MULTI-ACTIVE BETA-GLUCAN COMPLEX OF BIOTECHNOLOGICAL ORIGIN FOR ACTIVATING THE IMMUNE SYSTEM OF THE SKIN WITH AN IRRITATION-INHIBITING, PROTECTIVE EFFECT AND FOR SMOOTHING WRINKLES WITH IMMEDIATE EFFECT AND LONG-TERM EFFECT

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#### Introduction

The trend in modern cosmetics clearly signals a preference for constituents of natural origin which often leads to a wise combination of old traditions and modern production technologies.

Yeast products have a long tradition both in cosmetics and medicine. This is undoubtedly no coincidence, since the immune-stimulating action of the yeast cell walls has been exerting favourable effects in countless applications since time immemorial. It was not until the 60s and 70s that scientists discovered that a very special structure of the polysaccharides present in the yeast cell walls is responsible for this effect. This is beta-(1,3)-glucan. Similar structures were later found in fungi, too, and these are sometimes used in cancer therapy.

LIQUID Beta Glucan is a multifunctional active substance complex based on purified yet natural yeast cell walls obtained using biotechnology, and is especially suitable for the production of cosmetic skin care preparations. LIQUID Beta Glucan has some extremely interesting properties that are consistent with the lifestyle of the modern consumer.

### 2. Background to the active substance complex LIQUID Beta Glucan

Numerous scientific investigations and publications refer to the extremely interesting biological properties of the natural biopolymer beta-(1,3)-glucan (1,2,3). Both the physical and physiological behaviour of this natural substance make it an obvious choice for use as an active substance for cosmetic skin care purposes.

#### 2.1 Structure and origin of beta-glucan

In view of many years of documented experience in dermal application, baker's yeast (Saccharomyces cerevisiae) suggests itself as the natural starting material for obtaining beta-glucan as an active ingredient for cosmetics. The cell walls are composed essentially of beta-(1,3)-glucan, a practically insoluble polysaccharide which is responsible for the structure and mechanical strength of the cell (50-58%).

The yeast cell walls also contain other, more soluble polysaccharides known as mannans (17-22%), proteins (13-17%) and minerals (about 3%). The yeast cell wall accounts for about 20% by weight of the total cell mass.

Like cellulose, beta-glucan is basically composed of glucose units except that they have different chemical linkages, namely beta-1,3-glucosidic linkages (4) (Fig. 1). It is this special linkage that is responsible for the helical structure of beta-glucan, and for its extraordinarily high water-binding capacity.

Figure 1: Structure of the beta-1,3-glucosidic linkage of beta-glucan

### 2.2 Preparation and work-up using biotechnology

LIQUID Beta Glucan is a stable complex of purified yet natural yeast cell walls, suspended in a self-preserving butylene glycol/water carrier system. The production process involves the culture of a selected strain of baker's yeast (Saccharomyces cerevisiae) using biotechnology under controlled and standardised conditions followed by autolysis by the cell's own enzymes. The cell walls are then separated from the cell contents by centrifugation and undergo a careful, non-destructive purification process. The insoluble constituents of the yeast cell walls purified in this way are then taken up in a butylene glycol/water carrier system. Fig. 2 shows the electron microscope photograph of such a yeast cell wall.

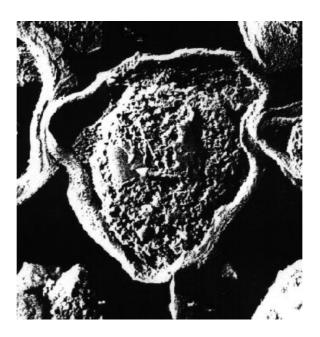


Figure 2: Yeast cell wall

### 2.3 Biological properties

An important function of the human skin is to shield the body and its internal organs from adverse external influences. Evolution has developed a defence and immune system for protecting us from infections, diseases and damage by other environmental factors. This defence system is based on the provision of macrophages, a type of cell (phagocytes) capable of eliminating foreign bodies such as microorganisms, dead tissue cells and also tumour cells (phagocytosis). Macrophages are found in all tissues of the human body. In the skin these are called Langerhans cells (2).

The efficiency of the immune system can be impaired by a number of factors. Natural ageing processes and environmentally induced accelerated ageing, for example due to UV light, as well as physical and psychological stress have been shown to have an adverse effect on the immune system (3).

