light radiation ( $\lambda = 400-500 \text{ nm}$ ) from **electronic devices, fluorescent and LED lights** generates an excess of ROS that favours chronic inflammatory states in the skin.



Visualization of ROS in fluorescent marked skin explants (A) not irradiated and not treated, (B) irradiated and not treated and (C) irradiated and treated with Bicoalgae® ω3. Green fluorescence indicates presence of ROS.



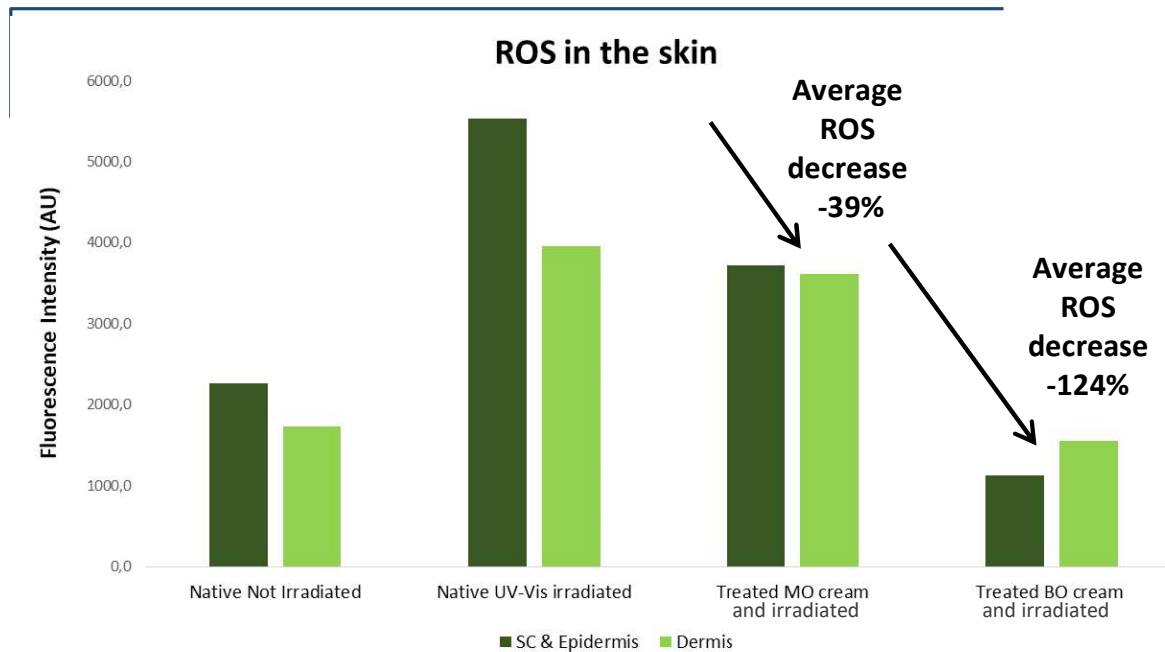
**Bicoalgae® ω3 prevents the generation ROS blue light induced**

**Experiment procedure:**

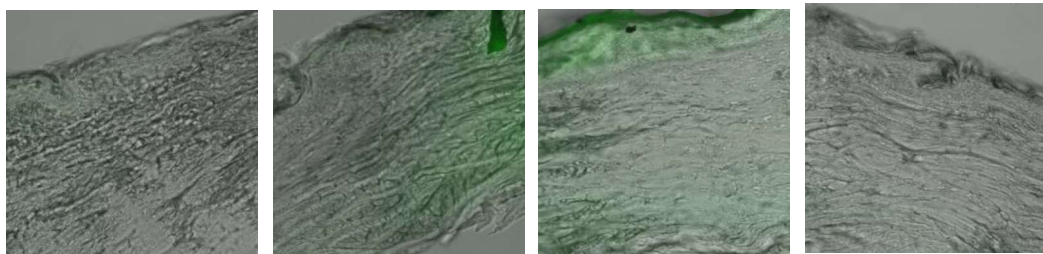
Skin samples treated and non treated with a cream containing 3% of Bicoalgae® ω3 for 24h were incubated during 30 min with dichlorofluorescein diacetate (DCFH-DA) at 40°C. DCFH-DA is a fluorescent marker that reacts with ROS becoming fluorescent. Afterwards the skin samples were irradiated with blue light ( $\lambda = 468\text{nm}$ ) during 4h. Control and test samples were observed by optical microscopy using fluorescence filters to evidence the presence of ROS and quantify them.

