Active Concepts
Tomorrow's vision... *Today*!®

ACB Wakame Bioferment Advanced

wakame cells
Detoxify mitochondria
powerful Antioxidant
nutrients + moisture + anti-aging
Collagen find a Solution! Oxygen
cellular longevity

Code Number: 20024

INCI Name: Undaria Pinnatifida Cell Culture Extract
INCI Status: Conforms
REACH Status: Complies
CAS Number: N/A
EINECS Number: N/A

Origin: Botanical
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation
Additives:
Preservatives: None
Antioxidants: None
Other additives: None
Solvents Used: Water
Appearance: Clear to Slightly Hazy, Yellow to Amber Liquid
Soluble/ Miscible: Water Soluble
Ecological Information: 100% Biodegradability
Microbial Count: <100 opg, No Pathogens

Suggested Use Levels: 0.5- 2.0%
Suggested Applications: Anti-Aging, Antioxidant

BACKGROUND

Stop band-aiding the problem and find a solution! Skin loses its luminescence over time and begins to have a dry, dull appearance due to the amount of toxins we encounter every day. It is critical that we provide the skin with nutrients, moisture and a way to detoxify. **ACB Wakame Bioferment Advanced** can efficiently detoxify the skin by targeting internal pollution, where the problem starts. Developed from cultured wakame cells, **ACB Wakame Bioferment Advanced** is the solution to cellular longevity. This product targets the mitochondria, which has been shown to contribute to the longevity of cells and minimize damage at a cellular level. **ACB Wakame Bioferment Advanced** can promote longevity while also providing powerful antioxidant benefits to personal care and cosmetic formulations.

SCIENCE

Mitochondria are understood to be the cause of aging. This theory of aging suggests that pollutants are formed as a byproduct of oxidative metabolism cause the damage that leads to aging. However, the majority of anti-aging ingredients don't address this issue. These products cover up the problem by using antioxidants to limit extrinsic oxidative damage or film formers to tighten the skin to mask signs of previous damage. This needs to change, due to the concentration and proximity of mitochondrial pollutants to DNA, which are more of a threat than external pollutants. In simplest terms, our mitochondria produce energy by moving proteins across the mitochondrial inner membrane. In essence, function like machines that use fuel to generate energy. In addition to generating energy, they also generate unwanted pollution.

Oxygen is involved in the electron transport and acts as the final electron acceptor. Research has shown that there are electron "leaks" that occur within the mitochondrial inner membrane that reduces mitochondrial efficiency. The leakages limit ATP synthesis, while damaging the mitochondria and the surrounding cellular environment.

Benefits of **AC Wakame Bioferment Advanced**:

- Anti-Aging
- Antioxidant
- Increase in Collagen Synthesis

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The leaks also leave cells more susceptible to destructive reactive oxygen species that were not coupled with protons at the end of the electron transport cycle. The combination of the mitochondrial leaks and the decrease in ATP synthesis not only limits efficiency, but also acts as pollutants that severely compromise the health and longevity of our cells. This is now understood to be the primary factor responsible for aging.

Much work has been done to show that live cell derivatives can be used to increase oxygen consumption to stimulate cellular respiration, proliferation and wound healing. However, researchers failed to address the issue of mitochondrial leakage that is directly related to aging and cellular longevity. One of the largest growing areas of anti-aging research today involves this concept of mitochondrial anti-aging.

Traditionially, live cell derivatives are produced by exposing cells to an environmental stress, such as UV irradiation. As referenced in the original patents by George Sperti, we have refined this method by also supplementing the growth media used to culture the wakame cells. We extract the cells from *Undaria pinnatifida* and grow them in media supplemented with Lactobacillus, which causes the stress that allows for prime production of secondary metabolites. These adaptations have allowed us to produe a unique cell derivative that is capable of optimizing electron transport to reduce mitochondrial leakage. This leakage results in the release of damaging free radicals and ROS that ultmately contribute to mitochondrial damage and aging.

Our findings illustrate that **ACB Wakame Bioferment Advanced** increase oxygen consumption, while simultaneously reducing the formation of ROS to increase overall mitochondrial efficiency. A DNA MicroArray Assay on cultured human fibroblasts confirm that **ACB Wakame Bioferment Advanced** is capable of enhancing oxidative phosphorylation, while limiting mitochondrial stress.

