

Potassium Undecylenoyl Hydrolyzed Wheat Protein: A New Surfactant With Dandruff Control Properties

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Summary

Undecylenic derivatives are well known in cosmetics and widely used; undecylenic soaps, amides and betaines have been already on the market for many years in antidandruff and mycotic growth control field.

Potassium Undecylenoyl Hydrolyzed Wheat Protein is obtained grafting undecylenic acid to hydrolyzed wheat protein. This surfactant maintains the properties of the molecules from which derives and can find several applications in personal care.

The work will illustrate the properties of Potassium Undecylenoyl Hydrolyzed Wheat Protein in dandruff control field.

The results of an *in vivo* test made using Piroctone Olamine as a control will be shown. Mycotic growth control capacity will be also described. Dermatological innocuity will be shown through different *in vivo* and *in vitro* tests.

Furthermore the sensitization power of this surfactant compared with classical antidandruff agents will be described using Interleukine-1 α secretion as a parameter to be evaluated.

Riassunto

I derivati undecilenici sono ben noti in cosmetica e largamente utilizzati; saponi, amidi e betaine derivati dall'acido undecilenico sono presenti sul mercato da molti anni come prodotti utilizzati nel controllo della forfora e delle infezioni fungine.

Potassium Undecylenoyl Hydrolyzed Wheat Protein viene ottenuto unendo l'acido undecilenico con proteina idrolizzata da grano. Questo tensioattivo mantiene le proprietà da cui deriva e trova diverse applicazioni nel personal care.

Il lavoro illustrerà le proprietà di Potassium Undecylenoyl Hydrolyzed Wheat Protein nel controllo della forfora.

Verranno mostrati i risultati di un test *in vivo* fatto usando Piroctone Olamine come riferimento. Sarà inoltre descritta la capacità del tensioattivo oggetto del lavoro di controllare la crescita fungina. L'innocuità dermatologica sarà valutata tramite diversi test *in vivo* ed *in vitro*. Verrà inoltre descritto il potere sensibilizzante di questa molecola rispetto ai classici agenti antiforfora utilizzando la secrezione di Interleuchina-1 α come parametro.

INTRODUCTION

The antifungal properties of short chain fatty acids were first recognized by the food industry, when it was noticed they inhibited fungal growth in baked goods. Soon it was found they also inhibited the proliferation of mold on cheese. Peck and Rosenfeld, in 1939 (1), observed that organic fatty acids present in sweat had fungicidal activity with no skin irritating effects, in contrast to existing preparations used for treating dermatophytoses.

These observations led to clinical trials including shorter chain fatty acids and undecylenic acid. Various formulations with undecylenic acid and its sodium, zinc or calcium salts were tried.

New studies have been made from then. All demonstrated the effectiveness of zinc undecylenate/undecylenic acid in treating tinea problems (2, 3, 4). Undecylenic acid and its derivatives are also well known for their dandruff and seborrhea control capacity. This makes them useful in every skin and hair disequilibrium.

As during the keratinization process a large quantity of enzymes coming from the Odland bodies (5) is exocited in the extracellular environment, Potassium Undecylenoyl Hydrolyzed Wheat Protein present on the skin can be split into its two components, that means undecylenic acid and hydrolyzed protein.

Free fatty acids are essential for the barrier effect of the skin (they keep skin supple, pliable and well moisturized) (5).

Hydrolyzed Proteins are able to form a continuous protective layer on skin and hair surface without influencing the cleansing or foaming capacity of formulations. This substantivity prevents penetration of other surfactants into the horny layer, avoiding so dryness and irritation. They give a soft and silky feel and contribute to the ability of the skin to absorb moisture.

To hair, proteins and derivatives have anti-static

effect (due probably to charge neutralization and moisture retention), are substantive, film forming, moisturizing and texturizing. They can also reduce damage resulting from permanent waving, oxidative dyeing, bleaching processes and UV radiations.

