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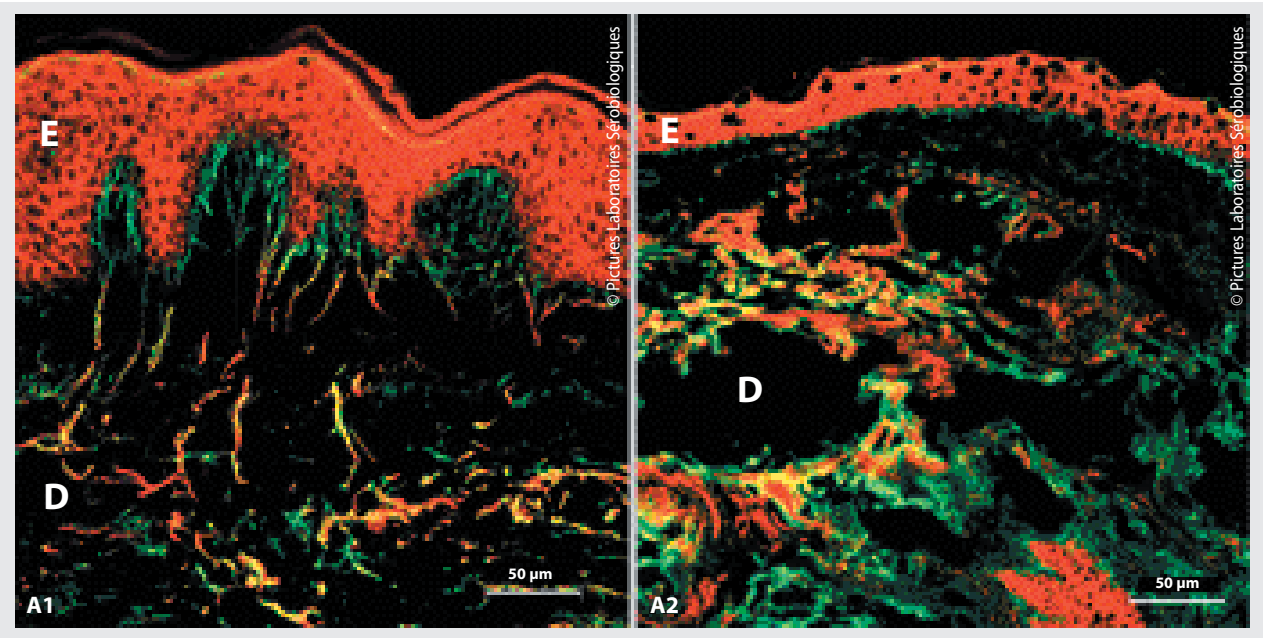
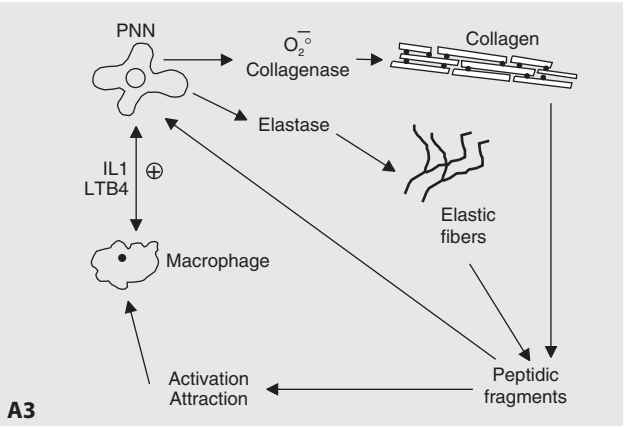
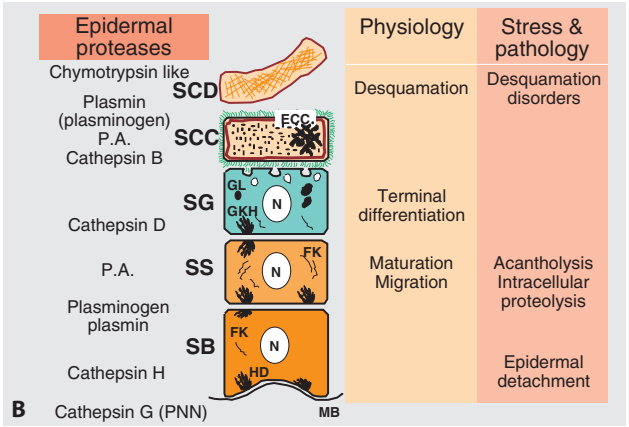
PROTEASYL® TP LS 8657

