

Original Article

Evaluation of pbserum Extreme Firmness' Efficacy on the Skin Firmness and Elasticity

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ABSTRACT

Introduction. Loss of firmness and elasticity of the skin is a complex phenomenon involving changes in the dermis, epidermis; and is a distinctive feature of skin aging. Peelings with recombinant enzymes may constitute an effective tool to improve the signs mentioned above because those enzymes combine keratinase PB333, collagenase PB220, DMAE, vitamin C, and hyaluronic acid. **Objective.** A study was carried out to evaluate the efficacy and tolerability of the pbserum Extreme Firmness Professional product in subjects between 24 and 70 years old to measure the firmness and elasticity of the skin before and after continued use of the product. **Material and Methods.** Following the instructions, participants applied the product containing PB333 keratinase, PB220 collagenase, DMAE, vitamin C, and hyaluronic acid weekly and avoided using other skin care products in the test area. The firmness and elasticity of the skin were evaluated using the Cutometer® dual MPA 580, the organoleptic properties and the result of the treatment, as well as adverse events and dermatological reactions. **Results.** The study's results showed that after 28 days of use, skin elasticity improved by an average of 11 % compared to baseline, which was statistically significant. Additionally, 96% of the subjects were satisfied with the results of the product on their skin, with no reports of adverse events. **Discussion.** Our findings prove, in a statistically significant way, an improvement in the skin's elasticity, hydration, and softness in the subjects who used the product compared to the control group. **Conclusions.** The study concluded that combining recombinant enzymes of keratinase PB333, collagenase PB220, DMAE, vitamin C, and hyaluronic acid effectively improves skin elasticity in people with sagging skin.

Keywords: elasticity, keratinase, collagenase, Cutometer dual MPA 580, clinical results

RESUMEN

Introducción. La pérdida de firmeza y elasticidad de la piel es un fenómeno complejo que involucra cambios a nivel de la dermis, la epidermis y es un signo distintivo del envejecimiento cutáneo. Las enzimas recombinantes pueden ser una herramienta efectiva para mejorar estos signos porque combinan queratinasa PB333, colagenasa PB220, DMAE, vitamina C y ácido hialurónico. **Objetivo.** Se llevó a cabo un estudio para evaluar la eficacia y tolerabilidad del producto pbserum Extreme Firmness Professional en sujetos entre 24 a 70 años de edad para medir la firmeza y elasticidad de la piel antes y después del uso continuado del producto. **Material y métodos.** Los participantes

aplicaron semanalmente el producto que contiene queratinasa PB333, collagenasa PB220, DMAE, vitamina C y ácido hialurónico, siguiendo las instrucciones proporcionadas y evitaron el uso de otros productos para el cuidado de la piel en la zona de prueba. Se evaluó la firmeza y la elasticidad de la piel mediante el Cutometer® dual MPA 580, la satisfacción con las propiedades organolépticas y con el resultado del tratamiento, así como los eventos adversos y reacciones dermatológicas. **Resultados.** Los resultados del estudio mostraron que después de 28 días de uso, la elasticidad de la piel mejoró en un promedio del 11%, en comparación con la línea de base, lo que fue estadísticamente significativo. Adicionalmente, el 96% de los sujetos se mostró satisfecho con los resultados del producto en su piel. No hubo reporte de eventos adversos. **Discusión.** Nuestros hallazgos comprueban, de manera estadísticamente significativa, mejoría en la elasticidad, hidratación y suavidad de la piel en el grupo de sujetos que utilizaron el producto, en comparación con el grupo control. **Conclusiones.** El estudio concluyó que la combinación de enzimas recombinantes de queratinasa PB333, collagenasa PB220, DMAE, vitamina C y ácido hialurónico es eficaz para mejorar la elasticidad de la piel en personas con flacidez cutánea.