BENEFITS

ACB Wakame Bioferment Advanced can increase oxygen consumption to help cells consume oxygen more efficiently thus making less oxygen available to form ROS. With cells working more efficiently, less toxins are produced, thus providing detoxification from within. Our research suggests that this product can deliver potent anti-aging benefits to minimize damage at the cellular level, where aging begins.

EFFICACY

In-vitro assays were conducted to assess the potential that **ACB Wakame Bioferment Advanced** has to induce changes in cellular oxygen consumption after 15 minutes and 24 hours of treatment. The results demonstrate that 1.0% **ACB Wakame Bioferment Advanced** is capable of increasing oxygen consumption by 26.8% after 15 minutes and 0.1% **ACB Wakame Bioferment Advanced** is capale of increasing oxygen consumption by 31% after 24 hours compared to the untreated control.

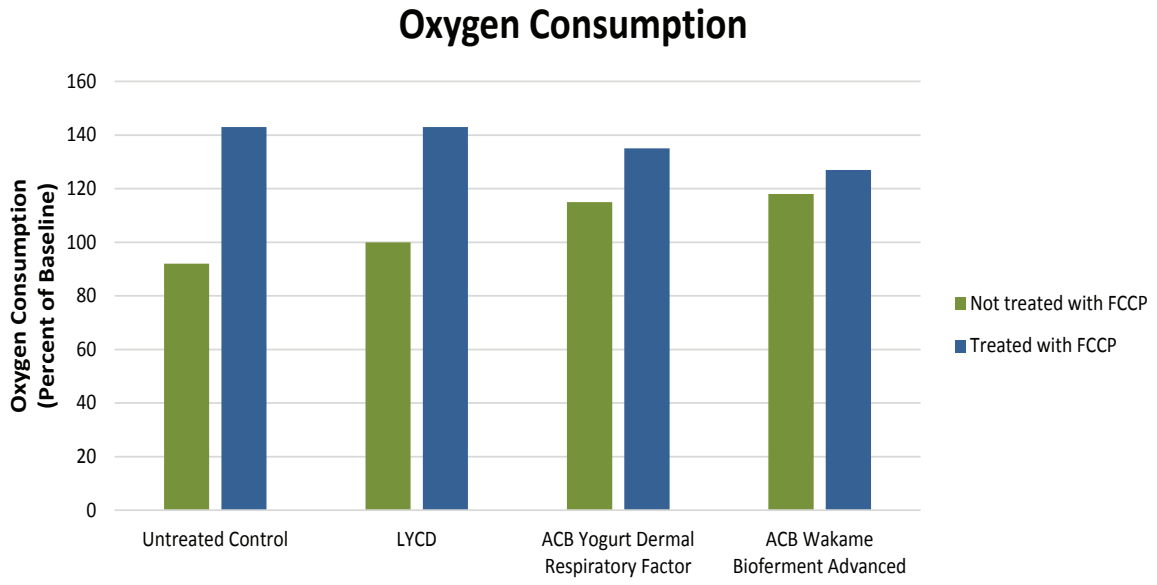

Figure 1. Results of the 24 hour Oxygen Consumption Assay.

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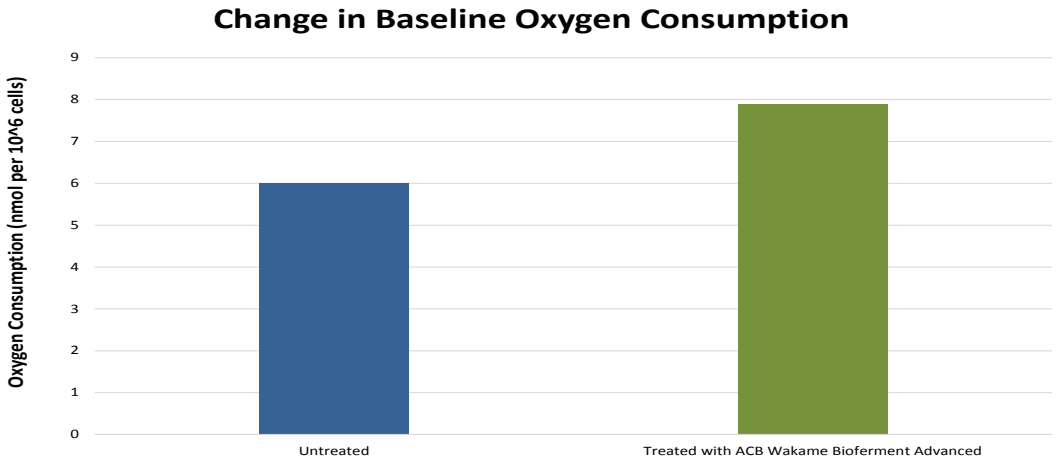

