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REVIEW ARTICLE

The future of enzymes in cosmetics

L. M. LODS*, C. DRES, C. JOHNSON, D. B. SCHOLZ and G. J. BROOKS

Brooks Industries Inc., 70 Tyler Place, South Plainfield, NJ 07080, U.S.A.

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Synopsis

The skin employs a host of protective mechanisms to defend itself against the ravages of the environment. One of the most widely studied protective mechanisms is the system of free radical scavengers. Free radical scavengers help to protect the skin by neutralizing dangerous substances that can be generated by sun exposure and pollution.

Two such protective substances – superoxide dismutase and peroxidase – were examined for their ability to reduce UV-induced erythema. The ability to reduce erythema is a measure of anti-irritant capabilities, which can also be thought of as free radical scavenging ability. There has been some research that shows that superoxide dismutase (SOD) and peroxidase work synergistically. The action of SOD, which neutralizes the superoxide anion, can sometimes produce hydrogen peroxide, which can have a detrimental effect on the lipid barriers of the skin. When peroxidase is present, it can work to neutralize the hydrogen peroxide, thus giving a full spectrum of free radical protection.

The present study employs a superoxide dismutase extracted from yeast. The peroxidase is found in an aqueous extract of fennel (*Foeniculum vulgare*). Minimal erythemal dose (MED) was determined on the panellists. Test compounds were then applied and then they were exposed to solar simulators in doses equivalent to their respective MEDs. Development of erythema was then measured via chromameter, and reduction in the development of redness was determined.

Résumé

La peau utilise un ensemble de mécanismes protecteurs pour se défendre contre les attaques de l'environnement. Un des mécanismes protecteurs les plus étudiés est le système de pièges à radicaux libres. Les pièges à radicaux libres aident à protéger la peau en neutralisant les substances dangereuses qui peuvent être produites par l'exposition au soleil et la pollution.

On a étudié chez deux de ces substances protectrices – la superoxyde dismutase et la peroxydase – la capacité à réduire l'érythème provoqué par les UV. La capacité à réduire l'érythème est une mesure des capacités apaisantes, qui peuvent aussi être considérées comme une capacité à piéger les radicaux libres. Certaines recherches montrent que la superoxyde dismutase (SOD) et la peroxydase fonctionnent en synergie. L'action de la SOD, qui neutralise l'anion superoxyde, peut certaines fois générer du peroxyde d'hydrogène, qui peut avoir un effet nocif sur les barrières lipidiques de la peau. Lorsque la peroxydase est présente, elle peut agir pour neutraliser le peroxyde d'hydrogène, apportant ainsi un spectre total de protection contre les radicaux libres.

La présente étude utilise une superoxyde dismutase extraite de levure. La peroxydase provient d'un extrait aqueux de fenouil (*Foeniculum vulgare*). On détermine la dose minimale donnant un érythème (MED) sur les sujets. Les composés testés sont ensuite appliqués puis on les expose à des simulateurs solaires à des doses équivalentes à leurs MED respectives. Le développement de l'érythème est ensuite mesuré avec un chromomètre, et on détermine la réduction du développement de la rougeur.

*To whom correspondence should be addressed. Fax: +1 908 561 9174

Introduction

We live in a world where there is danger from many sources – from sunlight, natural and man-made pollutants, and from oxygen, the element that keeps us alive. We are beginning to understand the mechanisms by which these dangers attack us. The skin, our largest organ, is the first line of defence separating us from the outside world. Oxygen, which is essential for most cellular processes, is highly toxic. However, the cells have a multitude of natural protective systems to ensure that the destructive events, which could be unleashed by oxygen, are under very tight controls. In many ways the ageing process can be defined as being 'oxidized' away.

The cells employ a multitude of protective mechanisms to defend themselves against oxidative stress. All the cellular components are susceptible to damage by free radicals, which diminish their efficient functioning. One of the most widely studied protective systems is that of the free radical scavenging enzymes – superoxide dismutase (SOD) and peroxidases (catalase, glutathione peroxidases, lactoperoxidases, etc.). In this study the ability of these enzymes to reduce UV-induced erythema when topically applied to the skin's surface has been examined. This can be thought of as a practical way of demonstrating the significance of their free radical scavenging ability from a cosmetic formulation. Much of the published research has shown that SOD and peroxidases work together. SOD neutralizes the destructive superoxide anion often to produce hydrogen peroxide, which is then neutralized by the peroxidase, thus giving a full spectrum of free radical protection.