Palabras clave: elasticidad, queratinasa, collagenasa, Cutometer® dual MPA 580, resultados clínicos

INTRODUCTION

The loss of skin's firmness and elasticity is a complex phenomenon that involves changes at the dermis and epidermis level, and it is a distinctive sign of skin aging.¹ Such aging is characterized by the disorganization of collagen and elastin fibers and the alteration of glycoproteins and proteoglycans, which are responsible for maintaining the structure and density of this cutaneous layer. These changes result in a thinner and less smooth dermis, contributing to an aged skin appearance.^{1,2}

Firmness loss can result from intrinsic factors such as chronologic aging and extrinsic factors such as diet, lifestyle, environmental pollution exposure, lack of hydration, exposure to irritant agents, and damage caused by sun exposure. All these factors act in synergy to accelerate the skin aging process and the mentioned loss of firmness.³ As a result, skin shows a flabbiness appearance that usually comes with decreased luminosity and loss of hydration.³

Keeping the skin's ability to recover its original shape after stretching and compressing and maintaining its tension and resistance to flabbiness are essential attributes of staying youthful and healthy skin.

In the last few years, treatments promoting skin health and beauty have become very interesting and have achieved technological advances.

Cosmetic products with recombinant enzymes (a combination of keratinase PB333, collagenase PB220, DMAE, vitamin C, and hyaluronic acid) have empirically stood out in cosmetic dermatology practice as one of the most effective options to improve skin's firmness and elasticity.²

Combining those five active ingredients intends to recover fibroblasts, restore collagen and elastin, achieve more hydrated and luminous skin, and create a natural barrier against extrinsic factors such as pollution and UV rays that can accelerate skin aging.^{4,5}

Keratinase is an enzyme with a keratolytic effect that increases permeability and improves penetration of other active ingredients. Its topic use aims to eliminate dead cells in the corneous layer without irritating the skin, promoting cell renovation naturally. This enzyme specifically degrades the peptidic links of the globular keratin structural chains. Using it topically also creates channels that allow active principles to penetrate the skin's deep layers.⁵

Collagenases participate in many processes and have also been used in therapeutics. They are enzymes that can break collagen's peptidic links to physiologic pH. They belong to the matrix metalloproteinases family; they participate in physiologic processes that support collagen's integrity and reordering.

Collagen is the most abundant fibrillar protein in the human body, and for its correct functioning, collagenases are indispensable. Its primary function is the normal collagen's rechange, but its activity increases to achieve the extracellular matrix remodeling during wound healing. In therapeutics, collagenases are used to treat burns and ulcers and to eliminate scar tissue. They usually participate in the dermis repair and collaborate with the granulation and re-epithelization stage.⁵

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Dimethylaminoethanol (DMAE) has been used because it is a potential precursor of acetylcholine orally for treating some conduct disorders. Its topic use is based on its antioxidant effect.

Advances in cosmetic dermatology with the combination of recombinant enzymes (like the ones pbserum Extreme Firmness offers) are an effective solution because they cover both intrinsic and extrinsic factors that contribute to improving skin's elasticity and luminosity; they also counteract the opacity associated with skin's aging.⁴

This comprehensive approach improves skin quality, giving the patients a younger and healthier look.

The main objective of this randomized, double-blind, and placebo-controlled study (conducted between February and March 2023 in one single research center) was evaluating the efficacy and tolerability of the cosmetic product pbserum Extreme Firmness Professional in 22 subjects between 24 and 70 years old, after 28 days of continuous use on face and neck. The hypothesis was that the product, used with recombinant enzymes, is effective in improving skin's firmness and elasticity.

MATERIAL AND METHODS

Patients

The study was conducted following the Good Clinical Practices and the Helsinki Declaration. All subjects gave their written informed consent before starting the study.

Both men and women between 18 and 70 years old were included, with all kinds of skin (sensible and non-sensible); all skin conditions were accepted (dry, normal, mixed, oily).

The selected patients must have normal lax skin, weak firmness, and flabbiness (IBSA scale, grade 2).

Regarding exclusion criteria, patients with skin diseases like rosacea or eczema in the area of testing were discarded, as well as subjects with relevant dermatological pathologies (atopic dermatitis, psoriasis, lupus, rosacea, ringworm, etc.), pregnant or breastfeeding women, transplanted patients, immunosuppressed, with known allergies to cosmetic or industrial products, with a previous history of photosensitivity, cardiovascular, gastrointestinal, neurologic, metabolic, hematologic or psychiatric diseases; patients under treatment with antibiotics, antihistaminic, corticoids, beta-blockers, retinoids, azelaic acid or any anti-acne treatment during 15 days before the study.