Figure 2. Mean results of the 15 minute Oxygen Consumption Assay.

An *in-vitro* ROS Formation Assay procedure was done to indicate the impact that **ACB Wakame Bioferment Advanced** has on intracellular reactive oxygen species (ROS) formation. Dermal fibroblasts were loaded with DCF and then treated with various test materials for 1 hour. At 0 and 1 hour intracellular fluorescence was measured to determine the amount of ROS formation associated with each test material. Over the course of the incubation, treatment with ascorbic acid was observed to significantly reduce ROS formation while treatment with H_2O_2 was observed to significantly increase ROS formation. Treatment with **ACB Wakame Bioferment Advanced** was observed to reduce ROS formation compared to the untreated control.

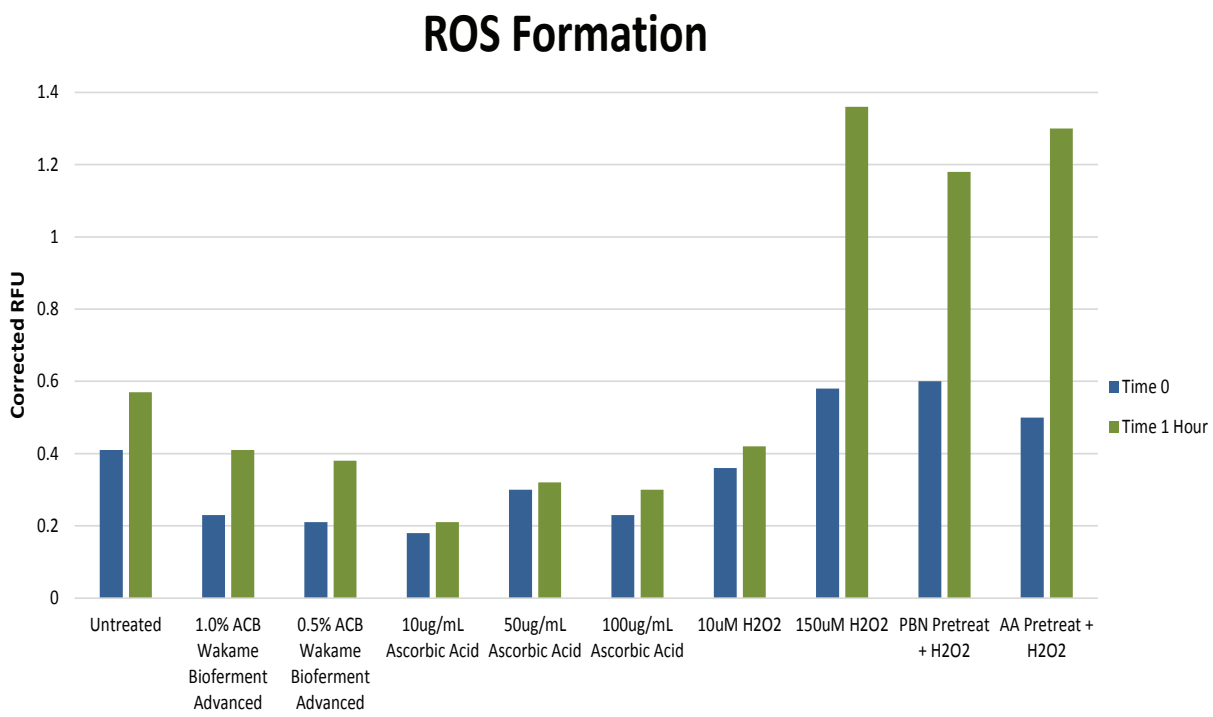

Figure 3. ROS Formation results.