*in vitro*  
*skin explants*

# OXIDATIVE STRESS UV – VIS INDUCED



**Bicosome® increase the efficacy of the microalgae extracts in the SC, Epidermis and Dermis**

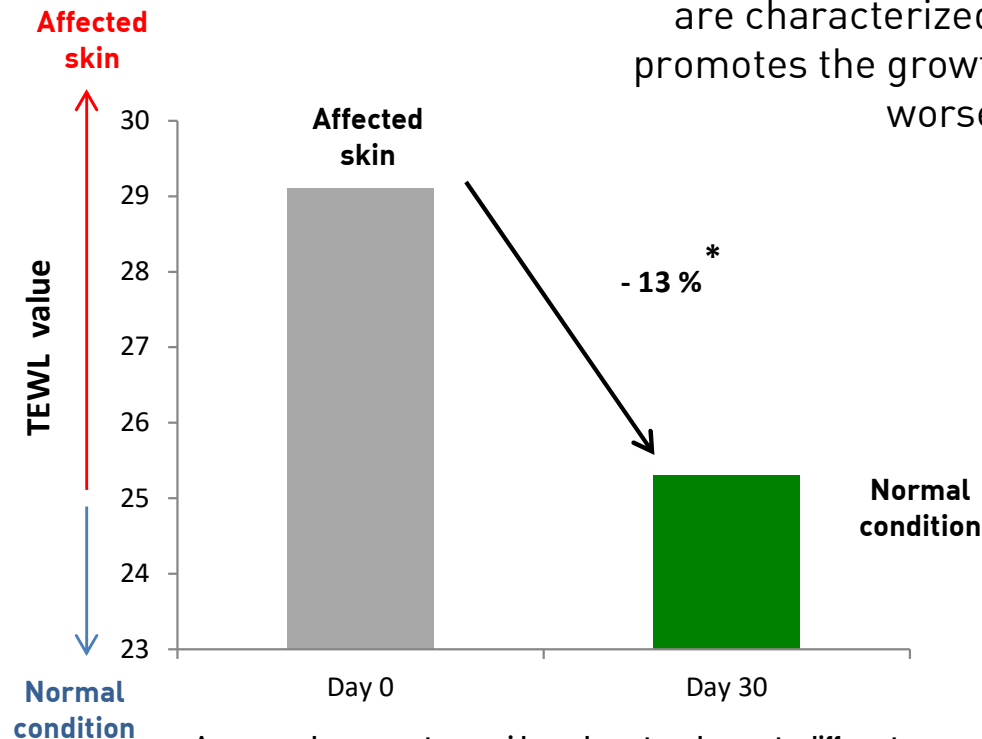


**Experiment procedure:**

First, skin samples treated with a cream containing 3% of Bicoalgae w3 (BO) or the microalgae extracts (MO) and incubated for 24h. Afterwards, skin samples treated with BO and MO creams and non treated samples were incubated during 30 min with dichlorofluorescein diacetate (DCFH-DA) at 40°C. DCFH-DA is a fluorescent marker that reacts with ROS becoming fluorescent. Finally, the skin samples were irradiated with UV-VIS using a SUNTEST CPS (Atlas, Illinois, USA) at 500 W/m2 during 30 min. Positive and negative controls and test samples were observed by optical microscopy using fluorescence filters to evidence the presence of ROS and quantify them.

# NORMALISATION OF SKIN BARRIER FUNCTION

*in vivo*  
20 volunteers  
inflamed skin condition



Average decrease transepidermal water loss at different experimental times (n=20, p value =0.000).