Measurements

The 5-grade of tissue laxity photographic scale was used⁶:

1. Normal state of the tissues, without laxity
2. Mild laxity
3. Moderated laxity
4. Moderated to severe laxity
5. Severe laxity

The product's efficacy on skin firmness and elasticity was evaluated with the Cutometer® dual MPA 580 (Courage

& Khazaka electronic GmbH, Germany), which measures the skin's mechanical deformation.

Skin firmness was measured on the first day, before the application of the product, and 28 days after continuous use of the product (subjective evaluation). A measurement with the Cutometer® dual MPA 580 was made on every visit to the center for ten cycles. Each cycle consisted of two seconds of suction and two seconds of relaxation to fatigue the skin.

The parameters subjected to statistical analysis were firmness (parameter F4) —the lesser the value, the higher skin firmness— and elasticity (parameter R6), where a lower value reflects more skin elasticity.⁷

The dermatologic test for tolerance consisted of a dermatologist visually evaluating the treatment areas to determine possible skin alterations before and after the treatment.

Alterations to evaluate were: desquamation, dryness, acne, erythema, spots, edema, vesicles, and other lesions on a 5-point scale (0: absence, 1: very mild, 2: mild, 3: moderated, 4: intense). The researcher had to register any other skin alteration. He also registered if the alterations were related to the product used according to the scale:

- | | |
|---------------|-------------------------|
| • Not related | • Probable |
| • Improbable | • Positively related or |
| • Possible | • Non-evaluable |

Patients made a subjective evaluation of the organoleptic characteristics of the product according to a hedonistic 5-point scale:

1. I like it a lot

2. I like it a little
3. I don't like it nor dislike it
4. I dislike it a little
5. I dislike it a lot

Patients answered a subjective questionnaire regarding the product's efficacy with 5 points:

5. Totally agree
4. Agree
3. Nor agreement nor disagreement
2. Disagreement
1. Definitely disagree

Likewise, the fulfillment of expectations regarding the treatment was evaluated with a specific questionnaire. For secondary events, a specific report form was to be filled.

Treatment

The electable subjects had a non-rinsed solution on the face and neck once a week for 28 days.

Subjects had to use the product according to the directions and avoid using any other skin care product in the study area during the study period.

Directions for use were the following:

- Retire the black cap from both vials
- Open the dust and the liquid vials with the opener
- Pour the liquid into the dust vial
- Close the vial with the pipette and shake it vigorously for 30 seconds
- Apply the solution on the face and neck with the pipette (avoiding eye contour)
- Gently massage the solution on the skin until its complete absorption.

Cleaning frequency was the doctor's decision based on the patient's skin type.

During measurements, subjects stayed between 20 and 30 minutes in a room with air conditioning between 18-25°C and a relative humidity of 35-65%.

The researchers supervised the treatment's application to ensure the protocol was followed.

Study design

- **Initial visit (day 0).** Twenty-four subjects were

included. Inclusion and exclusion criteria were reviewed. Consented informed formats were signed. Skin firmness and elasticity were evaluated with the Cutometer® dual MPA 580. A dermatologic tolerance test was done. Directions for applying and product for 28 days of treatment were given.

- **Final visit (day 28 of treatment).** Skin firmness and elasticity were evaluated with the Cutometer® dual MPA 580. A subjective questionnaire of the product's efficacy and organoleptic characteristics was applied. A dermatologic tolerance test was done. The fulfillment of expectations regarding the treatment was evaluated.

Statistical analysis

Descriptive statistics was done for the results of the biometric parameters on both treatment and control groups and for every time of study, including average, standard deviation, and absolute variation, compared to control and baseline. Graphics were also used to show the average trend of the response variables.

Atypical values were identified with a box diagram and third quartile. Values over three times the third quartile were considered critical atypical values (the context of these data was considered to decide if they had to be eliminated).

Mixed effects linear mixed models (LMM, linear mixed models) were adjusted to evaluate the treatment's effect concerning control at every experimental moment.

Multiple measurements taken along time (related between them) were considered, including aleatory effects for every subject. When data could not be adjusted by LMM, a Student's t-test was used (if data followed a normal distribution) or a Wilcoxon test if data did not follow a normal distribution.

