

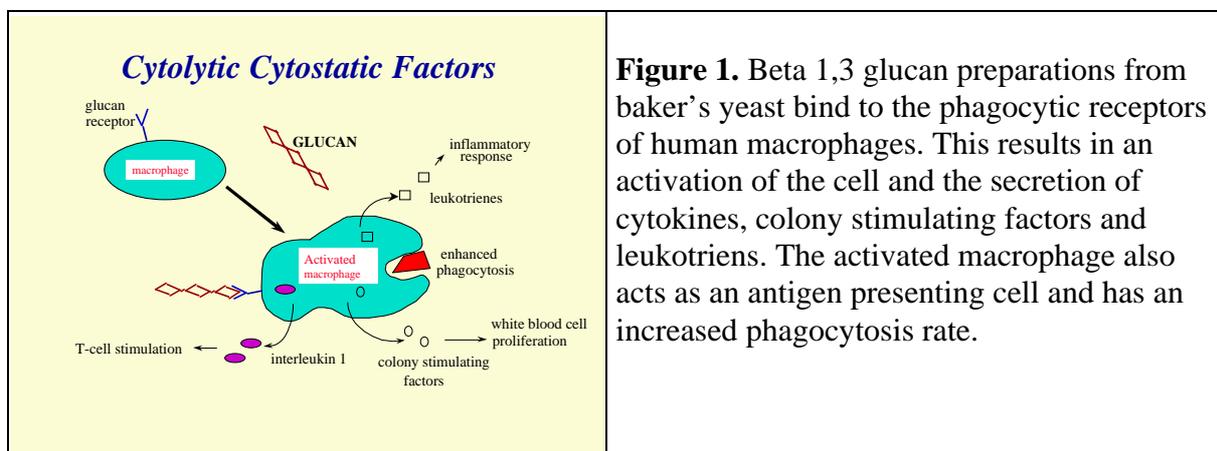
## Anti-Aging and Photoprotecting Effects of Carboxymethylated Glucan from Baker's Yeast

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UV irradiation, CM Glucan, polysaccheride, immuno protection

### Properties of Yeast Glucans

Glucan is a beta 1,3 linked polyglucose of high molecular weight and belongs to the class of compounds known today as biological response modifiers. Glucan from baker's yeast is a very potent stimulator of the immune system, with the ability to activate macrophages, neutrophils and other cells that carry specific beta-glucan receptors on their surface (1). Activation of these cells by glucan stimulates the nonspecific defense mechanisms of the host (Fig. 1). Therefore, glucan preparations have been extensively studied in oncology (2, 3), infectiology (4) and wound healing (5). In all these applications, different beta 1,3 glucan preparations from yeast have been shown to be very active. Other high molecular weight beta 1,3 glucans derived from different fungi, such as schizophyllan, lentinan and grifolan have been investigated in Japan in treating malignant tumors (6). However, polysaccharides, such as mannans, galactans and glucans from oat, barley and wheat have no such activity. The stimulatory actions of beta 1,3 glucan from yeast has been applied in immuno-compromised subjects. Since insoluble glucan preparations can produce undesirable toxicological properties, such as granuloma formation, clinical interest has focused on soluble glucan preparations. Recently, a Phase II clinical study showed the tolerability and efficacy of a new soluble yeast glucan preparation (7). Application of the product from 0.1-2.0 mg/kg before and after high risk surgery lowered postoperative infection rates.

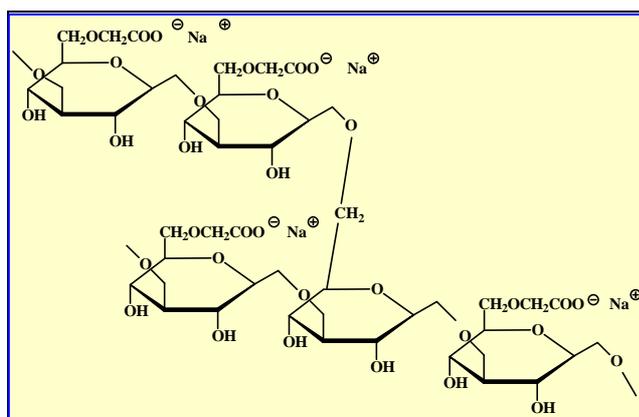


**Figure 1.** Beta 1,3 glucan preparations from baker's yeast bind to the phagocytic receptors of human macrophages. This results in an activation of the cell and the secretion of cytokines, colony stimulating factors and leukotrienes. The activated macrophage also acts as an antigen presenting cell and has an increased phagocytosis rate.

## The Skin's Immune System

The skin is the body's most important primary defense system, both as a physical barrier and as a metabolic and immunological biochemical-response system. At the epidermal level, the skin's immune system involves cytokines and the immunocompetent Langerhans cells and keratinocytes. The highly dendritic Langerhans cells which form a network throughout the entire epidermis are theorized to be the skin's own version of macrophages. Langerhans cells recognize antigens and cut them into small fragments. Subsequently, these activated cells migrate to the nearest lymph nodes, where the antigen fragments are presented to specific T-cells. These T-lymphocytes initiate either an antibody production by B-cells or the recruitment of macrophages to the injured skin site. Over the last decade, it has been shown that UV-irradiation can impair the number and viability of immunocompetent cells in the epidermis and thereby induce a suppression of the skin's immune system (8, 9). The specific role of UV-B and UV-A irradiation and the potentially limited effectiveness of commercial sun screens in immunoprotection of the skin has been the subject of recent investigations (10, 11, 12, 13).