Enzymes have recently been of great interest to the cosmetic scientist, as they have consumer appeal, resulting from their use in household products for improved performance. Can this consumer appeal be directed to the cosmetic industry, providing enhanced performance cosmetics to satisfy the ageing global population's quest for a youthful appearance?

Technically speaking, enzymes are poorly understood in the area of cosmetic product functionality. Developers and marketers are not yet fully aware of the difficulties of producing cosmetics containing safe but functional levels of enzymes. Until recently, there really were no enzymes developed for functionality, safety and stability in a cosmetic system. Now it is possible to use certain enzymes, which provide protection. They exist as natural materials and do not require non-renewable resources for their production.

Background on enzymes

Enzymes are effective cellular catalysts, which control the thousands of reactions occurring within the cell. These chemical entities were first discovered prior to 1833 by Payen and Persoz and called enzymes in 1878 by Wilhelm Kuhne. Then in 1926 it was established that they were special proteins with specific activity (Table I).

Enzymes are highly specific and complex protein catalysts that increase the rate at which reactions occur. At the temperature and pH usually present in the cells and extracellular matrices, most chemical reactions would not proceed fast enough to maintain cell viability without enzymes. Enzymes can increase the reaction rate anywhere from 100- to 1000-fold, and they are specific for the substrate that they use, hence there are no by-products and no side-reactions. Enzymes are much bigger than the substrate on which they work. A typical substrate molecule has a molecular weight of 250 and a medium size enzyme has a

Table I. Enzymes are catalysts

Responsible for coordinated chemical reactions in biological processes	Lower activation energy necessary for a reaction to occur	Not degraded by the reactions they catalyse

molecular weight of 100 000. The active site occupies only a small fraction of the enzyme molecule (Fig. 1).

Enzymes work by a shape recognition method, the substrate must form a complex with the enzyme. Two mechanisms have been proposed; the 'lock and key', and the 'induced fit', where the substrate creates its own site on a suitable portion of the enzyme (Fig. 2).

Six classes of enzymes have been identified (Table II). SOD and peroxidases belong to Class 1–oxidoreductases.

The protective enzymes

The use of enzymes in cosmetics has been advocated for many years. Proteolytic enzymes (bromelaine, papain, etc.) have been used for skin peeling and smoothing. There are

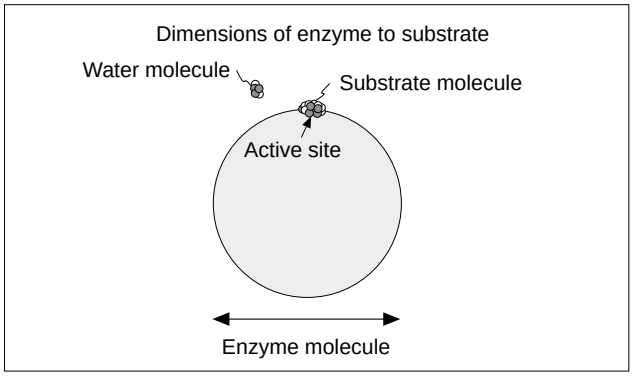

Fig. 1. Relative dimensions of the enzyme to the substrate.

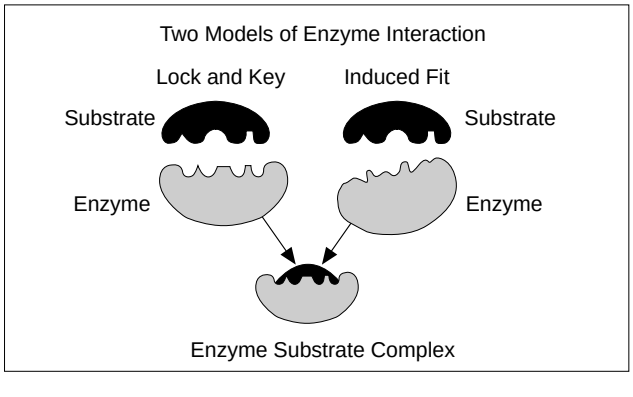

Fig. 2. How shape recognition works.