In the case of the data in the ordinal scale, a Wilcoxon signed-rank test of matched samples was used to evaluate the treatment effect on the response variable (comparing the experimental times) regarding the reference time because LMM cannot be adjusted to these data.

Besides, a matched sample Wilcoxon test was used to evaluate the treatment effect regarding the control at every experimental moment. The significance level was established at 0.05 (confidence interval of 95%) for every statistical test performed in the study.

RESULTS

Twenty-two subjects (19 women and 3 men) fulfilled the inclusion criteria and completed the study. Out of the 24 recruited subjects, 2 dropped out for non-compliance. The ages of the included patients were:

- for women, 4 under 50 years, 8 between 50 and 60 years, and 7 over 60 years
- for men, 2 were between 50 and 60 years old, and 1 was over 60

No patients reported adverse effects nor alterations in the tolerance dermatological evaluation.

Evaluation of efficacy on skin firmness

Twenty-eight days after continuous product use, firmness

improved by a median of 4% compared to baseline. This difference is not statistically significant, with a p-value over 0.05 (Graphics 1 and 2).

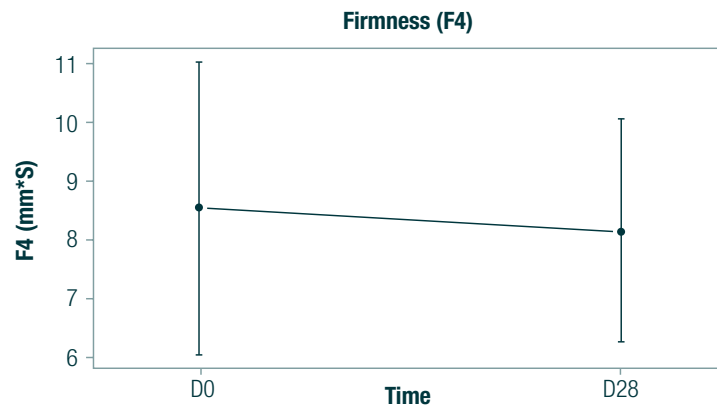
Evaluation of efficacy on skin elasticity

Twenty-eight days after continuous product use, elasticity improved by a median of 11% compared to the baseline.

This difference is statistically significant, with a p-value under 0.05 (Graphics 3 and 4).

The subjective evaluation of the product after 28 days of continuous use is shown in Graphics 5 and 6; the subjective evaluation of the product's efficacy after 28 days of continuous use is shown in Graphics 7 and 8.

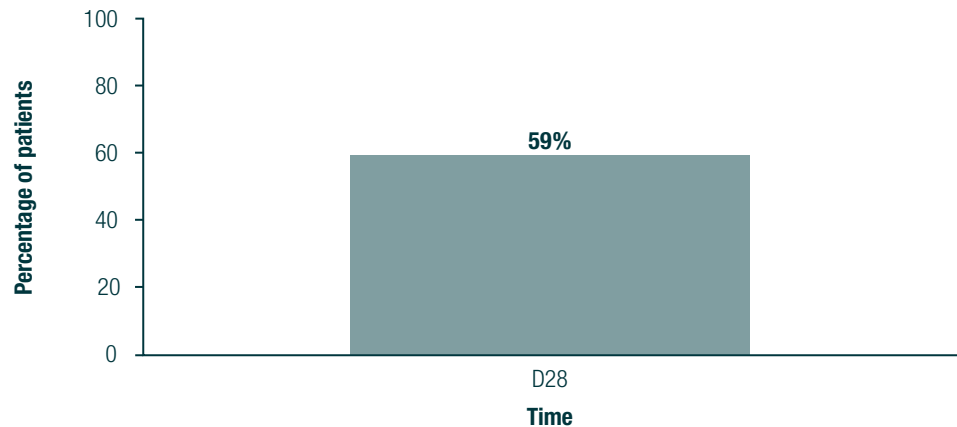
Graphic 1. Firmness determination



Parameter F4 $\pm$ SD at different experiment moments (D0 and D28)