BOTANICAL ANTI-ENZYME COMPLEX FOR FIRMNESS AND ELASTICITY







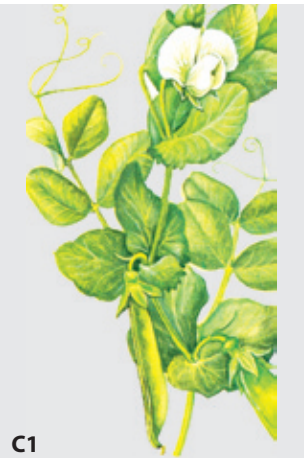


Fig. 1 – PROTEASYL® TP LS 8657 = Nature, Origin, Field of activity, Effects.

A = At the dermal level, the integrity of the protein network (elastic and collagen fibers) results from a balance between the rates of proteases (elastase, collagenase) and their inhibitors:
- *A1: illustrates young skin,*
- *an aged skin (A2) is, on the contrary, characterized by a degradation of the elastic and collagen network (excess of proteases and deficiency of inhibitors) resulting in a loss of tonicity and suppleness (see mechanism = A3).*

D = dermis, E = epidermis

B = At the epidermal level, skin's attacks induce an activation of the proteases, leading to cell damage (mechanism).

C = PROTEASYL® TP LS 8657 is a peptide obtained by extraction and purification from Pisum sativum L.

ACTIVE INGREDIENT FOR COSMETOLOGY
SKIN

Skin proteases and anti-proteases

1. Skin proteases

Proteases are enzymes which can degrade proteins by hydrolysis into peptides and constitutive amino acids. They can be divided into exopeptidases and endopeptidases, and into intra- and extracellular proteases.

1.1 Proteases in normal skin

• *Dermal proteases*

Dermal proteases renew structural proteins: elastic fibers and collagen of the extracellular matrix via the action of fibroblastic collagenases and elastases.

• *Epidermal proteases (Fig. 1C)*

These proteases play a fundamental role in epidermal cell differentiation, desquamation and moisturization.

1.2 Activation of skin proteases during aggressions

Skin aggressions (UV, chemical, immunological…) and cutaneous inflammation induce a major increase in proteases.

• *Dermal proteases (Fig. 1B)*

Polynuclear neutrophils (PNNs) activated by the inflammation secrete proteases (collagenase, elastase) lysing collagen and elastin. The released peptides, along with other factors (IL1, LTB4) also attract and activate PNNs and macrophages; the lytic reaction is boosted.

• *Epidermal proteases (Fig. 1C)*

The abnormally activated proteases cause some disturbances in normal desquamation, differentiation and moisturization. In advanced cases, an acantholysis (extrakeratinocytary lysis by destruction of the intercellular links leading the separation of the stratum spinosum cells) or an intrakeratinocytary proteolysis may be observed.

2. Inhibitors of skin proteases

The narrow balance between proteases and their respective more or less specific inhibitors, contributes to the balance between formation and turnover of cells and biomolecules within the extracellular spaces of the various cutaneous areas: hypodermis, dermis, epidermis, horny layers.

Proteasic inhibitors play an essential role in the regulation of many physiological processes.

3. Protease/protease inhibitor imbalance:

3.1 During skin aging[1, 2] (Fig. 1 B)

A rarefaction and a degradation of the dermal elastic network can be observed. The main causes are:

- a decrease of the biosynthetic activity of elastin and collagen by fibroblasts,
- an increase of elastolysis due to a relative increase of the elastasic and collagenasic activities and to a reduction of the natural inhibitors of elastase and collagenase.

The quantity of dermal collagen having strongly decreased, the ratio between collagen biosynthesis and its degradation by collagenases becomes negative with age. The result is a predominance of catabolytic activities.

In photoexposed areas, collagenases are especially activated (via cytokines and prostaglandins) and poor quality of (non cross-linked) non-functional elastin accumulates.

The receptors of fibroblasts become less interdependent on the elastic fibers and the collagen of the extracellular matrix, hence a decrease of skin elasticity and tonicity.

3.2 During skin aggressions (Fig. 1 C)

Activation of skin proteases due to acute environmental aggressions may cause major skin damages[3]. The repetition of such aggressions and the resulting proteolytic damages contribute to skin aging.