Countless clinical trials have shown that beta-glucans and, in particular, yeast beta-glucan have pronounced immune-stimulating properties (1,5). This is based on activation of the macrophages and Langerhans cells, counteracting age- and environmentally induced immune deficiencies. Beta-glucan has proved to be a non-specific stimulator of immune-competent cells (6).

The activation of macrophages or Langerhans cells by means of beta-glucan intensifies the formation of cytokines. Cytokines are hormone-like substances which activate additional functions of the immune system and support, for example, wound healing. As a result, topical application in a suitable form to stressed or damaged skin brings about both an accelerated regeneration and a so-called repair effect (3). This is also the basis of the anti-irritating properties of LIQUID Beta Glucan.

Reference has already been made to the extraordinarily high water-binding capacity of beta-glucan due to its helical structure. This property is proving to be particularly advantageous for the cosmetic use of natural yeast glucans. The result is optimum hydration of the skin. This leads after regular use to a pronounced improvement in skin elasticity and firming of the skin and simultaneous smoothing of wrinkles.

LIQUID Beta Glucan can thus be justifiably regarded as a multi-active complex of active ingredients. The activation of the immune system leads to various highly desirable effects which coincide perfectly with the requirements of the market for cosmetic products consistent with the modern lifestyle. One of these effects is the irritation-inhibiting property which is particularly favourable for stressed skin exposed to environmental pollution. Another is a protective effect which actively guards the skin from age-related and UV-induced skin damage (7,8). The smoothing of wrinkles as a result of improved hydration shows an astounding immediate effect. This property also meets the demands of today's lifestyle. The skin does not require a regular and lengthy pretreatment; the expected effect develops immediately after a single application!

- Effectiveness
- 3.1 Immediate effect after a single application
- 3.1.1 Protection against irritation and inhibition of irritation

The irritation-inhibiting effect of LIQUID Beta Glucan was determined on the basis of in vivo tests by AMA Laboratories Inc., New York, USA.

LIQUID Beta Glucan was incorporated in a test cream in a use concentration of 10%. The comparison preparation was a corresponding placebo cream without active substance. The test was carried out on the lower arms of 10 volunteers. Test zones on one lower arm were pretreated once daily for 7 days both with the test cream and with the placebo cream. The test areas were then treated with 0.5% sodium lauryl sulfate solution. On the other arm the test surfaces were treated with a mixture of the test cream and the placebo cream with 0.5% sodium lauryl sulfate in each case. The test areas pre-treated in this way were then treated in each case with test cream, placebo cream and distilled water under an occlusive patch. After 24 hours the patch was removed and the test surfaces were assessed visually for irritation (scale of 0 to 4). The results are shown in a graph in Fig. 3 and can be summarised as follows:

Both after pretreatment of the skin and with a simultaneous treatment together with 0.5% sodium lauryl sulfate, LIQUID Beta Glucan in a use concentration of 10% inhibits the induced skin irritation by an average of at least 55% compared with the placebo cream.

It can be concluded from this that LIQUID Beta Glucan exhibits both a protective effect and an irritation-inhibiting effect with respect to externally induced skin irritation.

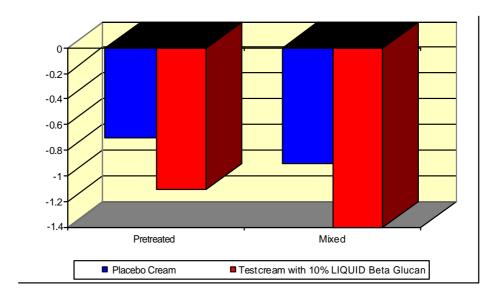


Figure 3: Protection against irritation and inhibition of irritation by LIQUID Beta Glucan

#### 3.1.2 Smoothing of wrinkles

The assessment of the immediate wrinkle-smoothing effect was carried out on the basis of in vivo tests by Dermatest in Münster, Germany.

LIQUID Beta Glucan was used in a concentration of 10% in a test cream. The effect was compared with a placebo cream without LIQUID Beta Glucan. The test was carried out in the cheek region/lower eyelid on 10 female volunteers. After a single application of the test and placebo creams, silicone impressions were prepared after 1, 2 and 8 hours for laser profile measurements. The Rz (DIN) values (mean surface roughness) were used to evaluate the results.

