

Anti-glycation skin care – a promising approach for young, mature and diabetic skin

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Abstract

Sugar molecules are the most important source of energy in cells. However, sugars are highly reactive compounds and they participate in many other processes that are not directly related to energy production. One of these is the generation of advanced glycation end products (AGEs), which seriously affect the youthfulness of our skin starting even at birth. Due to an elevated blood glucose level, diabetics in particular suffer from AGEs coupled with proteins. These cause the skin to become thin, inelastic and leave an uneven complexion and a yellowish, sallow look. Indeed, once formed, AGEs are hard to remove, so an important strategy for minimising the burden of AGE on the skin is the prevention of their formation. The most powerful detoxifying mechanism for modified proteins in the cells is provided by the proteasome. Here we showcase an active cosmetic ingredient consisting of extracts of olive leaves, jujube seeds and levan that goes by the name of PROTEOLEA®. It is specifically designed to reduce the accumulation of AGEs by preventing their formation as it combats ROS and by removing damaged proteins through activation of the proteasome. It contains powerful antioxidants derived from jujube and is standardised to the iridoid oleuropein, which is recognised as a potent enhancer of the proteasome activity.

Application of the active cosmetic ingredient showed significant AGE reduction compared to the placebo when assessed by an AGE reader, a non-invasive diagnostic instrument to determine fluorescent AGEs in the skin. A controlled user study of normal and diabetic participants confirmed a highly significant improvement in the various skin parameters associated with high AGE levels, which was more pronounced in the diabetics group as expected.

We conclude that by stimulating the proteasome's capacity to remove waste proteins and by preventing the formation of early glycation products through the elimination of ROS, the active ingredient serves as a safeguard against glycation.

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Introduction

Sugar – not just a source of energy – Advanced glycation end products (AGEs)

Sugar molecules are the most important source of energy in the cells. However, sugars are very reactive compounds so it is not surprising that they also participate in many other processes that are not directly related to energy production. While sugar molecules are well controlled by enzymatic reactions within the cells, on the outside, namely in the extracellular matrix, numerous unwanted chemical reactions of reducing sugars with proteins or lipids may occur. Such unwanted reactions are called glycation. Over the long term, all proteins present in the body will at some point react with sugars. The consequence is a complex cascade of subsequent reactions in which coloured, fluorescent and crosslinking reaction products are generated. These are called advanced glycation end products (AGEs)^[1]. In recent years, these have been attracting increasing attention in the fields of medicine and cosmetics. Due to the high concentration of collagen, the most affected area of the skin is the dermis.

How do AGEs affect the skin and how can they be measured?

AGEs are formed spontaneously. This formation can be exacerbated by oxidative stress, exposure to sunlight, smoking or high blood glucose levels. For that, it is no surprise that certain AGEs are important markers for the early diagnosis of diabetes. The HbA1c blood test for example shows the extent of glycation of haemoglobin. With this aid, clinicians can estimate the average blood glucose levels in a patient over the past 4 - 12 weeks. In the cosmetic context, crosslinking reactions have major effects, primarily on skin collagen. AGEs can reduce the elasticity of the skin and promote the formation of wrinkles^[2]. In addition, an increased level of AGEs can cause the skin to appear sallow and yellowish and leaves

an uneven complexion because of the inherent colouration of the reacted sugar molecules. This will inevitably lead to discomfort and low self-esteem.

As such, the reduction of proteins containing AGEs in the skin is an effective anti-ageing strategy that will lead to fully functional, rejuvenated and resilient skin.

Numerous studies were performed using an AGE reader as an instrument to determine the degree of glycation in the skin. This device measures the autofluorescence of AGE species noninvasively in vivo, directly from the skin of subjects^[3]. It is a tried-and-tested technique that was primarily developed to assess the risk of diabetes or cardiovascular disease. It has been shown that the level of autofluorescence closely correlates with age and increases by an average of 0.023 units per year in normal healthy subjects (figure 1)^[4].