PROTEASYL® TP LS 8657

CONCEPT OF ANTI-PROTEASES

Certain proteases inhibitors (elastase, collagenase, trypsin, cathepsin, plasmin…) offer interesting features. When in excess, they contribute to skin aging and damage by environmental aggressions.

The permanent remodeling of the skin requires a narrow balance between dermal and epidermal proteases and their inhibitors.

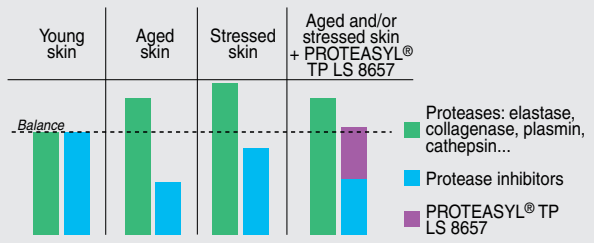


***Fig. 2** – Schematic representation of the dermal and epidermal anti-protease balance that led to the concept of PROTEASYL® TP LS 8657.*

DEFINITION / COMPOSITION

PROTEASYL® TP LS 8657 is a **new anti-protease** extracted from seeds of **Pisum sativum L.** (**peas**). First grown in the Near East, pea has been domesticated since Antiquity.

PROTEASYL® TP LS 8657 is a botanical peptidic active ingredient, prepared according to an exclusive extraction/purification technology. PROTEASYL® TP LS 8657 is supplied as a ready-to-use concentrated hydrosolute.

SKIN BENEFITS

1. **Anti-elastase, anti-collagenase and anti-free radicals properties**.
2. **Protector of dermal proteins: anti-aging effect**.
   Protects collagen and elastin against the deleterious effects of proteases activated during aging and stress. PROTEASYL® TP LS 8657 strengthens the activity rate of protease inhibitors, helping to restore the balance between proteases and anti-proteases (Fig. 2).
3. **Protection and repair of epidermal proteins**.
   Against damages induced by proteases activated by environmental aggressions (pollution, stress, UV…).
4. **Strengthening skin elasticity**.
   Anti-aging effect for slackened, atonic skin.
5. **Long-lasting hydroregulator**.
   Intervening according to a unique mechanism of action: inhibition of an excess of proteases within horny layers[4].

COSMETIC USE

PROTEASYL® TP LS 8657 can be used for:
- anti-age, protecting, repairing, skin defense care,
- elastifying, firming, moisturizing care,
- soothing, anti-irritating, anti-stressed skin, sun care,
- body care (stretch marks).

DOSAGE / SOLUBILITY / MODE OF INCORPORATION

1. Dose of use: 3 to 10%.
2. Solubility: soluble in water and insoluble in oils.
3. Mode of incorporation: PROTEASYL® TP LS 8657 is incorporated into the finishing process at 45°C, or at room temperature for cold processing.

ANALYTICAL CHARACTERISTICS

1. Aspect: light yellow limpid liquid, with a weak odor.
2. Specifications: upon request.

TOLERANCE

Good.

EFFICACY

Test summaries hereafter.

STORAGE

In its original packaging, at 15 - 25°C.

INCI NAME

Pisum Sativum (Pea) Extract.

MANUFACTURER

Laboratoires Sérobiologiques / Cognis France S.A.

[1] Robert et al.: Aging of Connective tissues. General considerations in *"Frontiers of matrix biology"*. S. Karger, Basel - Vol. 1, 1-45, 1973
[2] Bouissou et al.: Cutaneous aging. Its relation with arterio-sclerosis and atheroma. In *"Frontiers of matrix biology"*. S. Karger, Basel - Vol. 1, 190-211, 1973
[3] Eaglstein (W.H.) et al.: Ultraviolet radiation induced inflammation and leukocytes. *J. Invest. Dermatol.*, 72, 59-63, 1979
[4] Kitamura (K.) et al.: Research on the mechanism by which dry skin occurs and the development of an effective compound for its treatment. In *18th IFSCC Congress*, Venezia - Italy - Vol. 1, 131-166, 1994

PLASMIN INHIBITION (IN VITRO)

Aim / Protocol
Demonstration of the anti-plasmin effect of PROTEASYL® TP LS 8657. Plasmin is an epidermal serine protease, activated during skin aggressions. It induces epidermal destruction and acantholysis (= extra-keratinocyte lysis by destruction of the intercellular links leading to separation of stratum spinosum cells).
The effect of PROTEASYL® TP LS 8657 was studied on human skin, and visualized by histological staining of skin cross sections.