The results can be summarised as follows:

Rz (DIN) after 1 h: Test cream: 10.71% reduction

Placebo cream: 0.89.% reduction

82% better than placebo

Rz (DIN) after 2 h: Test cream: 17.60% reduction

Placebo cream: 9.08% reduction

94% better than placebo

Rz (DIN) after 8 h: Test cream: 28.42% reduction

Placebo cream: 11.32% reduction

151% better than placebo

The results are shown in a graph in Fig. 4. The wrinkle smoothing is clearly noticeable only one hour after application and increases considerably over the eight-hour test period.

The computer-aided image analyses of the skin relief of a female volunteer before and 8 hours after the treatment with a test cream with 10% LIQUID Beta Glucan can be seen in Fig.

5 and Fig. 6.

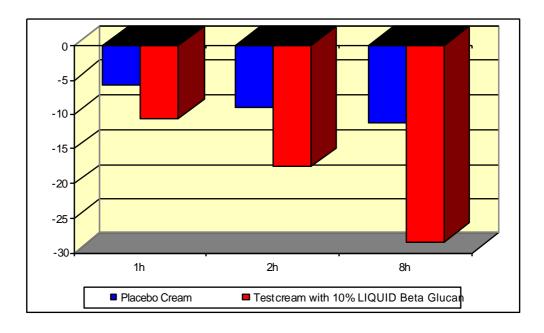


Figure 4: Wrinkle-smoothing effect of LIQUID Beta Glucan (Rz (DIN) 1, 2 and 8 h after application)

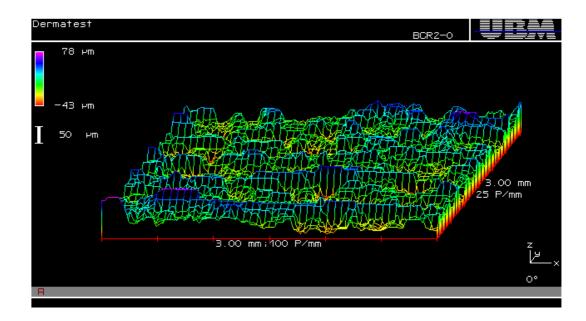


Figure 5: Skin relief before treatment

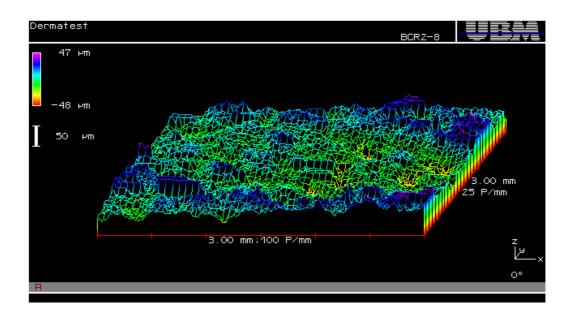


Figure 6: Skin relief 8 h after treatment with test cream with 10% LIQUID Beta Glucan

#### 3.2 Long-term effect after regular use

The effect of a test cream containing 5% LIQUID Beta Glucan compared with a similar placebo cream in terms of the improvement both in the elasticity and firmness of the skin and in its moisture content was tested in vivo on 5 female volunteers over the course of a 14-day application (twice daily) by Derma Consult in Alfter, Germany.

#### 3.2.1 Improvement in skin elasticity

The elasticity of the skin was measured with the Cutometer SEM 474. The test results obtained confirm that after regular treatment with the test cream, the skin was firmer than untreated skin or skin treated with placebo cream. The results are shown in the graph in Fig. 7 and can be summarized as follows:

Whereas skin firming of 8.0% was achieved with the placebo cream, skin firming of 32.4% was achieved after a similar treatment with the same cream base but which also contained 5% LIQUID Beta Glucan.

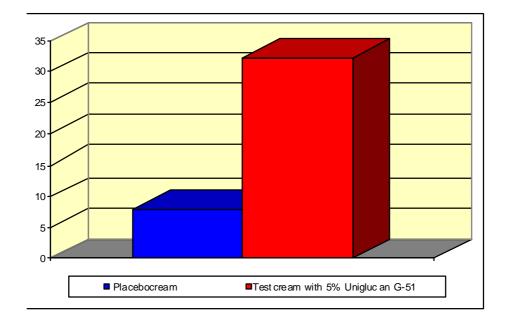


Figure 7: Improvement in elasticity of the skin due to LIQUID Beta Glucan (skin firmness)

#### 3.2.2 Improvement in the moisture content of the skin

The improvement in the skin moisture content was determined by capacitive means using the Corneometer CM 825. The use of the test cream led to an increasing improvement in the skin moisture content after 7 days' and after 14 days' treatment, both in comparison with the starting values and compared with the placebo treatment in each case. The results are shown in the graph in Fig. 8, and can be summarised as follows:

After 7 days' application of a test cream containing 5% LIQUID Beta Glucan, a 15.7% increase in the skin moisture content was obtained (placebo 4.8%); after 14 days' application, the increase in the moisture content rose to 28.1% (placebo 6.8%).