A constant, seemingly unstoppable increase in AGEs during the natural ageing process was observed, which was more pronounced in case of diabetics^[5]. Undiagnosed diabetics have elevated blood glucose levels, which directly increases the burden of AGE on the skin. However, after being diagnosed, diabetics should have a controlled blood glucose level and have even lower levels than the normal population. Taking into account that most diabetics are diagnosed late in their lives and that a certain level of blood glucose is always present, it is however unlikely that diabetics can ever achieve an AGE level comparable to the 'normal' age-matched population. For example, a 50-year old diabetic has already accumulated an AGE level equivalent to a 65 year old non-diabetic (figure 1, arrow).

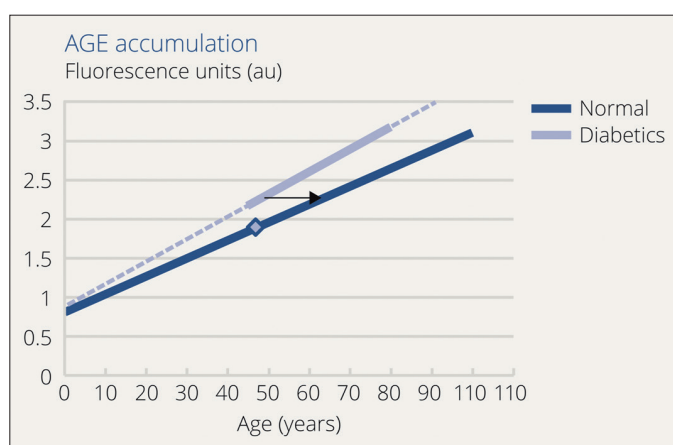


Figure 1: Increase in AGE with age. Skin autofluorescence, a marker for AGE, increases continuously after birth (normal, dark blue line^[4]). Diabetics have an increased AGE accumulation rate. The light blue line represents measured data from^[5] while the dotted line is an extrapolation. The diamond reflects the mean skin autofluorescence of the assessed collective described in this article at baseline.

Combatting ROS and stimulating the proteasome provides a unique strategy to counteract AGEs

The ability of skin cells to deal with glycated proteins is generally limited and it has not yet proved possible to develop active agents that are capable of returning existing AGE-affected biomolecules to their original state. Some small molecules like aminoguanidine can prevent the formation of AGEs by intervening in the first step, the Maillard reaction^[6]. They are even thought to be able to act as so called chain breakers in the early phase of AGE development. However, only a fraction of the reactive sugar species can be eliminated with such compounds^[7]. Hence, prevention of AGE-formation and limitation of damage is currently the only adequate way of arresting or at least attenuating AGE-related processes.

PROTEOLEA® is an active cosmetic ingredient comprising olive leaf extract, jujube seed extract and levan. It is designed to combat ROS, enhance the proteasome activity and provide the skin with additional moisture. The AGE-reducing effects of both jujube and olive leaf extracts have been demonstrated on molecular level elsewhere^[8,9]. We assume that anti-oxidative substances like jujubosides generally have AGE-reducing potential as they inhibit the formation of reactive sugar species and subsequent reactions of glycation products by eliminating ROS^[2].

The underlying action performed by the oleuropein in olive leaf extract is the enhancement of proteasomal activity^[10]. Although AGEs can be degraded by proteasomes to a certain extent, the proteasome activity is inhibited by AGEs and the level of defective proteins in cells thus becomes elevated^[11]. The proteasome is the cell's primary recycling bin for protein waste. It is essential to remove ineffective, old or damaged proteins to maintain proper cell function. However, a massive burden of AGE reduces the activity of the proteasome, which in turn leads to proteolytic stress and subsequent cell ageing and cell death.

Increased proteasome activity due to oleuropein activation can counteract the proteolytic stress caused by the accumulation of AGEs and thus help to normalise the cell functions.