Table II. Six classes of enzymes

Class 1	Oxidoreductases	Catalyse oxidation and reduction reactions	SOD Peroxidases Glutathione Catalase
Class 2	Transferases	Transfer chemical groups from one molecule to another	
Class 3	Hydrolases	Use water to cleave a single molecule into two molecules	
Class 4	Lyases	Split molecules using a non-hydrolytic process, leaving double bonds	
Class 5	Isomerases	Change isomeric structures	
Class 6	Ligases	Create a chemical bond to join two molecules using the energy from ATP	

problems associated with the general application of proteases to the skin's surface, as the action is difficult to control and the enzyme will cause irritation. One area where the topical application of enzymes has been shown to have significant benefits is in skin protection. Enzymes exist with excellent cosmetic stability. These enzymes have the ability to capture the free radicals encountered in the environment, caused by environmental pollution, bacteria, smoke, sunlight, etc. Here the enzymes can work successfully on the skin's surface.

Superoxide dismutase

Perhaps one of the most ubiquitous protective enzymes is superoxide dismutase (SOD). Mann and Leilin first discovered it as a blue/green protein, in 1938. However, its function as an enzyme, in that it was able to remove catalytically the superoxide radical, was identified by McCord and Fridovitch in 1968. Copper–zinc-containing SODs are highly stable enzymes and thus easily isolated. They are found in all eukaryotic cells such as yeasts, plants and animals. All the CuZnSODs isolated so far from eukaryotic cells contain two protein sub-units and have molecular weights of around 32 000. A β-barrel structure has been elucidated by X-ray crystallography. Each sub-unit is composed primarily of eight antiparallel strands of β-pleated sheet structure that forms a flattened cylinder, plus three external loops [1] (Fig. 3).

CuZnSOD works by dismutation, a process by which a dangerous highly reactive oxygen free radical is converted to a less reactive form. During cellular respiration it is important that the oxygen molecule be reduced to two water molecules by accepting four electrons. If the oxygen is only partially reduced, the product is the superoxide radical (Figs 4 and 5). Superoxide radicals are extremely toxic to cells as they will attack unsaturated fatty acids in the membrane lipids, thus damaging membrane structure and causing cell injury. The copper ions appear to function in the dismutation reaction by undergoing alternate oxidation and reduction. The zinc ions provide structure and stability to the enzyme [1].

In addition to the CuZnSODs, different types of SOD have been described, including bacterial and mitochondrial types based on manganese and iron (Ferrienzyme).

Fridovitch proposed that the superoxide radical is a major factor in oxygen toxicity and that SOD is an essential defence against it. Since then, more papers have been published

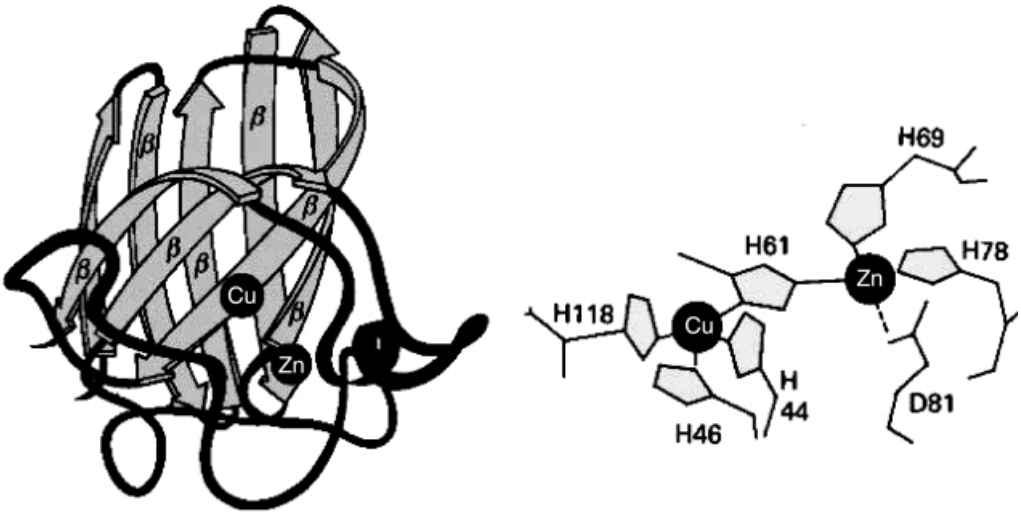

Fig. 3. Structure of CuZnSOD.