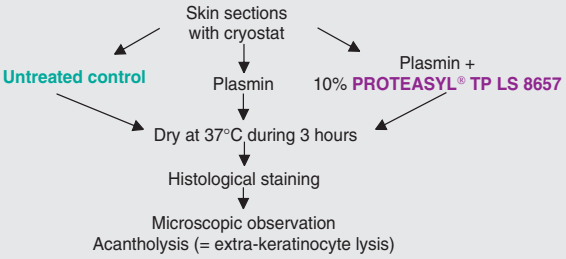


Fig. 3 *– Experimental protocol to study the anti-proteasic effect of PROTEASYL® TP LS 8657 in solution at 10% on epidermis.*

Results (Fig. 4)

Conclusion
Plasmin induces epidermal alterations ranging from minimal extra-keratinocyte lysis and acantholysis to total destruction of the epidermis.
PROTEASYL® TP LS 8657 at 10% almost completely neutralized the deleterious action of plasmin: skin treated with PROTEASYL® TP LS 8657 preserves its normal cell organization.

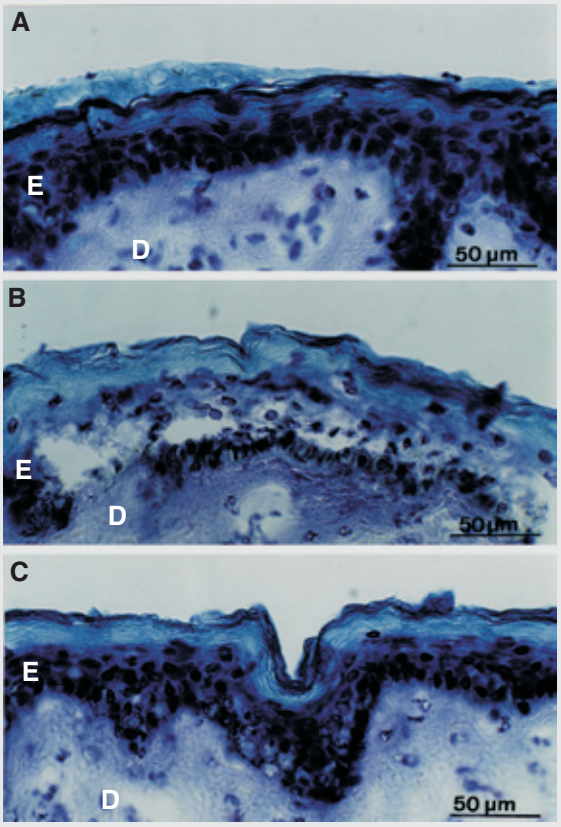


Fig. 4 *– Histological illustration of the effect of PROTEASYL® TP LS 8657 as an inhibitor of an excess of plasmin on human epidermis. Anti-stress effect.*
A *- Untreated control area.*
B *- Area treated with plasmin alone: destruction of the epidermis.*
C *- Area treated with plasmin + 10% PROTEASYL® TP LS 8657: perfect preservation of the epidermis. E = epidermis, D = dermis.*

ANTI-ELASTASE ACTIVITY ON HUMAN SKIN (EX VIVO)

Aim
Elastases are proteases that specifically destroy the network of elastic fibers, at a neutral pH.
Skin aging generally appears at around 40-50 years old and is characterized by:
- an increase in the elastase activity of dermal fibroblasts,
- a decrease in the concentration of elastase inhibitors regulating the turnover of elastic tissue.

Thus, a degradation of the dermal elastic network causes a deterioration of the functional properties of the skin: elasticity, suppleness and tonicity.

Protocol (Fig. 5)
Human skin biopsies.
Treatment:
- untreated control,
- elastase,
- elastase + 5% PROTEASYL® TP LS 8657.

Incubation during 2 hours.
Staining by the Weigert's technique.
Optical microscopy + image analysis.

Results (Fig. 6)
For a dose of elastase causing complete disappearance of elastic fibers, the addition of 5% PROTEASYL® TP LS 8657 significantly preserved:
- 50% of the papillary elastic fibers,
- 35% of the reticular elastic fibers.