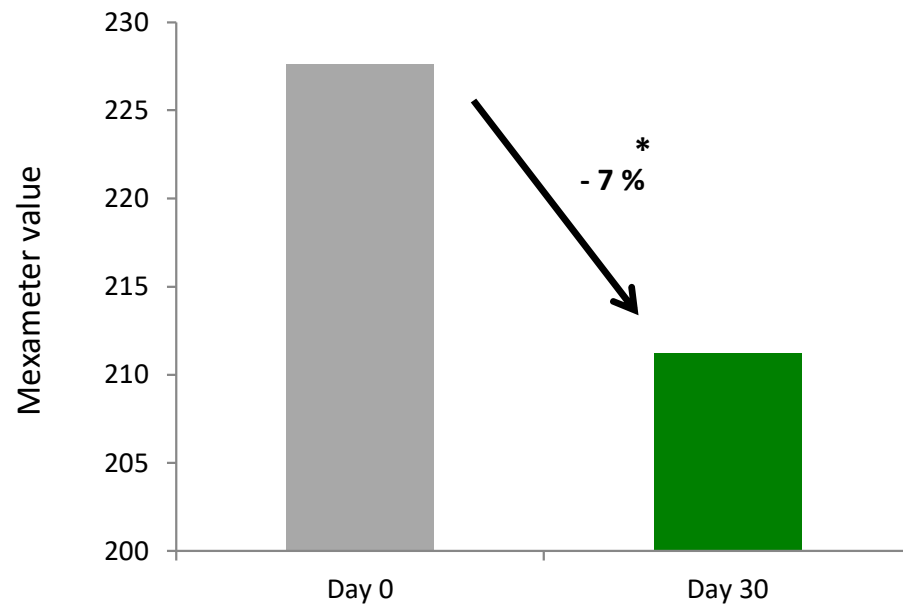
## Experiment procedure:

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. Transepidermal water loss (TEWL) was evaluated on the volunteer's forehead with the equipment Tewameter® TM 300 at the beginning and the end of the treatment..

# MELANIN REDUCTION

*in vivo*  
20 volunteers  
inflamed skin condition

Age spots are characteristic of chronic inflamed skin. The chronic state of inflammation can affect the function of melanocytes causing this age-related condition.<sup>6</sup>



Average result of Mexameter value in different experimental times (n=20, \* =  $p$  value = 0.05).



**Bicoalgae® ω3 cream improved hyperpigmented spots**

### Experiment procedure:

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. Melanin content was measured using a Mexameter at the beginning and the end of the treatment.

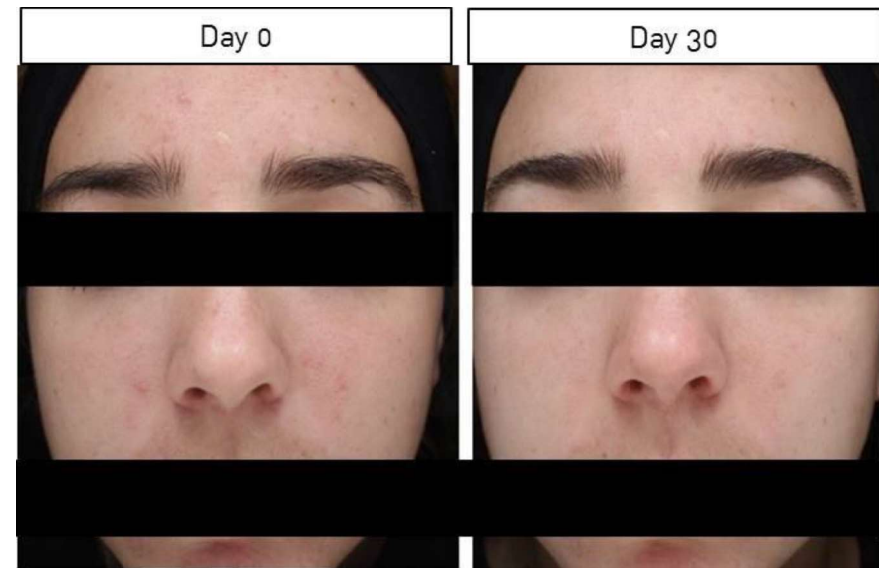
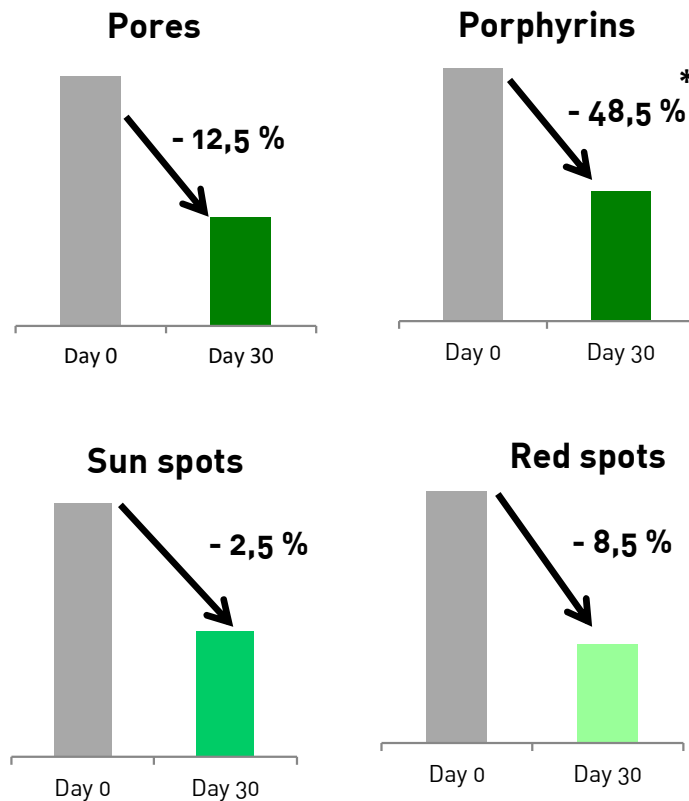
### Reference:

6.Zhuan & Lyga, 2014. Inflammaging in Skin and Other Tissues – The roles of Complement System and Macrophage. Inflammation & Allergy – Drug targets, 13, 153 – 161.

## 3D ANALYSIS BY VISIA® TECHNOLOGY

*in vivo*  
20 volunteers  
*inflamed skin condition*

Quantification of red spots, sunspots, pores and porphyrins give valuable information in the analysis of the improvement of inflammatory conditions.



**Bicoalgae® ω3** cream decreased number of surface bacteria, pores and skin spots.

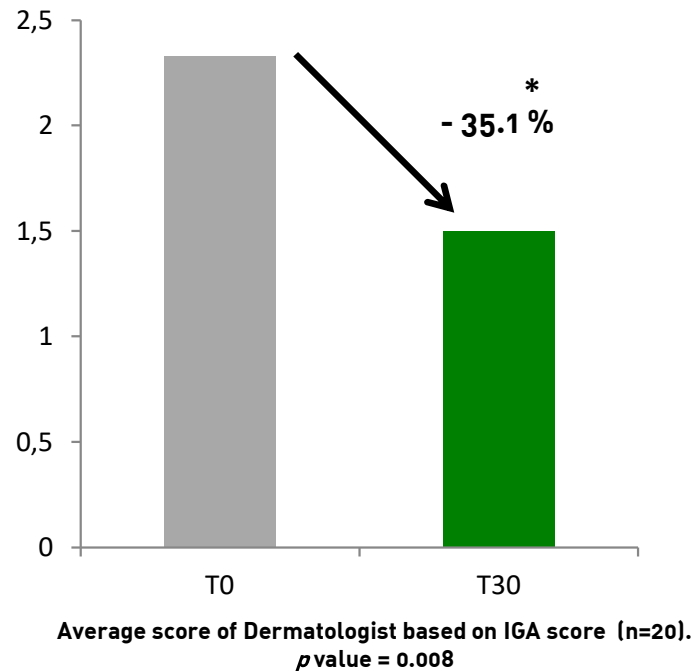
### Experiment procedure:

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. Visia® was used to evaluate changes in skin color hyperpigmentation (freckles, melasma), red areas (vascular disorders due to acne, rosacea, spider veins, inflammation, presence of large pores and porphyrins, at the beginning and the end of the study.



# INVESTIGATOR GLOBAL ASSESSMENT - IGA

*in vivo*  
20 volunteers  
inflamed skin condition



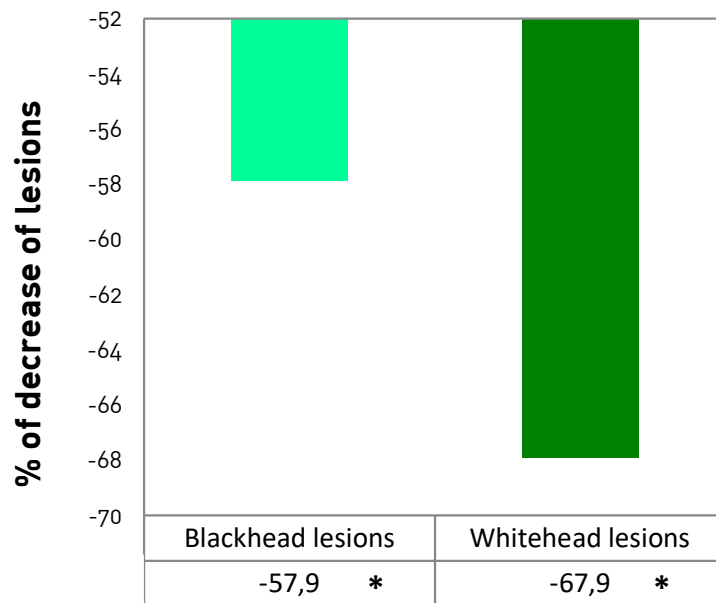
**Bicoalgae® ω3** cream improved volunteers inflammatory condition around 35.1% according with clinical evaluation.