An ORAC assay was also conducted on **ACB Wakame Bioferment Advanced** showing its capability of reducing the presence of Reactive Oxygen Species compared with Trolox, the vitamin E analog used as the control. By looking at the ORAC results, **ACB Wakame Bioferment Advanced** provides strong antioxidant properties at the low concentration of 0.05% and 0.1%.

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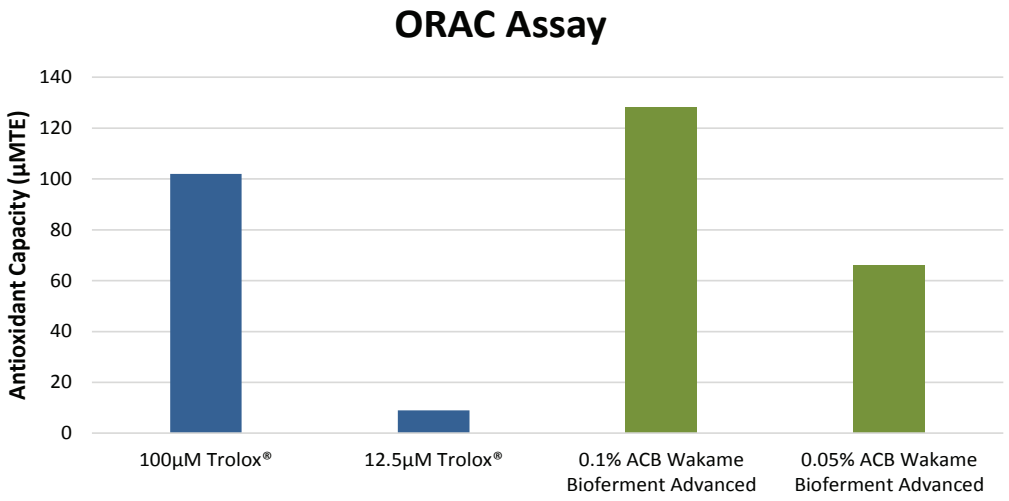

Figure 4. Antioxidant capacity of test material in comparison to Trolox.

Nitric Oxide (NO) has received attention in the past 15 years, due to this harmful effects on the environment. It then started to be studied for its harmful biological effects. NO can form reactive intermediates that can have an effect on protein formation and trigger damange that in turn can lead to aging. Effects of reactive oxygen species (ROS) and reactive nitrogen species can affect lipids, proteins and DNA and contribute to the aging process[1]. An *in-vitro* assay was conducted to assess the impact that **ACB Wakame Bioferment Advanced** has on intracellular NO formation.

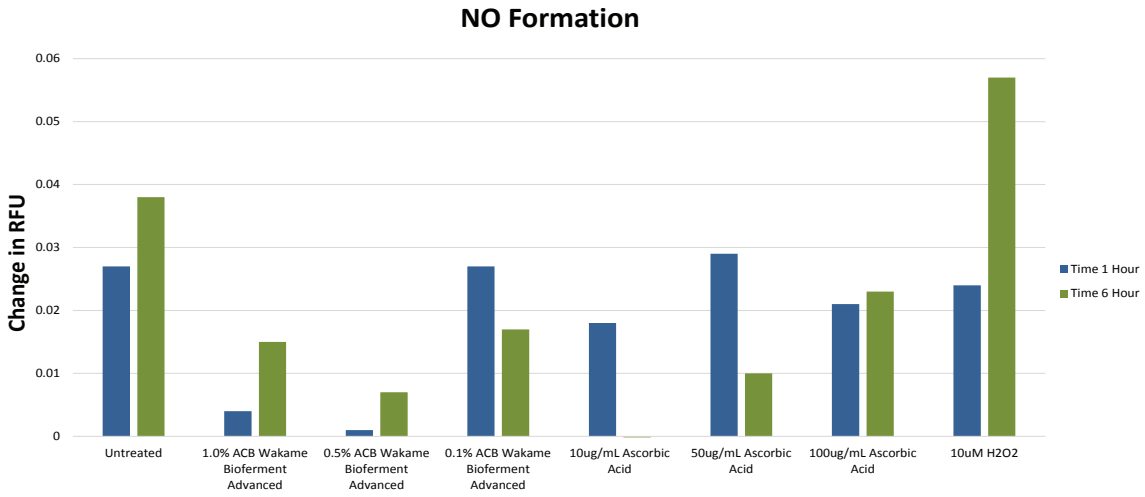

Figure 5. NO Formation shown in realative fluorescent units (RFU).