We postulate that by preventing the de novo formation of AGEs due to elimination of ROS by jujube seed extract and maintaining proper cell function by activation of the proteasome with oleuropein from olive leaf extract, the natural turnover of proteins - especially that of collagen - will slowly lower the total AGE level in the skin.



Figure 2: Source of ingredients for the described active ingredient. Extracts from jujube seeds (left) and olive leaves (middle) provide the necessary compounds to combat ROS (jujubosides) and activate the proteasome (oleuropein, right). Images with permission of iStockphoto LP.

Results

The skin autofluorescence of 20 study subjects (9 male, 11 female), aged 40-54 (average 48) was measured using an AGE reader (NOVALAB, France). The study subjects applied a day cream containing 2% PROTEOLEA® and a night cream containing 4% PROTEOLEA® on one forearm and the same formulations without active ingredients on the other arm (placebo) once daily. After 2 months of application, the AGE content in the skin was measured again. Skin autofluorescence values are expressed in arbitrary units (au).

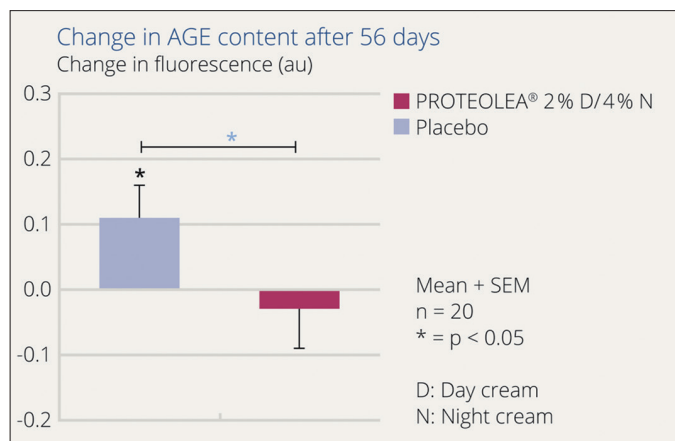


Figure 3: Reduction in levels of AGEs in skin. The burden of AGE was measured using an AGE Reader. After 2 months, there was a significant difference in the active-treated skin in comparison with the placebo. While there was a significant increase in AGEs by 0.11 units in the case of the placebo, the active ingredients completely neutralised this effect and showed a clear trend towards AGE reduction. ANOVA analysis.

In comparison with skin treated with the placebo, the active ingredients caused a significant reduction in levels of AGEs (figure 3). The baseline skin autofluorescence of subjects was exactly in the expected range of fluorescence units reported by Kotsier et al., 2010^[4] (1.88 au; see figure 1, diamond). While there was a significant increase in levels of skin AGEs in the areas treated

with the placebo, a clear trend towards reduced amounts of AGEs in the areas to which the active ingredient was applied could be witnessed. As we do not expect overt AGE breaking activity with the concept of ROS elimination and proteasome activation, we believe that this effect is a result of the natural collagen turnover in the dermis.

Improvement in uneven complexion and sallow look (controlled user test)

The reduction of AGE levels in skin also had a direct effect on its appearance. It is assumed that a reduction of glycated collagen is associated with an increase in the firmness and elasticity of skin. This results in a diminution of wrinkles and the generalised roughness of skin. Indeed, we have demonstrated impressive improvements to these parameters earlier^[12].

As the modified sugar molecules of AGEs have an inherent yellowish colouration, use of the active ingredient should also result in improved skin complexion – providing a healthy, rosy skin tone in place of a sallow, yellowish hue. In order to evaluate the effects that can be expected as a result of the reduction of AGEs, the test subjects were surveyed after using a face cream containing 2% active ingredients for 2 months. A study population of 34 people with Caucasian skin (40-63 years, average 50) was tested, including 12 age-matched diabetics, which were evaluated separately.