#### Experiment procedure:

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. The Dermatologist evaluate volunteers using IGA scale.

# DECREASE OF ACNE LESIONS

*in vivo*  
20 volunteers  
inflamed skin condition



Average score of Dermatologist based on IGA score (n=20).  
p value = 0.022 for blackheads and p value = 0.035 for whiteheads



The use of the **Bicoalgae® ω3** cream decreased relevantly both white and blackhead lesions.

#### Experiment procedure:

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. The Dermatologist evaluated comedogenicity by counting white and blackhead lesions before and after treatment.

## PATIENT GLOBAL ASSESSMENT - PGA

*in vivo*  
20 volunteers  
inflamed skin condition

PGA is a measurement instrument for patient subjective evaluation on the perception evolution of its condition. It uses a punctuation systems that goes from -2 (meaning relevant worsening) to + 3 (meaning intense improvement).

Patient score	
Average improvement	1.4 *

Average score of PGA score (n=20).  
p value = 0.001

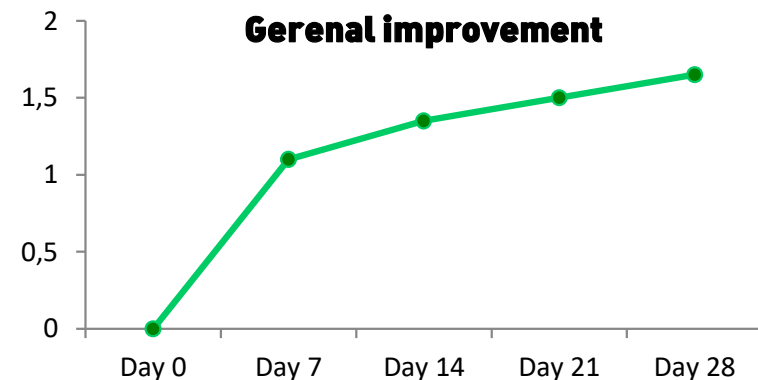
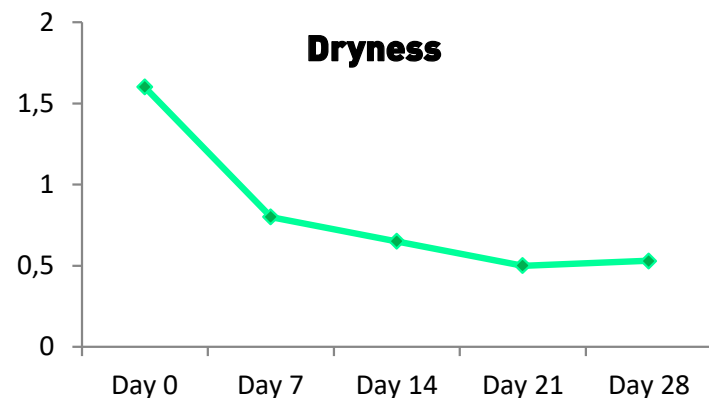
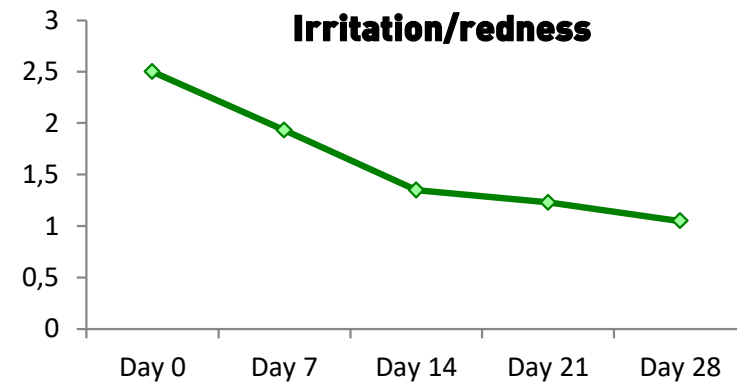
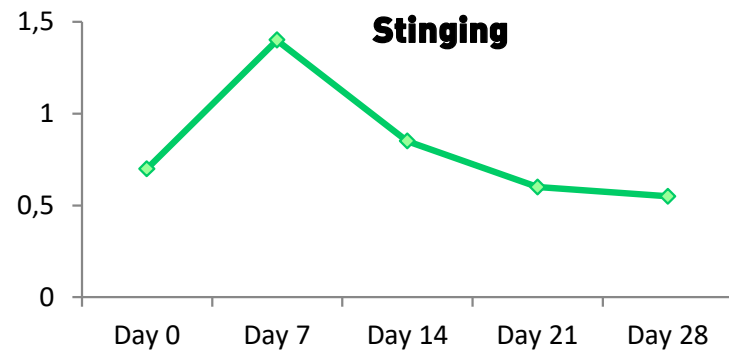
**Bicoalgae® ω3** cream raised volunteers perception on the improvement of their inflammatory condition.