These results are impressive confirmation of a pronounced long-term effect.

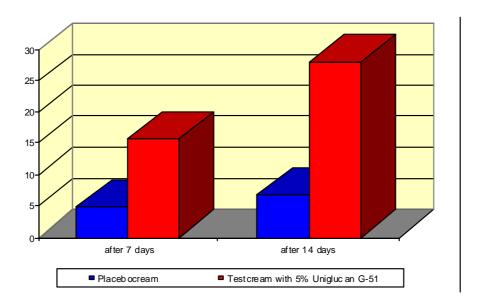


Figure 8: Improvement in the moisture content of the skin due to LIQUID Beta Glucan

### 4. Physiological properties

By deliberate policy, no animal tests were carried out to verify the non-toxicity of LIQUID Beta Glucan. The constituents can be regarded as non-toxic on the basis of literature values and those gained from experience. As expected, these findings were confirmed in the in vitro and in vivo tests described below.

### 4.1 Compatibility with mucous membranes

The mucous membrane compatibility of LIQUID Beta Glucan was determined by means of alternative in vitro technology. LIQUID Beta Glucan was tested in the undiluted state on a proven corneal model composed of cultivated human keratinocytes. The corneal model underwent an MTT test after a contact time of 10 minutes, 1, 3 and 24 hours (Acute Ocular Toxicity in vitro Test). The product was classified as non-irritating. These tests were carried out by SKINETHIC in Nice (France).

### 4.2 Skin compatibility

An closed patch test was carried out on the inside of the lower arm of 50 volunteers using LIQUID Beta Glucan as a 50% aqueous solution. None of the volunteers exhibited any skin changes whatsoever in the test region after 24, 48 or 72 hours. It can be concluded that LIQUID Beta Glucan in practical use will not lead to undesirable skin reactions caused by primary irritation or allergic hypersensitivity. These tests were carried out by Dermatest, a company engaged in allergy research in Münster (Germany).

#### 4.2.1 Mutagenicity

In order to rule out any potential mutagenic properties, LIQUID Beta Glucan underwent an AMES test. In view of the results of this test, LIQUID Beta Glucan can be classified as non- mutagenic. The company commissioned to carry out this AMES test was RCC, Itingen, Switzerland.

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#### 5. Use

The active substance complex LIQUID Beta Glucan based on yeast polysaccharides is a contemporary constituent, obtained using biotechnology, available to producers of skincare cosmetics. It has anti-irritating properties that are particularly beneficial to stressed skin, and smoothes out wrinkles on more mature skin. The astounding immediate effect can be emphasised as particularly useful for the modern consumer. The extraordinarily skin-friendly properties of LIQUID Beta Glucan leave a lasting, extremely pleasant feel after use. LIQUID Beta Glucan is suitable for incorporation in skin care products and in immune- stimulating skin protection preparations of all kinds (sunscreens, after sun, body lotion, anti-ageing products).

LIQUID Beta Glucan is water-dispersible and therefore suitable for incorporation in all types of emulsions, lotions, gels etc.

On the basis of the efficacy studies described in section 3 and the experience already gained since then, we recommend a use concentration of between 3 and 10% for LIQUID Beta Glucan.

Product from Switzerland

#### 6. Characteristics

Composition LIQUID Beta Glucan is an active substance concentrate composed of

yeast polysaccharides obtained using biotechnology, and a carrier

system based on butylene glycol/water.

LIQUID Beta Glucan contains no preservatives (self-preserving).

Description Free-flowing paste

Analytical data See specification.

Solubility Can be diluted with water.

Insoluble in lipids

Storage 15°C - 25°C (see safety data sheet)

Shelf life 2 years (see specification)

Processing LIQUID Beta Glucan is relatively stable and can be processed

without difficulty under the usual conditions for the preparation of

cosmetics.

Biodegradability A biodegradability of 93% was determined with the OECD 310 D

"Closed Bottle Test". LIQUID Beta Glucan can thus be regarded as

very readily biodegradable.

Identification INCI name CAS No.

Butylene Glycol 107-88-0 Yeast Polysaccharides (Beta Glucan) 9012-72-0

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