Potassium Undecylenoyl Hydrolyzed Wheat Protein can be therefore regarded as a mild surfactant able to supply skin and hair with their physiological components (proteins and fatty acids), biodegradable and effective.

Potassium Undecylenoyl Hydrolyzed Wheat Protein has the molecular structure reported in figure 1.

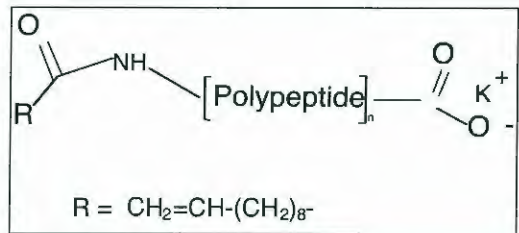


Fig. 1

It can be synthesized from castor oil by pyrolysis or by pyrolysis of methyl ricinoleate obtained by methanolysis of the oil. Castor oil is obtained from the plant of *Ricinus Communis*. Undecylenic acid is also a physiological component of human skin and hair.

In Potassium Undecylenoyl Hydrolyzed Wheat Protein, the presence of the undecylenic group and the peptide residue do not impart only cleansing characteristics and skin compatibility but also functional properties.

MATERIALS AND METHOD

Potassium Undecylenoyl Hydrolyzed Wheat Protein solution was provided by Zschimmer & Schwarz Italiana, Tricerro.

Other surfactants, Piroctone Olamine and Zinc

Pyrithione solutions were provided by the same company. Reagents were analytical grade from Sigma Aldrich.

MIC tests were previously carried out by Biolab and Microna using standard protocols.

Dandruff control and in vivo innocuity tests were performed by ISPE Laboratoires while *in vitro* test by ABICH, always using standard international protocols.

Dandruff control capability

Dandruff, the most common and annoying scalp disease, induced by a disequilibrium of cutaneous homeostasis, is worsened by growth of saprophyte microorganisms.

Pytyrosporum ovale is common believed to be involved in this phenomenon; although it is observed also when dandruff is not present, it has a double concentration than in non-affected scalp (6) in the skin areas with dandruff. Furthermore, *Pytyrosporum ovale* is able to cleave sebum and give rise to free fatty acids. The lipoperoxides they originate are frequently responsible for irritation that can make worse dandruff phenomenon (6).

MIC (Minimal Inhibitory Concentration) of Potassium Undecylenoyl Hydrolyzed Wheat Protein towards *Pytyrosporum Ovale* and *Malassezia Furfur* was determined in a serial

dilution test in liquid broth at pH 5.5. Table I shows the obtained results (7).

Microorganisms	MIC in %
<i>Pytyrosporum ovale</i>	2.5
<i>Malassezia Furfur</i>	3.5

An in vivo test was also performed comparing different samples. Piroctone Olamine, a well-known antidandruff agent, was used as a reference. Sample examined were the following:

Sample A = Sodium Laureth Sulfate (10.5% active matter) plus Potassium Undecylenoyl Hydrolyzed Wheat Protein (4% as it is)

Sample B = Sodium Laureth Sulfate (11.25% active matter) plus 0.75% Piroctone Olamine.

Sample C = Sodium Laureth Sulfate (10.9% active matter) plus Potassium Undecylenoyl Hydrolyzed Wheat Protein (2.5% as it is) plus 0.15% Piroctone Olamine.

30 volunteers aged between 18 and 60 years with dandruff problem had to use one of the three samples at home at least three times a week for about one month. Dandruff scales were weighed before and after the treatment. Means values and standard deviations were calculated. Figure 2 shows the obtained results (8).

