

# Clinical evaluation of the efficacy of bee venom as cosmetic active

## Abstract

**Objective:** Clinical evaluation of the efficacy of a treatment for facial skin through a cream which contains Bee Venom (BV).

**Methods:** BV was added to a cream for treating facial skin as an active component. Dosification of the melittin contents of BV was previously done. A medical cosmetologist performed the skin evaluation of 30 volunteers, who passed a BV irritation test and non-inclusion criteria. The clinical evaluation was carried out by evaluating the appearance of the state of skin by means of different indexes and quantifying fifteen attributes of the skin with 9-point scales, before starting the treatment, at 14 and 28 days.

**Results:** Participants were sorted in two clusters (C1 and C2) taking into account their skin state at the beginning of the study. Clusters were differentiated in age and photodamage, resulting that C2 participants were with better skin conditions. During treatment, both clusters increased their hydration, smoothness, firmness, lightness, youthful appearance, healthy appearance and general state of the skin. Moreover, C1 presented a decrease in depth of facial wrinkles and C2 an increase in elasticity and facial oval definition. The volunteers showed an excellent tolerance to treatment and high scores of satisfaction.

**Conclusion:** The methodology that was used resulted suitable in classifying facial skin of the volunteers as well as assessing the treatment outcomes using a cream with BV during 28 days. The BV, as an active cosmetic, contributed to improve the state of facial skin.

**Keywords:** clinical evaluation, Bee venom, cosmetic efficacy, emulsions, claim substantiation, statistics.

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## Introduction

Bee Venom (BV) consists of a complex mixture of several components that include proteins, peptides, amino acids, phospholipids, sugars as well as volatile and mineral components. Melittin is the main component of BV. Its presence is between 40 and 60% and it is used to characterize trade products of cosmetic use which contain BV.<sup>1,2</sup> BV can be produced by all bee species, highlighting the *Apis mellifera* which is used in apiculture worldwide.

Several studies have shown the powerful pharmacological effects of *Apis mellifera*'s BV, including the following: antibacterial, antiviral, anticancer, anti-inflammatory and anticoagulant.<sup>3-6</sup>

Also, a series of studies have endorsed the BV use of *Apis mellifera* for skin treatment with possible cosmetic effects. Among them, the evaluation of antioxidant effects, anti-inflammatory and cytotoxic properties.<sup>7,8</sup>

On the other hand, BV also has effects on different cells which can be related to skin care. Han et al.<sup>9</sup> reported BV action on human fibroblasts against UV radiations.<sup>4</sup> Han et al.<sup>9</sup> studied the effects of BV on keratinocyte migration in vitro, concluding that this active might be applied topically to accelerate wound healing.<sup>9</sup> Han et al. 2015a investigated the anti melanogenesis of BV action, finding that it might be used to treat pigmentation disorders.<sup>10</sup> By means of a clinical study, You et al. evaluated the effects of a product with BV in patients with atopic dermatitis.<sup>4</sup> Also by means of a clinical study, Han et al. 2015b studied the effect of an anti wrinkle serum with BV, concluding

that prolonged treatments with cosmetics containing BV might be considered efficient and safe.<sup>11</sup>

Bee venom is included in the database of ingredients of European Commission (Cosmetic Cosing) for cosmetic use. It is described as the venom collected from the honeybee, *Apis mellifera* consisting chiefly of melittin, to which astringent and skin protecting actions are attributed.<sup>12</sup>

Therefore, BV is used as an active component in several cosmetic formulations such as: facial creams, balsams, masks and serums according to its protecting properties against bacteria and skin inflammation.<sup>4</sup>

The efficacy of cosmetic products might be considered as an achievement of the main proposed objectives during their development, which is an important factor for the potential sales of any product. Different kinds of testings can be held in order to research on: the efficacy of cosmetics considering the effect which is aspired to assess, the population to whom the product is targeted, and the methodology that is going to be applied.<sup>13</sup> Among the methodologies used to assess the efficacy are the sensory and instrumental methods which allow to evaluate different aspects of the skin state and its annexes.

One of the sensory methods that is used to assess efficacy is the clinical evaluation performed by experts (dermatologists or cosmetologists), that used suitable senses and procedures to objectively assess the skin state, hair, etc. of the volunteers.<sup>14</sup> The parameters are evaluated by clinical observation and/or scoring. They

can be quantified by comparison with the initial results or with an untreated control or a placebo or a reference product.<sup>15</sup>

Numerous studies have used different methods for assessing efficacy or have studied its correlation with complementary methods.<sup>14</sup>

The aims of the present study was to assess the BV as potential ingredient of a cream for treating facial skin using clinical evaluation.

## Materials and methods

### BV procurement

Four different BV samples were collected between May and July 2019 from *Apis mellifera* hives located in an apiary in the northeast region of Uruguay. Each sample that was collected corresponded to bee venom from 5 neighboring hives. At the hive entrance, a BV Bee Whisper 0412 collector (IGK Electronics, Bulgaria) was used to collect the sample. A slight electrical impulse discharge (1.8V) was applied when the bees covered the entire surface of the collector frame and were in contact with the wire screen. This generated an immediate response from the mellifera bees that stung the surface of the crystal collector. After 40 minutes of working time, the device was automatically turned off. Later, the bee venom was scraped off the glass with a sharp scraper and a pool was made with all the extracted venom. The bee venom was stored at -18°C until later analysis.