In addition to the above efficacy data, we also wanted to assess the effects that **ACB Wakame Bioferment Advanced** has on collagen synthesis. Fibroblasts are the main source of the extracellular matrix peptides, including the structural proteins, collagen and elastin. Procollagen is a large peptide synthesized by fibroblasts in the dermal layer of the skin and is the precursor for collagen. As the peptide is processed to form a mature collagen protein, the propeptide portion is cleaved off (type I C-peptide). Both the mature collagen protein and the type I C-peptide fragment are then released into the extracellular environment. As collagen is synthesized, the type I C-peptide fragment accumulates into the tissue culture medium. Since there is a 1:1 stoichiometric ratio between the two parts of the procollagen peptide, assaying for type I C-peptide reflects the amount of collagen synthesized. Type 1 C-peptide was assayed via an ELISA based method.

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The ELISA indicates that **ACB Wakame Bioferment Advanced** is capable of increasing the expression of procollagen type 1-C peptide in the fibroblast cell culture model. **ACB Wakame Bioferment Advanced** was shown to be comparable to Magnesium Ascorbyl Phosphate in increasing the synthesis of Collagen Type 1. These findings suggest that **ACB Wakame Bioferment Advanced** is useful in cosmetic preparations to stimulate

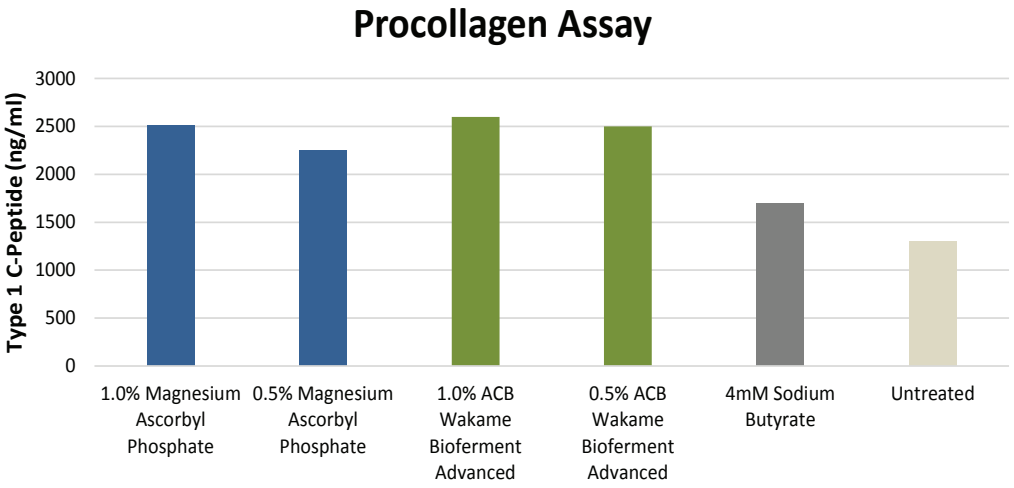

Figure 6. Comparison of the effects on procollagen levels following treatment.

Collagen I is a major component of the dermis and provides structural support and elasticity to the skin. *In-vitro* testing was performed to determine if **ACB Wakame Bioferment Advanced** is capable of inducing collagen I synthesis. Three varying concentrations of **ACB Wakame Bioferment Advanced** were compared to positive and negative controls and the results were determined via an ELISA assay. These findings indicate that **ACB Wakame Bioferment Advanced** is capable of increasing collagen I synthesis in a dose dependent manor.