$$O_2 \xrightarrow{e^-} O_2^{-\cdot}$$

Fig. 4. Generation of superoxide radical.

$$O_2^{-\cdot} + O_2^{-\cdot} \xrightarrow{2\,H^+} H_2O_2 + O_2$$

Fig. 5. Effect of superoxide dismutase.

on SOD than on any other enzyme. In the 1980s, SOD was described in the popular press as a 'Lazarus drug', when it was advocated as treatment for heart attack victims. Studies have shown that, as one ages, the levels of SOD in the tissues diminish. A recent article in *Science* showed that when fruitflies had more SOD in combination with catalase (to mop up the by-product of dismutation, hydrogen peroxide), that they lived 33% longer and enjoyed their lives better, as measured by their ability to walk. The fruitflies' proteins were protected from oxidation [2].

Based on the results of research into the effectiveness of SOD, an excellent case can be made for using it in all cosmetic formulations, to promote, at least, younger looking skin. In fact, L'Oreal quickly recognized the importance of this enzyme and obtained a patent for a marine source of SOD in 1973 for its general use in cosmetics (EU patent no. 2 287 899).

A yeast-derived CuZnSOD was developed by Brooks Industries back in 1987 (Biocell SOD–YeastCuZnSOD–powder with $\approx$600 units SOD activity). This was made from yeast, which had been fermented in a copper/zinc-rich nutrient media. The CuZnSOD was found in specific protein fractions isolated from the yeast cells, which were purified to provide a stabilized level of activity. Tests have shown that this form of 'yeast protein

bonded SOD' had excellent stability at 45 °C in aqueous solution, when compared to pure forms of SOD, which showed very poor stability. Extensive safety testing on YeastCuZnSOD indicated that it is non-irritating and non-sensitizing in both the powdered form and as a 1% active YeastCuZnSOD liposome (Brookosome SOD–INCI name: phospholipids and superoxide dismutase–liposome with ≈ 6 units of SOD activity). There have been reports of irritation using the pure form of SOD. It is believed that the yeast matrix proteins help to stabilize the enzyme under typical cosmetic use conditions, and play a critical protective role in reducing irritation potential.

Recently, tests have been carried out on the YeastCuZnSOD in a liposomal form, which have shown that it has excellent *in vitro* antioxidant activity, better in fact than most other popular cosmetic antioxidants, such as tocopherol (Vitamin E) and the polyphenols from green tea.

1. Lactate dehydrogenase activity (LDH)

This is an *in vitro* test, carried out by Stephens & Associates, Carlton, TX, U.S.A., using a human skin equivalent to measure the activity of LDH, the enzyme responsible for reduction of NADH by pyruvate to NAD+ and lactate. This activity is dramatically suppressed by UV light. After irradiation with 3.0 MEDs, the YeastCuZnSOD allowed the most units of LDH to remain intact in the cells, allowing normal cell catabolism to occur (Fig. 6).

2. Prostaglandin E2 synthesis (PGE2)

This was an *in vitro* test, carried out by Stephens & Associates, using a human skin equivalent to measure the activity of PGE2, a lipid soluble essential fatty acid derivative