At the start of the test, more than a quarter of the normal study population was dissatisfied (moderate, high) with their skin complexion and referred to a sallow look (figure 4, left). After a 2-month application of active ingredients, only 10% felt dissatisfied in respect to these parameters. More impressively, the fraction of completely satisfied people (none) was 5 times greater, going from below 10% before application to more than 50% after application in the case of uneven complexion and from 32% to 76% in the case of a sallow look. This improvement was highly significant statistically.

The combination of jujube and olive extracts is particularly suitable for diabetic skin

In the diabetic study population (figure 4, right), as expected, a larger fraction was dissatisfied with the complexion at the beginning of the study. However, upon application of the active ingredients, all the diabetics were satisfied and 50% were even completely satisfied, although no-one was at the beginning of the study. A similar result was observed for the assessment of the sallow look. Here, the completely satisfied fraction (none) was 4 times greater, going from 17% to 67%, and at the end of the study, all study participants were satisfied (slight, none). A significant improvement in both parameters was already evident when the product was applied for 1 month (data not shown).

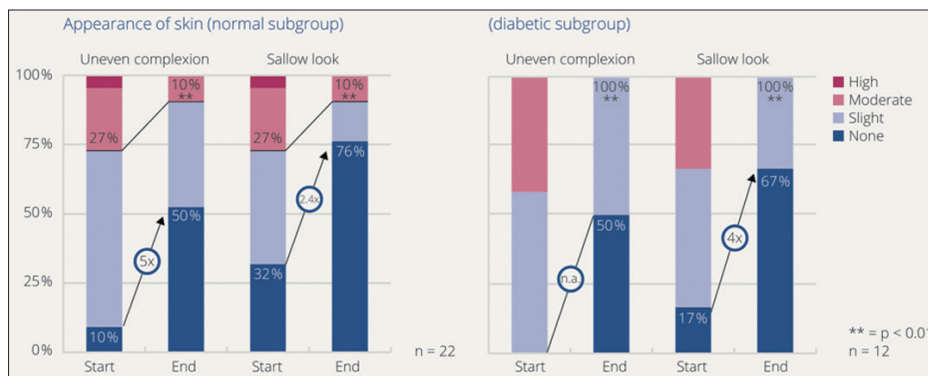


Figure 4: Reduction in the signs of AGE loaded skin. In a normal healthy collective (left), more than 25% of the study participants were dissatisfied with their skin complexion and described a sallow look to the skin. After 2 months' application comprising 2% active ingredients, only 10% described moderate dissatisfaction with the skin parameters. In the diabetic group (right), almost half of the collective was concerned about an uneven complexion and one third about a sallow look. After completion of the study, all diabetics were satisfied with their skin appearance. The fraction of completely satisfied study participants increased dramatically in both groups. All improvements were highly significant statistically ($p < 0.01$; Wilcoxon signed-rank test).

Conclusion

PROTEOLEA® is an effective active cosmetic ingredient that ameliorates the consequences of skin with a burden of AGE

In this 2-month trial, it was demonstrated that the active ingredient can prevent and even reverse the progressive accumulation of advanced glycation end products (AGEs). There was a significant reduction of 0.14 fluorescence units (au) after 2 months in comparison with the placebo. Based on the reference values provided in [4], this would be equivalent to skin being rejuvenated by 6.8 years (assuming an increase of 0.023 fluorescence units per year). We demonstrated a comparable rejuvenating effect for the active ingredient earlier with regard to the reduction of wrinkles around the eyes^[12].

The reduction in skin autofluorescence indicates that AGE accumulation can even be reversed, taking advantage of the natural collagen turnover in the dermis, although this effect is apparent as a trend only. This means that the natural structure of the skin was preserved from the point in time at which use of the active ingredient commenced. The ageing of skin as a result of the accumulation of AGEs is not something that only appears in mature skin; it is an effect that begins immediately after birth (figure 1). The active ingredients can thus delay the effects of ageing even in the skin of younger individuals. As a result of using the product, premature ageing of the skin can be prevented by safeguarding the proper functioning of the skin. It is especially useful in the daily skin care of diabetics who have a higher burden of AGE than the normal population.

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