

효소 화장품 개발을 위한 효소 안정화 기술

**(Stabilization of Enzyme  
for Application to Cosmetic Products)**

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**ABSTRACT**

Development of stabilized enzyme was attempted for cosmetic applications. Papain, a proteolytic enzyme, was stabilized through conjugation with a soluble carbohydrate biopolymer, SC-glucan<sup>TM</sup>. With a novel structure of the conjugation site, stability of the enzyme was significantly enhanced such that more than 90 % of the initial activity retained after a month storage at 45°C, while no activity were detected in native enzyme or enzyme simply mixed with SC-glucan<sup>TM</sup> after the storage. Conjugation with SC-glucan<sup>TM</sup> not only extended the half-life of the enzyme on storage at higher temperature, but was also found to protect enzymes against some components contained in cosmetic products for skin care. Cosmetic lotion containing 1 % papain conjugate was more effective and less irritative in exfoliating stratum corneum of human skin than the lotion containing 5% lactic acid, one of the current popular exfoliating agents.

## INTRODUCTION

Enzymes are essential biocatalyst in all living organisms, mediating nearly all biochemical reactions. Based on the biological roles, enzymes have been finding many applications in our daily life for decades. Lipase has been used in laundry and personal care industries for decades, protease as a digestive agent, and enzymes have become indispensable part in manufacturing many of the fine chemicals largely owing to its high reaction specificity. Much attention has currently been paid to other uses of enzymes, for example, for therapeutic purposes. Enzymes are used as oral drug for several inflammatory diseases, and it seems likely that, will be used against some cancers in a few years (Wrba & Pecher, 1996; Holmer, 1998). Enzyme therapy for light diseases is more at hand, and among them are those for dermatological disorder and aesthetic improvement (Brooks, *et al.*, 1997; Yarosh, *et al.*, 1999)

Cosmeceutical, whose meaning is a drug-like cosmetic, is a newly coined word and has recently gained much popularity among the industry and consumers (Umbach, 1995). It can be more precisely interpreted as a cosmetic material that shows a therapeutic effect comparable to, though not same as, that of pharmaceuticals. Recent boom of cosmeceutical products such as those containing  $\alpha$ -hydroxy acids (AHA) or retinoid made research scientists in the cosmetic industry develop novel active ingredients and identify their beneficial effects against skin problems such as unusual pigmentation and for aesthetic improvement as removing fine wrinkle (Fox, 1997).

Enzymes have been also known to be potential active agents for cosmeceuticals because some of them possess beneficial efficacy on skin. Worldwide demand for enzymes in cosmeceutical applications were estimated to reach \$11 million in 2003, up from \$6 million in 1998 (Martineau and Misch, 1999). Based on the essential roles of enzymes in skin

homeostasis, some commercial enzymes have been introduced to cosmetic products. For example, some proteases including papain, bromelain, and trypsin were used as functional ingredients of skin care products to hydrolyze keratin proteins in the stratum corneum of the skin. The controlled removal of old skin surface can trigger skin repair, and bring to the surface a layer of smoother, softer skin (Forestier 1992). Superoxide dismutase (SOD) is known to have a high capacity of removing free radicals on skin that is one of the main causes for skin aging (Brooks, 1997; Forestier, 1992). What is an attracting characteristic of enzymes to the cosmetic scientists is that they are specific and hardly cause side effect such as irritation, which is very critical in cosmetics (Dres, *et al.*, 1998). Since a cosmeceutical though it have therapeutic efficacy is still a cosmetic product, even a light irritation that is observed only by those with sensitive skin should not be permitted. A recent survey indicated that more than 70% of consumers who experienced irritation would never buy the product again. In addition, regulatory environment about the safety of cosmetic products is becoming harder. Recent controversy about the usage of AHA in cosmetics is a good example (Bunk, 1998).