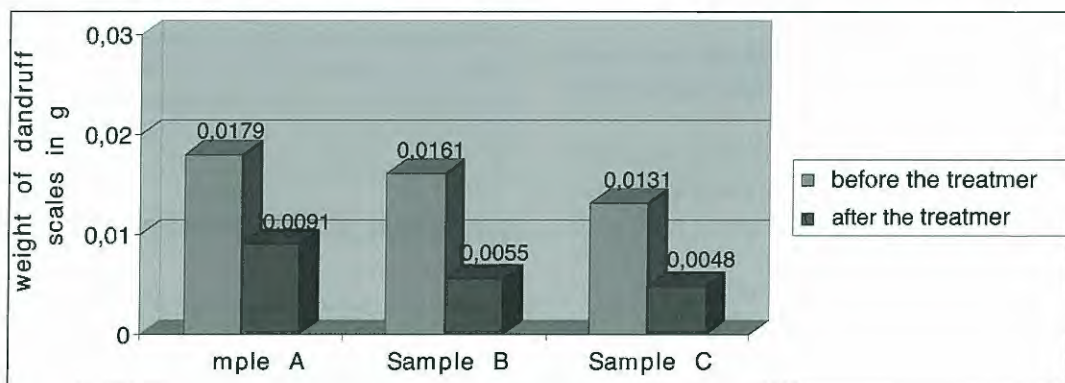


Fig. 2

The effectiveness of the three samples was statistically compared and all they showed an effective reduction of dandruff scales weight ($p < 0.1$).

The three samples were then statistically compared by mean of Analysis of Variance and no differences in their activities were found ($p > 0.1$).

Skin and hair disorders

Many microorganisms are involved in skin and hair disorders.

Pityrosporum ovale is believed to be involved in many skin disorders, such as seborrheic dermatitis, folliculitis, confluent and reticulate papillomatosis and psoriatic lesions (9).

Tricophyton and *Epidermophyton* are fungi responsible for skin, hair and nail disorders. They attack keratins and cause many pathologies. We remember tinea capitis, typical of children, tinea barbae, tinea corporis, tinea pedis (the most widespread in Italy) that is typical for sportsmen and people who attend swimming pools and showers in crowded places and gives the so called athlete foot, tinea favosa (typical for Africa and Medium Oriente), tinea cruris (for inguen) and tinea unguium (10).

Candida Albicans is also a problematic microorganism.

MIC (Minimal Inhibitory Concentration) of Potassium Undecylenoyl Hydrolyzed Wheat Protein was determined in a serial dilution test in liquid broth at pH 5.5 with different microorganisms. Table II shows the obtained results (7,11).

Microorganisms	MIC in %
<i>Pytyrosporum ovale</i>	2.5
<i>Malassezia Furfur</i>	3.5
<i>Ephidermophyton</i>	3.5
<i>Tricophyton Mentagrophite</i>	3.5
<i>Candida Albicans</i>	1.0

Skin with mycosis or excessive bacterial development is often irritated; a mild detersion becomes important in order to prepare the skin surface to pharmaceutical treatments and to restore its natural acidity.

Potassium Undecylenoyl Hydrolyzed Wheat Protein maintains the skin compatibility characteristics of hydrolyzed proteins and can therefore help the skin to be well moisturized.

Dermal innocuity

Skin irritation was evaluated through a patch-test on 20 volunteers (12). The method consists in an occlusive application of the product by means of Finn chamber (aluminium cells of 20 microliters volume) on subjects' volar part of the forearm. The irritating activity was clinically evaluated by observing the erythema induced by the product:

> 30 minutes after application (immediate irritative power)

> 48 hours after application (irritative power)

The substance was tested at a concentration of 22% as it is.

The immediate irritative power and the irritative power were separately evaluated.

The irritative power was evaluated considering:

- the number of reactions that the product caused related to the total number of subjects

- the severity of the irritative reactions.

Potassium Undecylenoyl Hydrolyzed Wheat Protein was void of any irritation potential.

Hypoallergenicity was evaluated through a patch test on 20 volunteers (13).

The method consists in an occlusive application of the product by means of Finn chamber (aluminium cells of 20 microliters volume) on subjects' volar part of the forearm for 48 hours.

After the removal of the occlusion, the cutaneous reactions induced by the product are evaluated. These evaluations are performed 24 and 48 hours later.