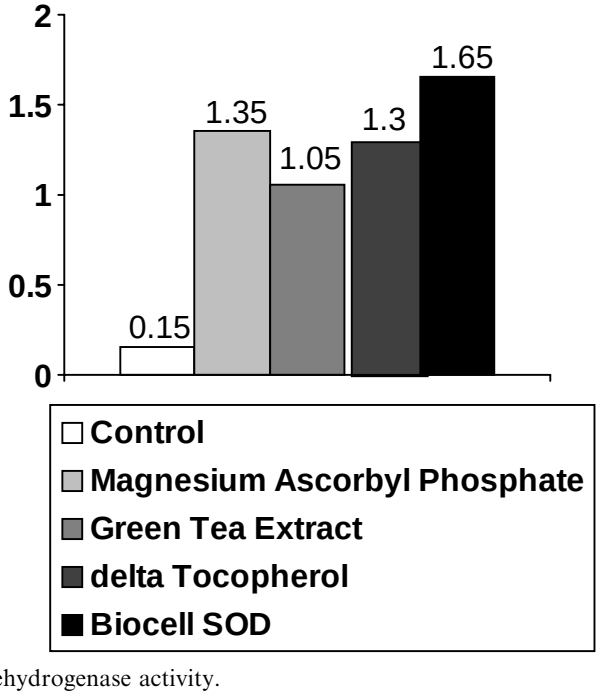

Fig. 6. Lactate dehydrogenase activity.

(contains a five ring group), which mediates the inflammatory response which results in erythema. Irradiation using 3.0 MEDs causes significant production of PGE2. The YeastCuZnSOD was found to inhibit the synthesis of PGE2, decreasing visible erythema levels (Fig. 7).

3. Inhibition of erythema using skin reflectance spectrophotometry

This was an *in vivo* test run on two different formulations, a gel and an emulsion. Visible light was used (400–700 nm). The YeastCuZnSOD worked better from the emulsion than the gel. It was better than tocopherol and ascorbyl palmitate at preventing erythema (Fig. 8).

In summary, it is believed that this enzyme has an important role to play in cosmetic formulations, as it has the ability to protect against the damaging effects of UV light by providing enhanced protection by helping to prevent photoaging. Additionally, it will help to protect against the damaging effects of environmental pollutants. The protein component will provide skin smoothing and moisturization properties and provide a natural affinity to the skin, which will help to resist removal through washing, swimming, etc.

Peroxidase rich fennel seed extract

Hydrogen peroxide is formed in many aerobic cells as a result of the dismutation of free radical oxygen. In addition, there are also several enzymes that produce hydrogen peroxide directly (e.g. glycollate oxidase, D-amino acid oxidase, etc.). Hydrogen peroxide can be produced during photosynthesis in plants, and as a result of photochemical reactions in seawater. It has also been measured at low levels in rain and river water. Hydrogen peroxide is a weak oxidizing agent and can inhibit glycolysis. It can cross cell membranes and once inside the cell it can react with ferrous ions to form the highly dangerous hydroxyl radical, which is probably the origin of many of the toxic effects attributed to it. It is also possible that UV light can cause homolytic fission of hydrogen

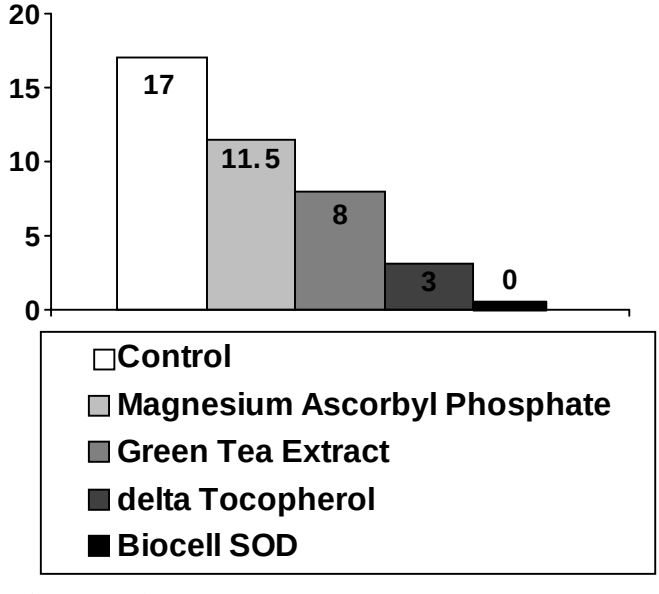

Fig. 7. Protaglandin E2 synthesis.