Conclusion
PROTEASYL® TP LS 8657 exhibited a good anti-elastase activity on skin.

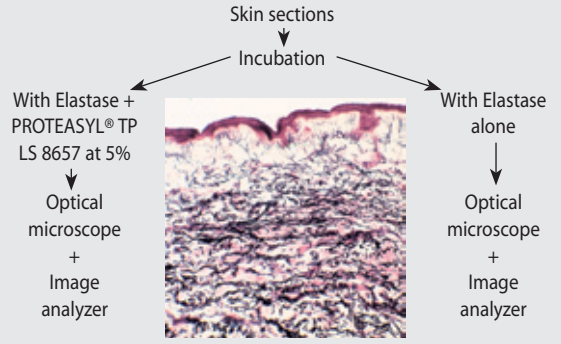


Fig. 5 *– Protocol.*

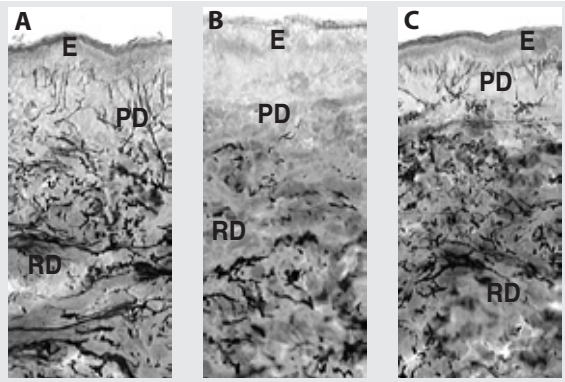


Fig. 6 *– Cross section of human skin illustrating the protecting effect of 5% PROTEASYL® TP LS 8657 against the destroying effect of elastase on the dermal network. Three experimental conditions A, B and C:*
A*: Untreated control area: integrity of the dermal elastic network.*
B*: Skin treated with the elastase enzyme alone: almost total destruction of the elastic network.*
C*: Skin treated by elastase + 5% PROTEASYL® TP LS 8657: good preservation of the papillary and reticular dermal elastic network.*
E = epidermis, PD = papillary dermis, RD = reticular dermis.

MOISTURIZING ACTIVITY ON THE HORNY LAYER OF HUMAN SKIN (EX VIVO)

Aim
Evaluation of the moisturizing effect of PROTEASYL® TP LS 8657 by measurements of dielectric conductivity on the horny layer (stratum corneum) of human skin (Tagami's method).

Protocol
Skin biopsies: the epidermis is separated from the dermis and the horny layer is isolated.
The samples of horny layer are tested under 3 differents conditions:
- untreated, control area,
- area treated with a placebo gel,
- area treated with a gel containing 5% PROTEASYL® TP LS 8657 (1 mg/cm^2).

Three treatments with the hydrogels have been made within 30 minutes of interval. Conductivity measurements were made up to 24 hours after the last application in standard conditions (t = 20°C, RH = 40%).

Results (Fig. 7)

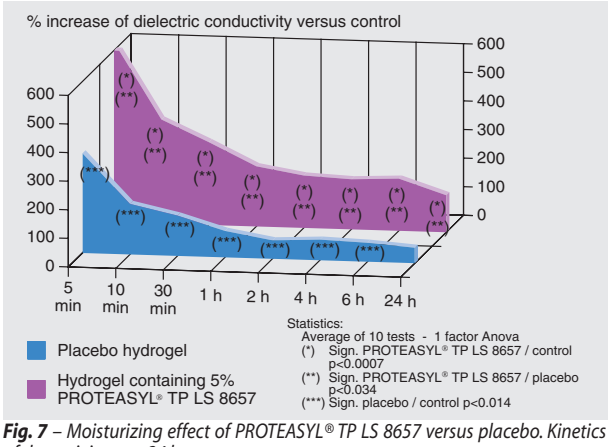


Fig. 7 *– Moisturizing effect of PROTEASYL® TP LS 8657 versus placebo. Kinetics of the activity over 24 hours.*

Conclusion
The moisturizing effect of PROTEASYL® TP LS 8657 was significantly higher than the placebo up to 24 hours after application: 60% higher for all time periods. It was long-lasting even after 24 hours.

IMPROVEMENT OF SKIN ELASTICITY (CLINICAL STUDY)

Aim / Protocol
The improvement of skin elasticity with a cream containing 3% PROTEASYL® TP LS 8657 was compared to that of a placebo cream.