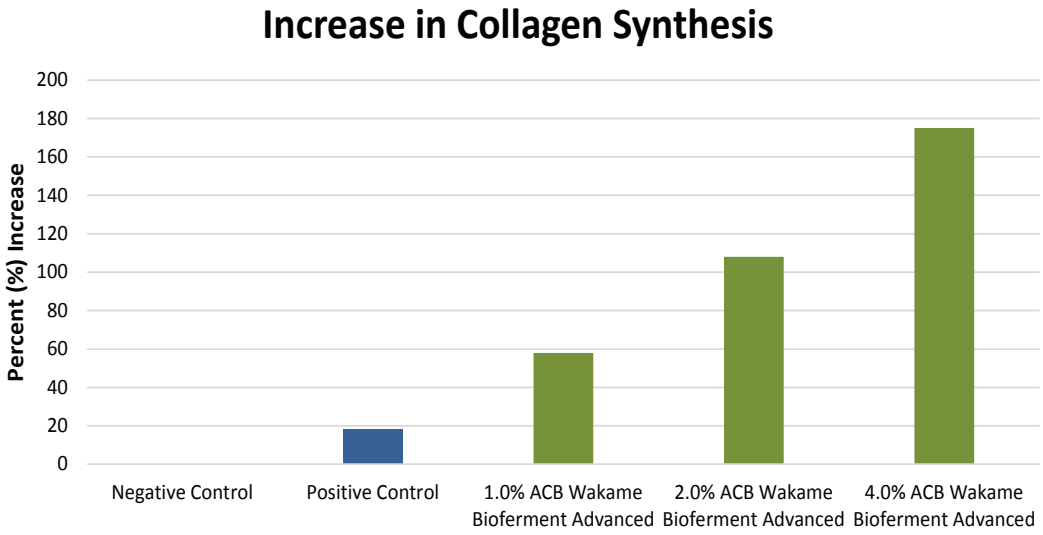

Figure 7. Comparison for percent increase in collagen I synthesis.

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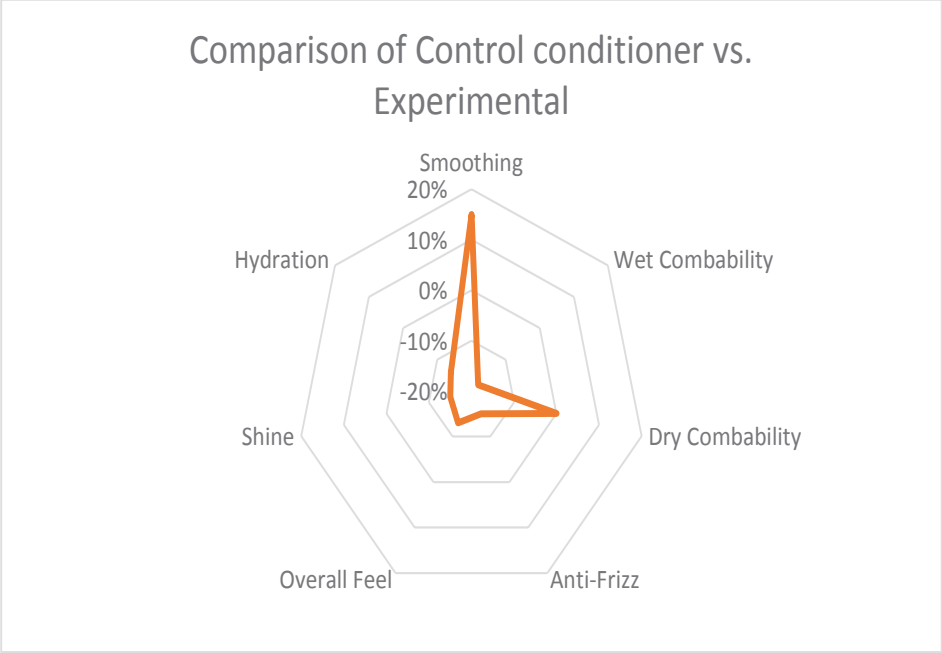

Figure 8. Hair Assessment results for sensory characteristics.

Additionally, a half head study was conducted to determine the comparison of a control conditioner vs. **2.0% ACB Wakame Bioferment Advanced** in the experimental conditioner. The results of the assessment indicate that when used in a conditioner **ACB Wakame Bioferment Advanced** is capable of improving smoothing more than the control conditioner. Furthermore, the subjects reported a significant increase in smoothness and overall apperance of the hair.

References:

1. Drew B, Leeuwenburgh C. Aging and the Role of Reactive Nitrogen Species. Biochemistry of Aging Lab - New York Academy of Sciences. 2002; 959:66-91.

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