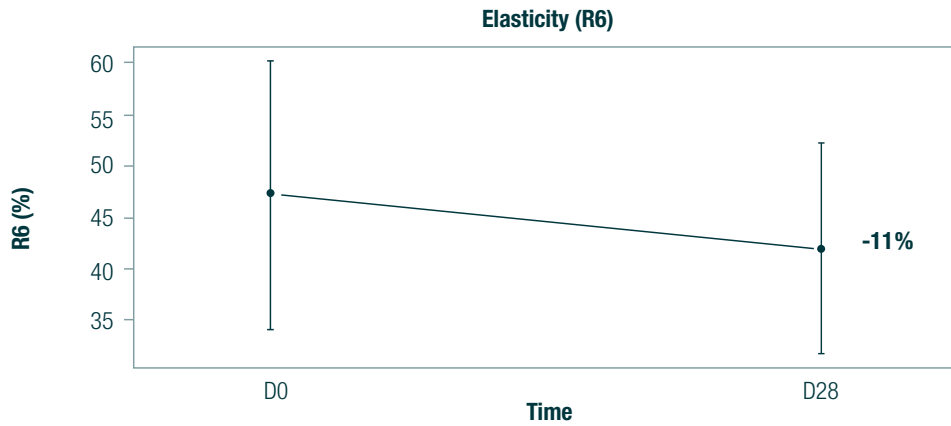
SD: standard deviation; D0: day zero; D28: day 28

Graphic 2. Percentage of subjects with improvement in firmness



Percentage of panelists with firmness improvement (D28)

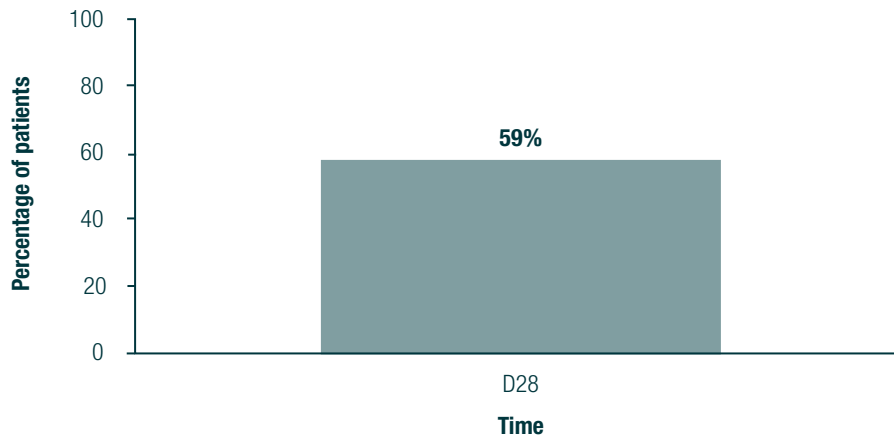
Graphic 3. Elasticity determination



Parameter $R6 \pm SD$ at different experiment moments (D0 and D28)

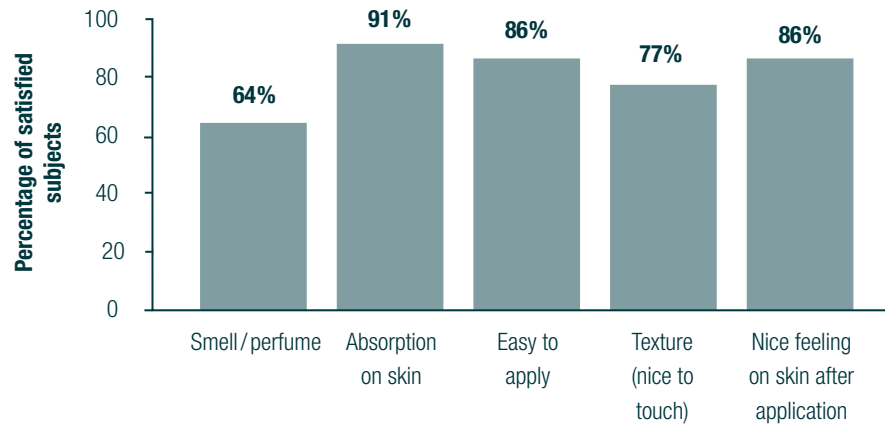
SD: standard deviation; D0: day zero; D28: day 28