With all the benefits and potentials of enzymes as cosmeceutical materials, problems of complex formulating and delivery requirements delay commercializing enzyme ingredients. One thing included in the obstacles should be overcome before an enzyme become popular as a general functional ingredient in cosmeceutical products. Proteins including enzymes are relatively unstable due to the physiological requirements, and still more in *in vitro* conditions. In the aqueous environment, most enzymes fail to maintain activities in a week at room temperature. Furthermore, a simple cosmetic product consists of more than 10 components including oils and surfactants, which also often disturb structural conformation of enzyme and consequently deactivate it. Therefore, the stability of enzymes must be required to be improved for their cosmetic applications by a reliable strategy, which

make an enzyme in a cosmetic product maintain activity during the period of market distributions, sales, and consumer's usage, and finally provide beneficial efficacy on skin when consumers apply the product. Many strategies have been pursued to obtain more stable enzymes (Gianfreda and Scarfi, 1991). Among them, immobilization of enzymes is a technique extensively studied since the late 1960s, and now widely employed both in fundamental studies in biochemistry and in practical applications in biotechnology. By definition, an immobilized enzyme is a protein physically localized in a certain region of space or converted from a water-soluble mobile state to a water-insoluble immobile one. Most of them are not suitable for cosmetics in large part due to the rigidity or hardness of the immobilized state of enzyme, which interferes action of enzymes on skin environment and sensory properties of the final cosmetic products. Instead of such immobilization, attachment of enzymes to a soluble polymer matrix such as dextran (DX), polyacrylic acid and polyethylene glycol (PEG) has received a lot of attention for stabilizing various proteins among stabilization methods(Inada et al., 1995). However, long-term stabilities of the modified enzymes in aqueous or cosmetic formulation were unsatisfactory for industrial application.

We report here that an enzyme specifically stabilized with SC-Glucan™, a biopolymer produced by fermentation, showed marked stability which can be introduced into cosmetic products.

## **MATERIALS & METHODS**

## **RESULTS & DISCUSSION**

### ***Enzyme stabilization by conjugation with SC-glucan™***

Papain is a protease and has long been known to stimulate skin turnover rate by exfoliating old keratin cells of stratum corneum, the utter-most layer of skin. Papain was conjugated with SC-glucan™ as well as PEG and DX, in order to estimate the stabilizing effect. After conjugation with SC-glucan™, recovery yields of enzyme activity were in the range of 85~95 %. Fig. 1 shows the stability profiles of the papain conjugates with each polymer at 25°C.

All three polymers were able to increase stability of papain compared with that of native enzyme, but the effect of SC-glucan™ was most promising, maintaining more than 95% of the initial activity after a month storage. The stabilization effect of SC-glucan™ was more clearly shown at 45°C (Fig. 2). More than 90% of the initial activity remained after a month storage, while about 27% and 42% remained with PEG and DX, respectively. However, simple mixing of SC-glucan™ and papain was not effective on stabilizing the enzyme [data not shown].

### ***Stability of SC-glucan™-conjugated papain in a cosmetic formulation***

Oils and surfactants included in typical cosmetic formulations are generally known to disturb enzyme structure and often lead to the enzyme deactivation. Thus, the enzyme should be also confirmed to be protected against these ingredients before it is applied to cosmetic products. Practically, the SC-glucan™ conjugated papain was incorporated into a cosmetic lotion base containing stearic acid, cetyl alcohol, polysorbate, dimethicone, glycerol, methylparaben, butylene glycol, etc. Almost all of papain activity was retained in the lotion containing conjugated enzyme. The enzyme activity of the lotion was found to maintain at

45°C for over three months (Fig. 3). On the contrary, native papain in the lotion was severely inactivated in a few days.