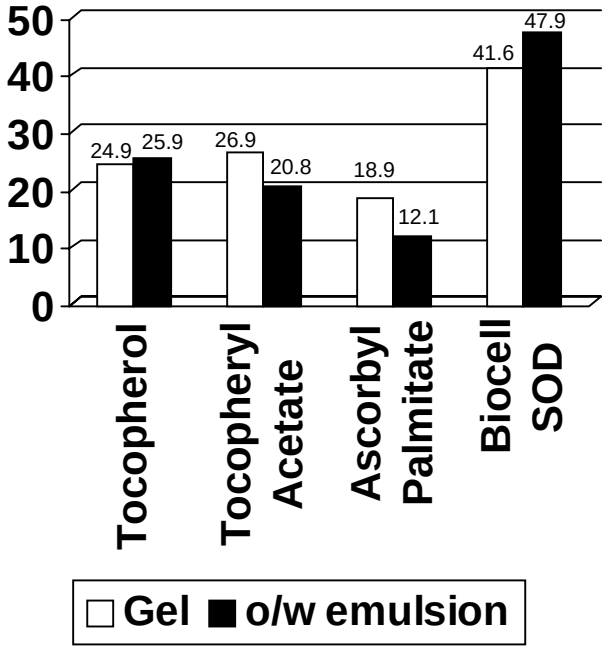

Fig. 8. Inhibition of erythema–*in vivo*.

peroxide, generating two hydroxyl radicals, which can then attack DNA causing single strand breaks and DNA protein cross-links [1].

Two types of enzymes have been identified in the cells to remove hydrogen peroxide; these are the peroxidases and the catalases.

Plants often contain haem-containing peroxidases, these are frequenty considered 'non-specific' and are capable of acting on a wide variety of substrates, including hydrogen peroxide. Similar non-specific peroxidases have been found in animals, such as the lactoperoxidases (milk and saliva–oxidize the thiocyanate ion), myeloperoxidase (contributes to phagocytosis) and thyroid peroxidase (probably oxidizes the iodine ion). The most studied non-specific peroxidase is horseradish peroxidase, obtained from the roots of the horseradish plant (*Amoracia*). The iron in the haem ring of the 'resting' peroxidase is in the ferric ion form [1].

Plant and animal tissues generally contain their catalases in subcellular organelles known as peroxisomes. Most purified catalases have been shown to consist of four protein sub-units, each of which contain ferric ion bound to its active site. Each sub-unit usually contains one molecule of NADPH bound to it, which helps to stabilize the enzyme [1].

Peroxidase activity has been observed in roots and stem tissues of a wide variety of plants. The fennel seed extract is made by a gentle aqueous extraction process to ensure that enzymes are not broken down. It complies with the CLSC (Japan) monograph for fennel extract and has a measurable peroxidase activity which has been standardized at 200 units/ml. This fennel seed extract is an excellent cosmetic material, being extremely low in odour and a very pale yellow/green liquid. A combination of *in vitro* toxicity testing and human tests has shown that this material is practically non-irritating and non-sensitizing.

The reaction mechanism by which the hydrogen peroxide is reduced to water is not fully worked out, but can be summarized as shown below:

$$\text{peroxidase-Fe(III)} + H_2O_2\ k1 \rightarrow \text{compound 1}$$
$$\text{compound 1} + H_2O_2\ k2 \rightarrow \text{peroxidase-Fe(III)} + H_2O + O_2.$$

k1 second order rate constant
k2 second order rate constant

The formation of compound I leads to characteristic changes in the absorption spectrum of the molecule. The iron is oxidized to Fe(V) and two molecules of hydrogen peroxide are needed to impact on each active enzyme site. Compound I will also oxidize alcohols to aldehydes (for methanol and ethanol), formic acid to carbon dioxide and nitrite ion to the nitrate ion [1].

In vitro and *in vivo* efficacy tests have recently been run. As far as the ability of the fennel extract to protect against lipid peroxidation was concerned, it showed much better protection than tocopherol. It is believed that the reason for this is that the enzyme keeps on working as long as there is substrate, whereas tocopherol is 'used up' and needs to be regenerated. Obviously, that mechanism for regeneration exists inside the cell, however, on the skin's surface it probably does not.