Graphic 4. Percentage of subjects with elasticity improvement

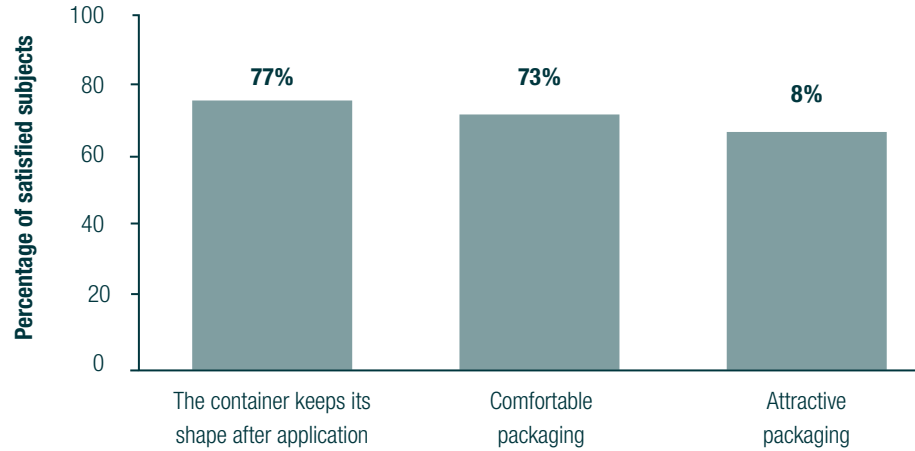


Percentage of panelists with elasticity improvement (D28)

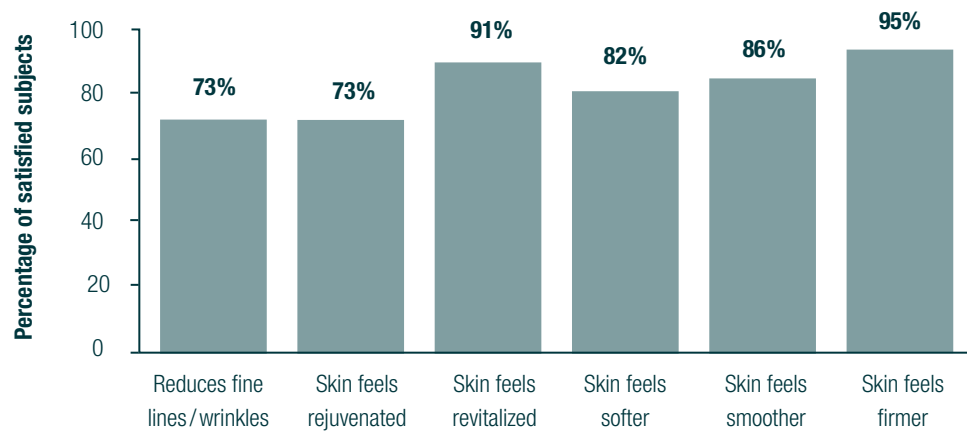
Graphic 5. Global appreciation of organoleptic characteristics



Graphic 6. Global appreciation of the package characteristics



Graphic 7. Evaluation of perceived efficacy



Graphic 8. Evaluation of perceived efficacy 2



Thirty-two percent of the subjects were very satisfied with the product's results on the skin, 64% was satisfied and 5% were indifferent.

Regarding general product expectations, 95% of the subjects were satisfied.

DISCUSSION

Our findings prove, in a statistically significant way, an improvement in skin elasticity, hydration, and softness in the group of subjects who used the product compared to the control group.

After continuous product use, elasticity increases by a median of 11% compared to the instrumental efficacy baseline.

After answering the questionnaires, subjective evaluations showed a high degree of satisfaction 28 days after continuous product use.

Ninety-six percent of the panelists were satisfied with the results of the product on the skin, and 87% manifested the intention of buying it. The product was well tolerated, and no adverse events were reported.

CONCLUSIONS

These results demonstrate the efficacy and safety of pbserum Extreme Firmness Professional in improving skin firmness and elasticity in subjects with flabbiness and lack of skin firmness. Cutometer® dual MPA 580 is a reliable method to measure skin firmness and elasticity. There is a need for subsequent prospective studies with biopsies.

CONFLICT OF INTEREST

Jorge López Berroa Works at Proteos Biotech SL, manufacturer of pbserum Extreme Firmness Professional; the rest of the authors declare no conflict of interest.