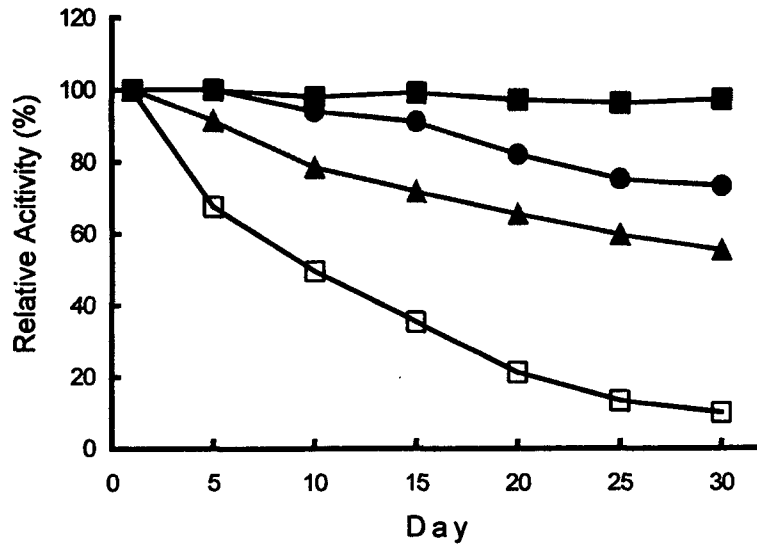
### ***In situ activity of SC-glucan™ conjugated papain on skin***

No matter how stable enzyme is in the cosmetics, it may be useless if the activity can not be expressed *in situ* on skin. It was tested whether papain could express its activity on skin as the conjugate form with SC-glucan™. The activity was assayed by measuring the rate of removal of stratum corneum (Pierard & Pierard 1993). When daily applied to human skin, the cosmetic lotion containing 1 % conjugated papain was more effective in exfoliating the stratum corneum than the lotion containing 5 % lactic acid (Fig. 4 and 5). Fig. 6 represents microscopic view of skin surface treated with papain conjugate.

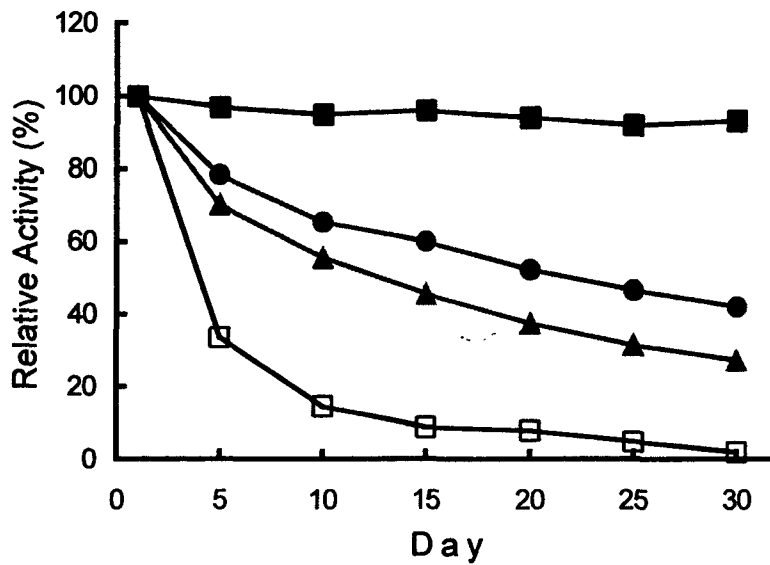
In addition to exfoliating efficacy, papain conjugate with SC-glucan™ showed marked safety compared with AHA (Fig. 7). After a month treatment of the papain conjugate, no sign of irritation such as redness was observed. On the contrary, only 1 % lactic acid caused clearly visible sign of redness just in a week treatment.