A number of different compounds have been studied as ingredients in sun care products to counteract these undesirable effects of UV-irradiation. Beta 1,3 glucan preparations from yeast have shown very promising effects regarding the improvement of the skin's self defense mechanisms.



**Figure 2.** Chemical structure of carboxymethylated glucan (CM-glucan) from baker's yeast. On average, three out of four glucose units of the beta-(1-3), (1-6)-linked polymer are modified at position 6.

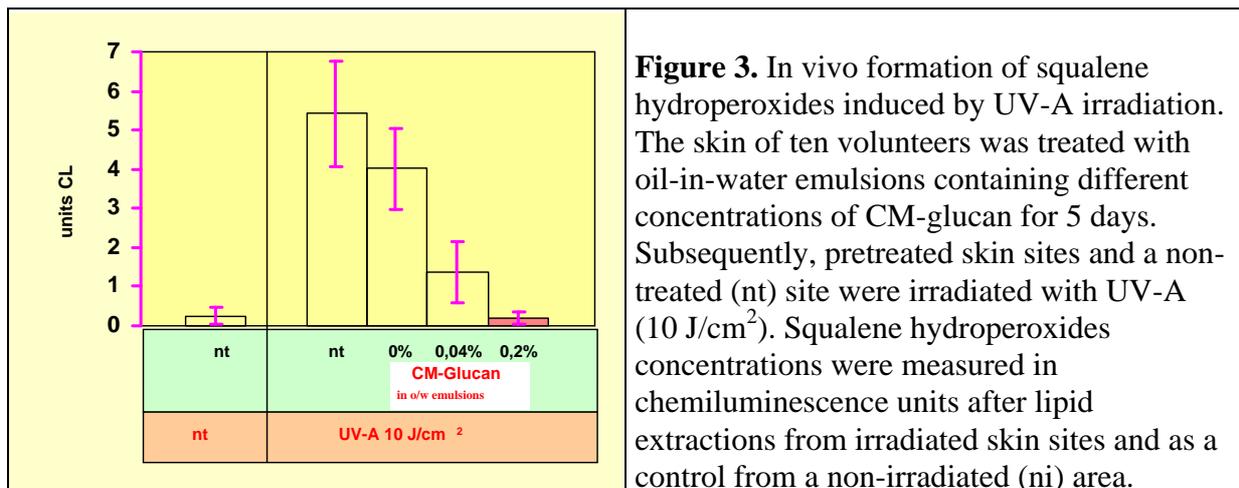
## Carboxymethylated Beta-1,3-Glucan: An Exciting Cosmetic Ingredient

Water-soluble carboxymethylated beta-1,3-glucan (CM-glucan) from yeast (Fig. 2) has gained a lot of interest over the last few years as a new cosmetic ingredient and has been included in many different products on the market (sun care, wound healing and sensitive skin).

Initial in-vitro studies have shown that CM-glucan with a degree of substitution of 0.75 is able to protect fibroblasts and keratinocytes from the depletion of antioxidant molecules induced by UV-A irradiation. The protection against oxidative stress in human skin cells could be demonstrated by measuring intracellular glutathione and ferritin concentrations as endpoints (14). The polysaccharide also non-specifically stimulates the keratinocytes

proliferation (15) which results in an increase of the renewal rate of the stratum corneum in-vivo (16).