Double blind clinical study in 10 female volunteers from 51 to 65 years old, having a slackened skin with a loss of elasticity on the external side of the arms and forearms.

Twice daily randomized treatment for 3 weeks:
- placebo cream on one side,
- cream with 3% PROTEASYL® TP LS 8657 on the other side.

Quantitative measurements of skin elasticity by vertical extensiometry (Cutometer) after repeated constraints. Comparative study of the effect of PROTEASYL® TP LS 8657 12 hours and 24 hours after the last application, after the 3 weeks of treatment.

Results (Fig. 8)

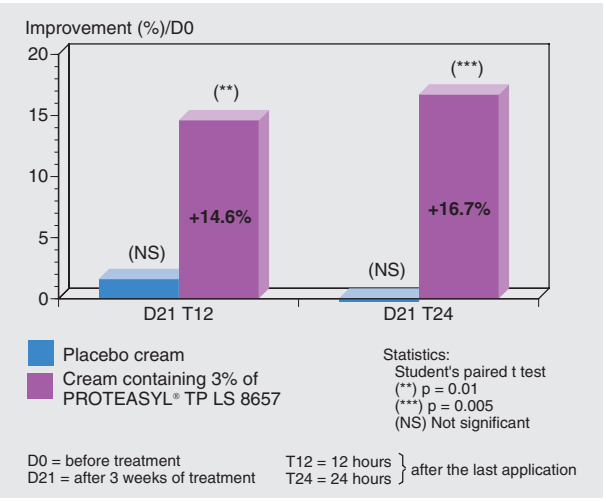


Fig. 8 *– PROTEASYL® TP LS 8657: strengthening effect of skin elasticity versus placebo.*

Conclusion
The cream containing 3% PROTEASYL® TP LS 8657 significantly improved skin elasticity 12 hours (+14.6%) and 24 hours (+16.7%) after the last application, after 3 weeks of treatment. The placebo cream had no effect under the same conditions.

IMPROVEMENT OF SKIN FIRMNESS (CLINICAL STUDY)

Aim
Laboratoires Sérobiologiques have developed an exclusive device to evaluate skin firmness, based on the cutimeter's model: the **dermofirmometer**.

This device looks like a skin fold clipper, it is equipped with a sensor position measuring the skin fold thickness and then calculating its compressibility, related to skin firmness.

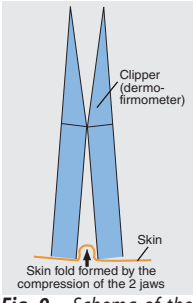


Fig. 9 *– Schema of the dermofirmometer.*

Protocol
Double blind clinical study on 10 female volunteers, aged from 46 to 64 years old, having a loss of skin firmness on their forearms.
Bi-daily randomized treatement during 6 weeks, in the morning and in the evening, with:
- a placebo cream on one forearm,
- a cream containing 5% PROTEASYL® TP LS 8657 on the other forearm.

The cutaneous compressibility was evaluated before and after the 6 weeks of treatment, with the dermofirmometer.

Results

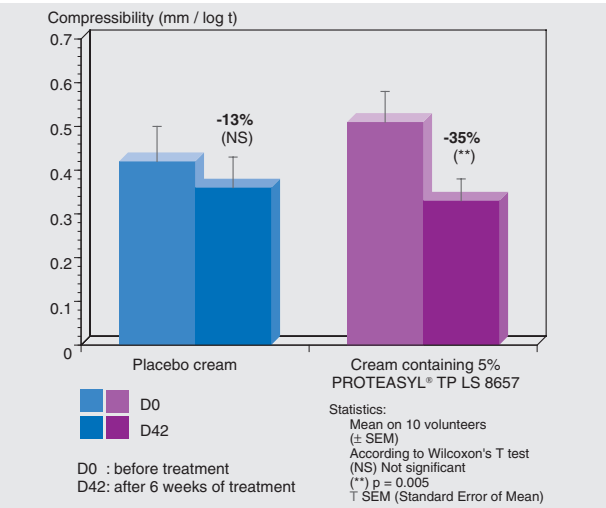


Fig. 10 *– PROTEASYL® TP LS 8657: results after 6 weeks of treatment.*

Conclusion
After 6 weeks of treatment, the cream containing 5% PROTEASYL® TP LS 8657 has increased, by +35%, the skin firmness of the forearms.

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