## ***Conclusion***

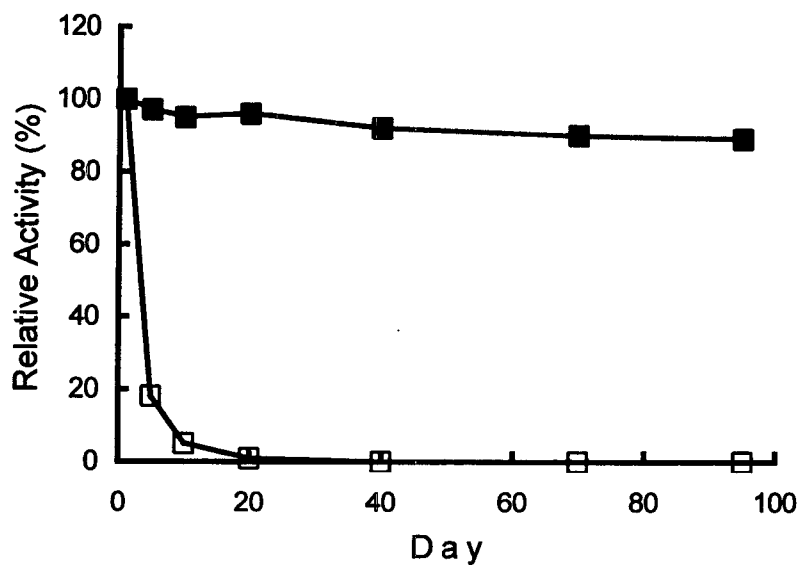
Conjugation of papain with SC-glucan™ showed significantly enhanced enzyme stability in both aqueous and cosmetic emulsion systems. The conjugated enzyme was soluble form, which is suitable for cosmetic applications in sensory properties. Daily skin treatment of a cosmetic lotion containing the papain conjugate showed keratolytic and skin renewal activity without any visible sign of irritation. Based on these results, it can be concluded that the conjugation with SC-glucan™ is a promising method of stabilizing enzyme for cosmetic applications.



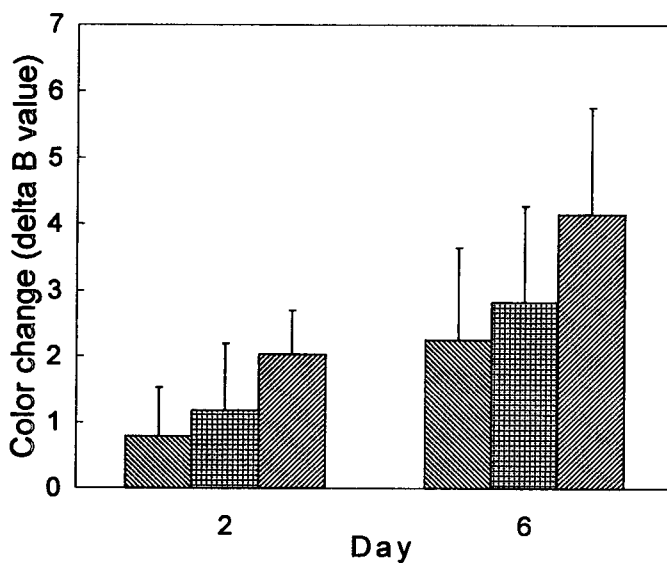
**Figure 1.** Stability profile of papain when stored at room temperature (25°C). Symbols are: (■) conjugated with SC-glucan™; (●) with dextran 250000; (▲) with polyethylene glycol 10000; (□) native papain.



**Figure 2.** Stability profile of papain when stored at 45°C. Symbols are: (■) conjugated with SC-glucan™; (●) with dextran 250000; (▲) with polyethylene glycol 10000; (□) native papain.



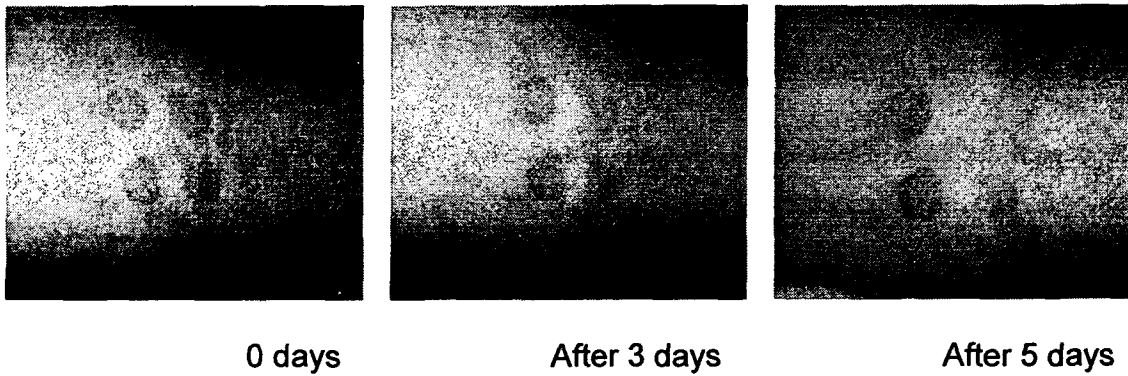
**Figure 3.** Stability profile of papain stored at 45°C when formulated into a skin lotion. Symbols are:



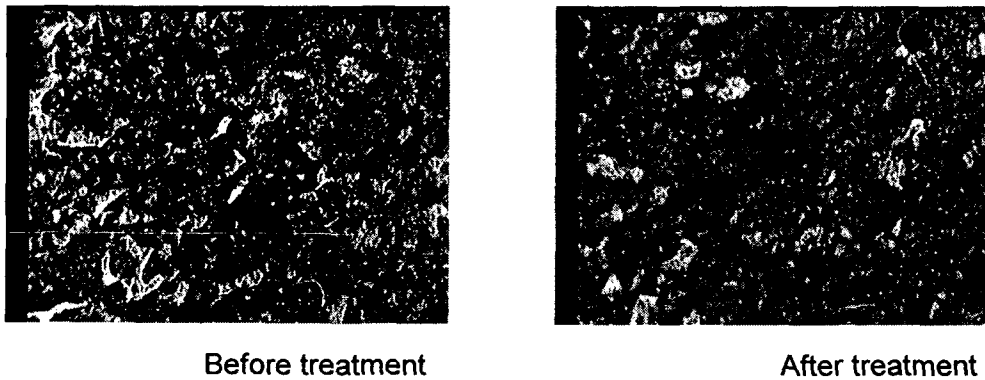
(■) papain conjugated with SC-glucan™; (□) native papain.

**Figure 4.** Stratum corneum removal activity of SC-glucan™ conjugated-papain was evaluated by the dihydroxyacetone test on human skin. The changes in skin color according to stratum corneum exfoliation were determined by chromometry (Pierard 1993). The columns in the figure represent: (■) Cosmetic lotion base; (■) Cosmetic lotion with 5% lactic acid; (■) Cosmetic lotion with 1% papain conjugate with SC-glucan™.

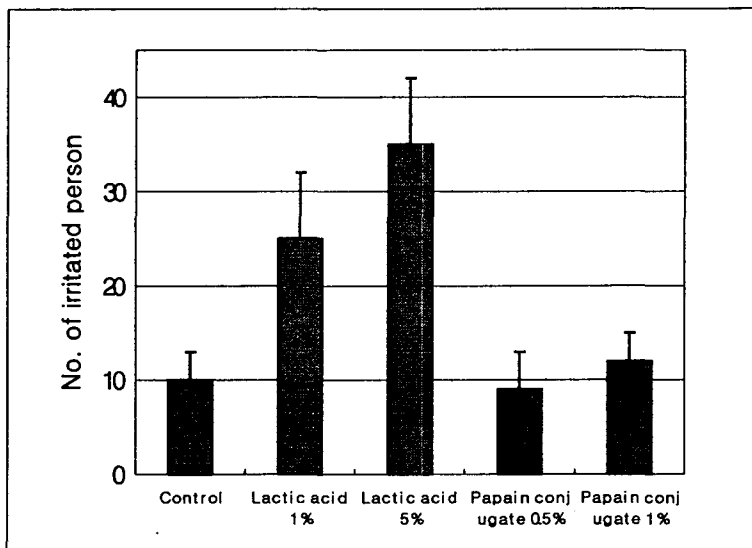




**Figure 5.** Stratum corneum removal activity of SC-glucan™ conjugated-papain was evaluated by the dihydroxyacetone test on a human skin. The cosmetic lotion containing 1% papain conjugate with SC-glucan™ was daily applied to right two circular zones pigmented by dihydroxyacetone treatment, and the left two circles were treated with the cosmetic lotion base without the enzyme conjugate.



**Figure 6.** Comparison of microscopic view of skin treated and untreated with lotion containing 1% papain conjugate.



**Figure 7.** Comparison of irritation during stratum corneum removal between SC-glucan™ conjugated-papain and lactic acid. The cosmetic lotions containing papain conjugate with SC-glucan™ and lactic acid were daily applied to arms of 100 volunteer for a week.

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