### Experiment procedure:

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. The patients evaluated their condition together with the Dermatologist using PGA scale.

# PATIENT EFFICACY SELF-ASSESSMENT

Additionally, patients weekly evaluated the improvement of their main inflammatory symptoms.



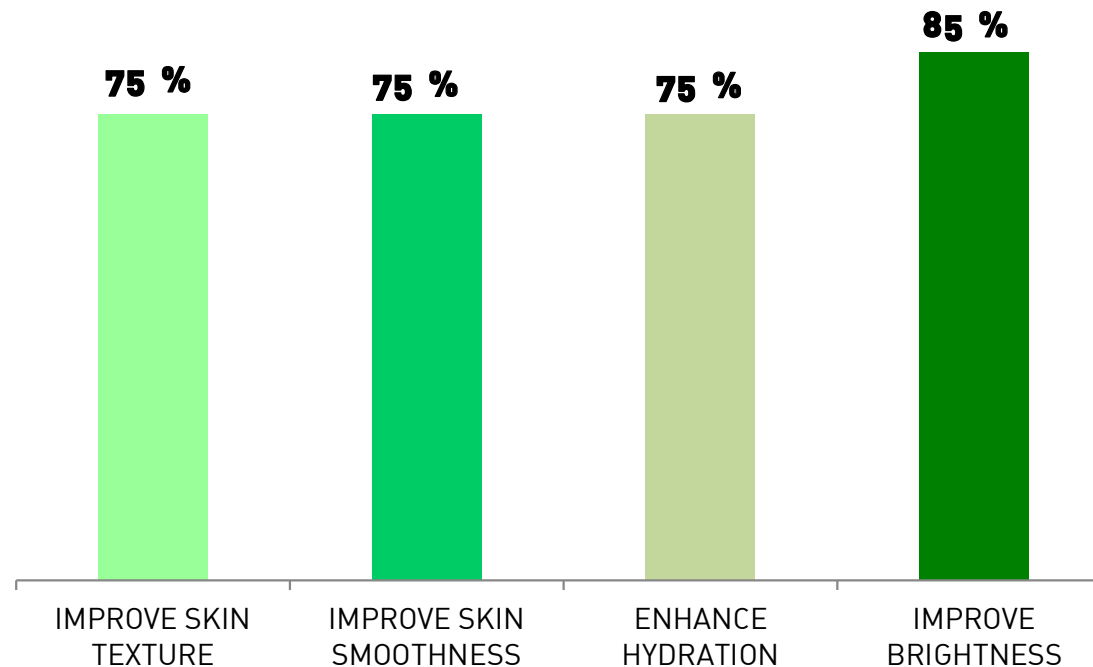
**Experiment procedure:**

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® w3 at 3% twice a day, morning and evening, during 30 days. Volunteers answered a questionnaire using a scale from 0 to 5, where 0 strongly disagrees and 5 strongly agrees to evaluate stinging, irritation, dryness and general improvement of the treatment at different days during the study.