The repeated control of the treated areas allows to trace the presence and/or the activity in elicitation of cutaneous reactions of common allergens.

The allergic potential is expressed as a percentage of allergic reactions and it is evaluated considering:

- the number of reactions observed
- severity, type and duration of reactions.

Potassium Undecylenoyl Hydrolyzed Wheat Protein tested at 22% as it is can be classified as not sensitizing.

In vitro methods are an interesting alternative system to traditional *in vivo* tests to evaluate biological properties of cosmetic ingredients and products, according to the current European cosmetic rules that ask manufacturers to assess the product safety, without employing animals. Citotoxicity tests can be carried out in order to evaluate *in vitro* the citotoxicity of cosmetic ingredients on keratinocytes cultures. The *in vitro* test on skin-derived cells is a simplified but yet very informative model of the reactions that may occur *in vivo*.

The test was performed on a 3D epidermis obtained from epidermal keratinocytes seeded on a collagen matrix and grown in a serum-free medium to reach a multilayer conformation with a differentiated stratum corneum at the surface. The products to be tested is placed in contact with the 3D epidermis for 24 hours. Sodium Lauryl Sulfate is used as a reference.

At the end of the exposure period, the epidermis layers are disrupted with the enzyme trypsin and the cytotoxicity is evaluated through the quantification of surviving cell percentage.

Surviving cells are able to cleave a dye (MTT) in order to have a coloured solution that can be spectrophotometrically evaluated.

The absorbance is measured at 570 nm on a microplate reader, with background reading at 690 nm.

The results of the MTT test are expressed in terms of viability:

$$\% \text{ Viability} = \frac{\text{OD}_{570} \text{ treated cultures} \times 100}{\text{OD}_{570} \text{ untreated control cultures}}$$

Potassium Undecylenoyl Hydrolyzed Wheat Protein tested at 4% as it shows a percentage of cell survival higher than 100% (14).

***In vitro* sensitization potential**

Under normal physiological conditions, resting skin keratinocytes produce usually a number of cytokines. Under stressful conditions, such as exposure to ultraviolet light, tumor promoters or chemical agents, epidermal keratinocytes release the inflammatory cytokines interleukin-1 α (IL-1 α), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α) as well as interferon- α (IFN- α) and Interleukin 6 (IL-6) which trigger cutaneous inflammation. Consequently, the appearance of these cytokine markers in the cell culture medium suggests the initiation of an inflammatory cascade and therefore, IL-1 α and TNF- α are excellent indicators of skin inflammation.

In this study, the sensitization potential of Potassium Undecylenoyl Hydrolyzed Wheat Protein was compared with the classical anti-dandruff agents. In the study IL-1 α has been determined using ELISA test kit.

The test allows the titration of cytokines in the cell medium by reading the absorbance at defined wavelengths, after specific identification with colorimetric reaction based on antibodies. Sodium Lauryl Sulfate (SLS) 0.5% was used as a reference.

Potassium Undecylenoyl Hydrolyzed Wheat Protein (PUHWP) was compared with solutions of 0.75% Piroctone Olamine and 1% of Zinc Pyrithione.

Tab. III

pg/ml IL-1 alfa				
	SLS 0,5%	Potassium Undecylenoyl Hydrolyzed Wheat Protein (PUHWP) 4%	Piroctone Olamine 0,75%	Zinc Pyrithione 1%
2 h	42	24	29.5	15
6 h	52	39	44	28
18 h	789	42	76	60

Table III shows the obtained results.

Potassium Undecylenoyl Hydrolyzed Wheat shows the lowest inflammatory potential, mainly increasing time contact. It is less toxic than traditional antidandruff agents and it can be used at higher concentrations.

CONCLUSIONS

Surfactants are nowadays not only surface modifiers, dirt removers and/or foam boosters but also multifunctional molecules.

Potassium Undecylenoyl Hydrolyzed Wheat Protein offers a mild and effective solution for dandruff problem and skin/hair disorders.

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