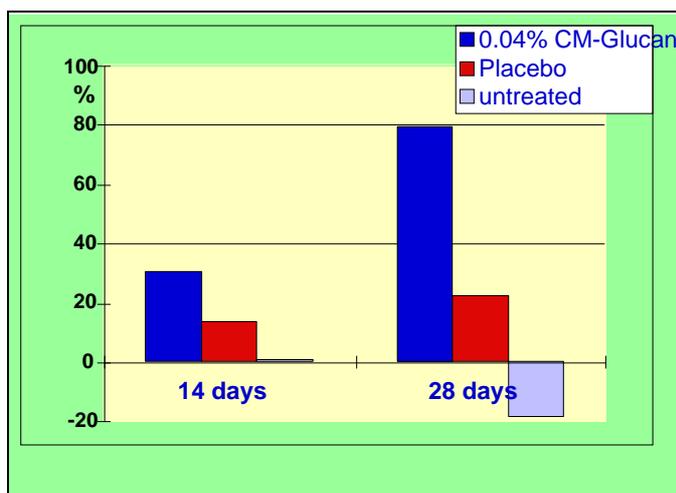
To evaluate the in-vivo efficacy of the polysaccharide to protect skin against oxidative stress induced by UV-A radiation, the non-invasive technique of squalene hydroperoxides determination was applied (17). Squalene is one of the main component of the sebum and is particularly susceptible to photooxidation. Colin et al. (17) showed that even low dose UV-A irradiation of the skin leads to the formation of squalene hydroperoxides. However, the application of certain free radical scavengers to the skin can protect these lipids against peroxidation. In our study, three oil-in-water emulsions (containing 0.2%, 0.04% and 0% of CM-glucan) were applied twice daily on the forearms of ten volunteers. On the fifth day, the pretreated skin and a non-treated area were exposed to UV-A irradiation ( $10 \text{ J/cm}^2$ ). Subsequently, skin lipids were extracted with 1 ml of ethanol from all irradiated areas and as a control also from non-irradiated skin. Squalene and squalene hydroperoxides were then determined in these extracts by HPLC techniques. A very wide range of squalene concentrations could be detected in the skin of the different volunteers. However, in all subjects, the UV-A irradiation led to a substantial increase in the concentration of squalene hydroperoxides. The pretreatment of the skin with the different formulations resulted in a reduction of the squalene peroxidation (Fig. 3). The addition of only 0.04% CM-glucan to the oil-in-water emulsions strongly inhibited the squalene peroxidation. An almost complete protection against UV-A induced oxidation could be observed in this test with the product containing 0.2% CM-glucan.



### CM-Glucan and Photoaged Skin

Prophylactic and therapeutic care of photodamaged skin is of considerable importance since photoaging and also photocarcinogenesis are a rapidly growing concern in developed nations throughout the world as a result of increased life span and hence of larger populations of elderly individuals. The aging process of the skin can be divided into intrinsic changes and extrinsic influences (photoaging) resulting from chronic exposures to UV-irradiation and other environmental hazards. Intrinsic aging leads only very slowly to obvious changes in the

skin. In contrast, photoaging results in a marked deterioration of the skin appearance namely increase of wrinkles, enhancement of roughness, loss of firmness and mottled pigmentation. In the dermis, a loss of elastin fibrils and soluble collagen can be observed. As a result, the skin appears wrinkled and has lost its elasticity. Topically applied tretinoin has been successful in the treatment of some of the visible signs of photodamaged skin (18). However, the marked skin irritation caused by tretinoin excludes the use of this retinoid from cosmetic applications. We thus studied the potential „anti-aging“ effects of carboxymethylated glucan from baker’s yeast. An oil-in-water emulsion containing 0.04% CM-glucan and the corresponding placebo product were applied to the forearms of ten volunteers (age 61-75) twice daily over a period of 28 days. To simulate photoaging to some extent, the skin of these volunteers was exposed to a sun simulator at 0.75 MED twice weekly during these 28 days. The elasticity of the skin, determined as skin firmness with a cutometer, was used to characterize the skin condition at days 1, 14 and 28. The irradiation of the skin at 0.75 MED led to a clear reduction of the skin firmness after 28 days (Fig. 4). The application of an oil-in-water emulsion (placebo) counteracted the photoaging process of the skin slightly. However, the incorporation of only 0.04% CM-glucan into the same emulsion led to a statistically significant (Wilcoxon matched pairs signed rank test;  $p \leq 0.05$ ) improvement of the skin firmness after 28 days. A small change for the better could already be observed after 14 days, though that early effect was not statistically significant.



**Figure 4.** Treatment of photoaged skin with an oil-in-water emulsion containing 0.04% CM-glucan. The forearm skin of ten volunteers (age 61-75) was treated with the emulsion containing carboxymethylated glucan or with the corresponding placebo product over a period of 28 days. To simulate photoaging, the skin of the volunteers was irradiated twice a week at 0.75 MED during this period. The skin firmness was measured with a cutometer at days 1, 14 and 28.

### Anti-Irritating Effects of Carboxymethylated Beta 1,3 Glucan

In a number of experiments carboxymethylated beta 1,3 glucan has shown to significantly reduce skin irritations caused by detergents, alcohol and retinoic acid (Züllli, unpublished data). Castelly et al. studied the clinical efficiency of mitigating contact dermatitis with a formulation containing CM-glucan and Ginkgo biloba extract (19). Contact allergy is a complex phenomenon in which the skin’s immune system is involved. Usually, allergic contact dermatitis is expressed as erythema, oedema (inflammatory responses) and pruritus. The mentioned formulation was very effective in reducing these signs of allergic contact

dermatitis induced by the preselected allergens (nickel sulfate, balsam of Peru, fragrance mixture and methyl-isothiazolinone).

## Conclusion

The data presented shows that CM-glucan is able to stimulate skin cells, most likely through a receptor mediated process, to produce endogenous products against oxidative stress and other environmental hazards, such as irritants. In addition, long term application of CM-glucan at low concentrations can revoke a reduced skin firmness level in aged or photoaged skin. Despite of the fact that polar agents of high molecular weight remain on the skin's surface, CM-glucan does induce biochemical effects within the skin. However, the exact molecular mechanism of CM-glucan's activity and skin penetration has not been fully elucidated and warrants further investigations.

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