# SELF ASSESSMENT USE TEST

*in vivo*  
20 volunteers  
inflamed skin condition

Volunteers rated **Bicoalgae® ω3** cream sensorial impact in their skin



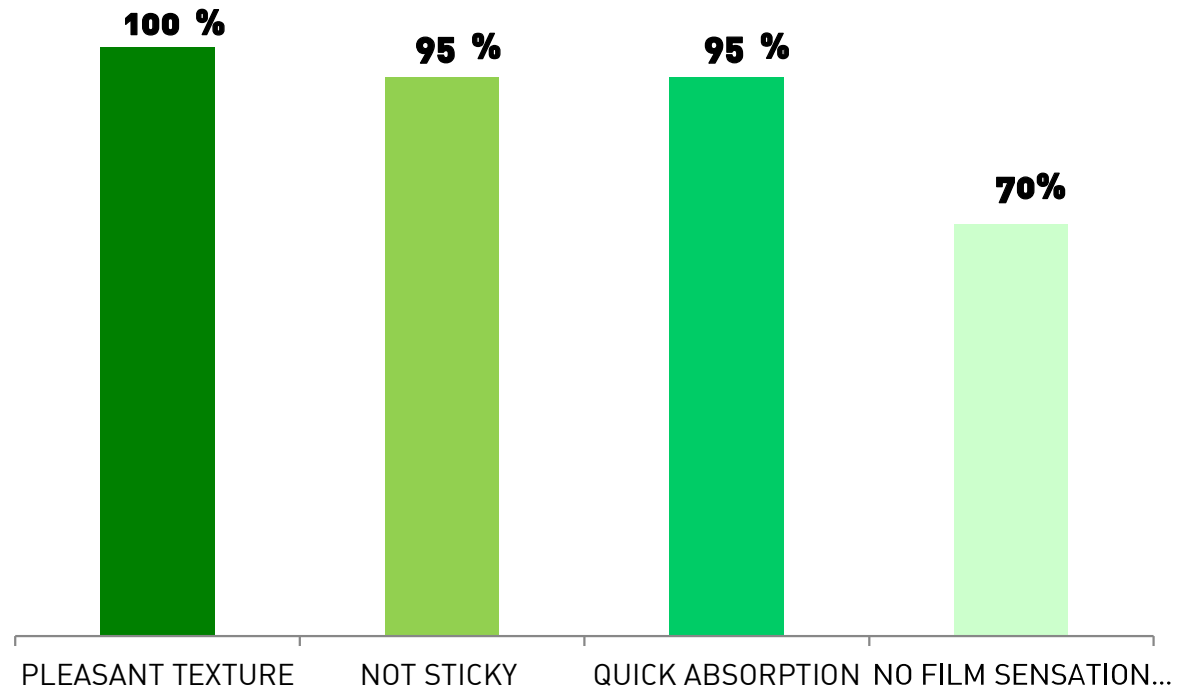
#### **Experiment procedure:**

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. Weekly volunteers answered a questionnaire to evaluate the sensorial impact of the formula in their skin.

# PRODUCT COSMETICITY USE TEST

*in vivo*  
20 volunteers  
inflamed skin condition

Volunteers also evaluated **Bicoalgae®** ω3 cream cosmeticity.



#### **Experiment procedure:**

Twenty (20) adult volunteers, average age of 36 years old, with chronic mild to moderate inflammatory acne and/or rosacea used a cream containing Bicoalgae® ω3 at 3% twice a day, morning and evening, during 30 days. Weekly volunteers answered a questionnaire to evaluate the product cosmeticity.

# SUMMARY OF RESULTS

- ❖ **Decreases** the level of the **age-related cytokines IL-1β and IL-8** in inflamed skin models.
- ❖ **Prevents the generation of ROS induced by blue light.**
- ❖ The incorporation of the MO extracts in **Bicoalgae® ω3** **increased their efficacy in reducing ROS** in the Stratum Corneum, Epidermis and Dermis.
- ❖ The clinical study showed **35% improvement** in volunteers general inflammatory condition in 30 days. The use of BO cream **reduced TEWL, hyperpigmented lesions, surface bacteria, pores and skin red and sun spots.**
- ❖ Volunteers self assessment showed good perception in the **improvements of stinging, redness, dryness, skin texture, smoothness, brightness and hydration.** Volunteers also approved product cosmeticity and sensoriality.

**BICOALGAE® ω3** is a SUPER SKIN FOOD that works **proving microalgae EPA and DHA rich extracts deep in the skin**