1. Reduction in lipid peroxidation

The tests (Peter Pugliese & Associates, Bernville, PA, U.S.A.) were run using 2% of the extract (four units of peroxidase activity), 2% of the liposome (made by adding 10% phospholipids to the fennel extract and microfluidizing to 120 nm particle size) and 5% tocopherol. The test for lipid peroxidation was based on using methyl linoleate treated with 2,2′-Azobis (aminopropane) dihydrochloride and an oxygen electrode was used to measure oxygen consumption as a measure of protection over a 24-min period.

The results are interesting in that the liposomal formula gave immediate complete protection, but lost activity with time, whereas the extract itself gave a steady result (Fig. 9).

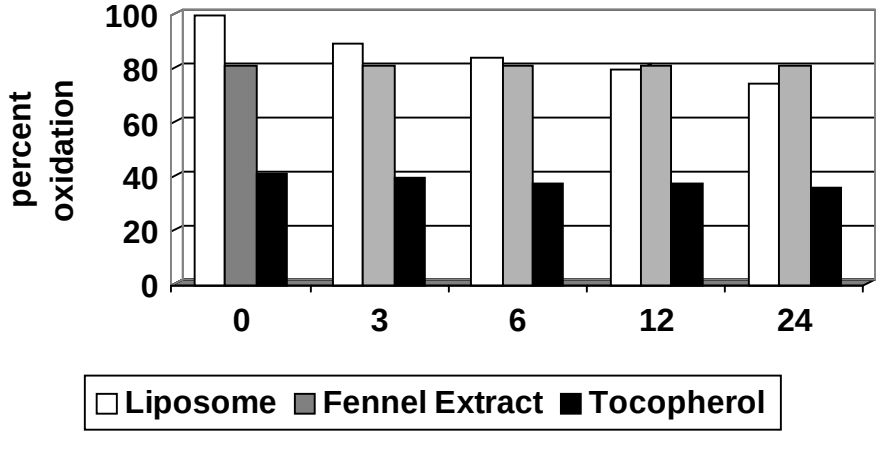

Fig. 9. Reduction in lipid peroxidation.

2. Reduction in erythema–in vivo

This test (AMA Laboratories, New City, NY, U.S.A.) was carried out from a formulation on a six-person panel, using the FDA method for evaluating sunscreens. The addition of 2% fennel extract (four units of peroxidase activity) gave a significant improvement in protection (Fig. 10).

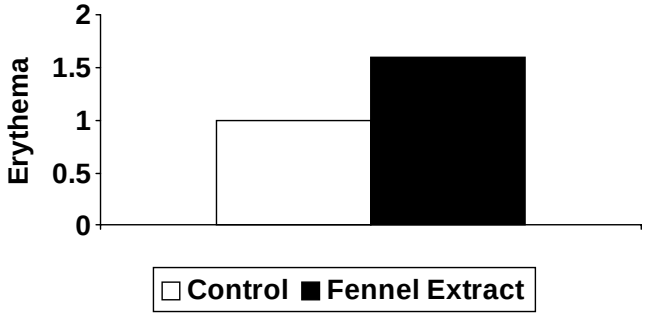

Fig. 10. Reduction in erythema.

Conclusion

As humans age, the enzymes critical to protecting the body from oxidative stress, SOD and catalase, become less and less. Hence as people get older, they age faster and the body's ability to protect itself is reduced. Can life span be extended and youthful appearance maintained? The recent work which showed that higher levels of both SOD and catalase in fruitflies extended the quality and length of their lives should certainly reveal two very important facts: (1) humans live in a hostile oxygen-rich world where the body is slowly 'oxidized' to death; and (2) to achieve sufficient protection is essential and very difficult.

As the world's population ages, help can be given to supplement natural defence systems by helping to protect the skin from the ravages of time and environmental attack by formulating cosmetics containing the safe protective enzymes – SOD and peroxidases.

This paper has discussed at length the cosmetic attributes of just two enzymes, which can be used in cosmetic formulations, where the safety, functionality, stability and formulation issues have been addressed. It has been shown that in this instance the word 'enzyme' can be meaningful to the consumer. There is an ideal opportunity to do more to promote healthy, youthful looking skin by using protective enzymes in cosmetic formulations. Consequently, as the educated ageing consumer of the developed countries becomes acutely aware of the many hostile aspects of life in their environment, their awareness of protective enzymes will be connected to positive results coupled with longer happier lives.

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