### BV analysis

The analysis of melittin in bee venom was performed by HPLC using a RPC18 (250x4.6mm, 5µm) column and a 220nm UV detection system according to Rybak-Chmielewska and Szczêsna publication.<sup>16</sup> Melitina Sigma (lot 076M4044V, 93.63%) was used as a reference compound. The chromatographic separation was performed at 25°C. It was also used a linear elution program formed by 5%B-80%B per 40 min; 80%B-5%B from 40.1 to 41 min; 5%B from 41 to 45 min. The flow was of 2 ml/min and the injection volume of 50µL.

The solvents were: A, 0.1% of trifluoroacetic acid (HPLC grade, J.T. Baker) in water and B, 0.1% of trifluoroacetic acid (HPLC grade, J.T. Baker) in acetonitrile (HPLC grade, Merck)-water (80:20).

### Cosmetic base

An O/W emulsion was made whose composition included: aqua, cetearyl alcohol, glycerin, mineral oil, glyceryl stearate, cetareth 20, dimethicone, phenoxyethanol-ethylhexylglycerin, di sodium EDTA, with the incorporation of BV 0.006%. The ingredients used are authorized by MERCOSUR and European Union legislations.

### Efficacy assessment

#### a. Recruiting

30 Caucasian women were recruited with a good state of health and between 35 and 66 years-old. Volunteers had to fulfill the following non-inclusion criteria: pregnancy or breastfeeding, dermatologic affections, medical history of previous adverse reactions to cosmetics, use of drugs which could produce abnormal cutaneous response as well as localized or generalized dermatologic diseases.<sup>14</sup>

The volunteers read and signed an informed consent to participate in the study, endorsed by the Ethic Committee of Chemistry Faculty, Universidad de la República. The clinical studies were performed considering MERCOSUR Technical Regulation under the verification of good clinical research practices and the Declaration of Helsinki.<sup>17,18</sup>

An information survey of volunteers was performed. It included: age, marital status, level of studies, menopause, skin diseases, hormonal medication and habits (smoking, alcohol, diet), use of cosmetics for hygiene and skin treatment as well as the use of sunscreen protection.

#### b. Test of cutaneous tolerance

A clinical skin tolerance test (patch test) of a BV solution was applied to volunteers under medical surveillance in order to ensure that cosmetics with BV did not cause adverse reactions. With a syringe, a 0.1ml of BV solution to 0.001% was applied onto a 1.0cm<sup>2</sup> square piece of filter paper. The filter paper was applied to the forearm of the volunteers with a hypoallergenic occlusive tape.<sup>19</sup> The occlusive adhesive patch was kept during 48 hours. 48 hours after its application, the patch was removed and 30 minutes later the region of the application was evaluated. A second reading was performed at 96 hours.

#### c. Clinical evaluation of the skin state of the volunteers

Participants were asked to arrive to the interview with their skin clean. Subsequently, the volunteers were interviewed by a Medical Cosmetologist who examined their skin using LED lamp light with a magnifying glass. Photographs of their faces were taken under standardized conditions. The Cosmetologist classified the participants: a) according to phototype, using Fitzpatrick classification, b) determining the biotype of each volunteer considering Baumann skin type and c) evaluating the photoaging, through a Glogau scale.<sup>20</sup>

Moreover, the 15 attributes of each of the volunteers according to their facial skin condition were evaluated by a Medical Cosmetologist through a structured 9-point scales: wrinkles (1=no wrinkle to 9=many wrinkles), depth of wrinkle (1=less deep to 9=very deep), facial expression lines (1=none to 9=many), skin spots (1=none to 9=many), hydration (1=very dry to 9=very hydrated), smoothness (1=rough to 9=smooth), elasticity (1=less elastic to 9=very elastic), firmness/tonicity (1=less firm to 9=very firm), lightness (1=opaque to 9=very luminous), color uniformity (1=less uniform to 9=very uniform), oval face definition (1=less defined to 9=very defined), skin pores (1=few pores to 9=many pores), healthy skin appearance (1=very bad to 9=very good), youthful appearance (1=very bad to 9=very good) and general state (1=very bad to 9=very good).<sup>14</sup> Evaluations were performed in a room in which temperature and humidity were controlled (22±2°C and 60±10% of relative humidity).<sup>14</sup>

#### d. Product testing

Afterwards, a plastic pot with 50grams of product was given to the volunteers. Participants were instructed to use the products in the following way: The cream should be applied twice a day, preferably in the morning (after facial hygiene and before makeup) and at night (after facial hygiene and before going to bed). The cream should be applied to the face, and can also be applied to the neck and neckline, in the way that other creams are usually applied.

#### e. Clinical evaluation of treatment efficacy

At 14 and 28 days that treatment started, the volunteers were interviewed again by a Medical Cosmetologist who, using the same scales, reevaluated the 15 attributes related to the state of facial skin. Evaluation was performed under the same conditions in which the state of skin had initially been evaluated.

In addition, when the study was finished, the volunteers were asked to show their general satisfaction with the treatment through a

structured 9-point hedonic scale (1=dislike very much to 9=like very much) and a tolerance evaluation to the treatment with a structured 9- point (1=very bad to 9=very good).

**f. Data analysis**

Initially, it was determined the average and standard deviation (SD) of the data of the 15 attributes used to evaluate the skin condition. A Principal Component Analysis (PCA) was performed on these data to evaluate relationships between the variables studied and between the variables and the volunteers. Then a Hierarchical Cluster Analysis (HCA) was performed using Euclidean distance and Ward’s method Agglomeration to find groups of volunteers with differences in their initial skin condition.

Through Chi square test was determined if there was a significant difference between the participants of the clusters on the following data: sociodemographic, physiological, habits of cosmetic use and in regards to Fitzpatrick, Baumann and Glogau classification as well.

Subsequently, another analysis of variance (ANOVA) was done upon the data of the 15 attributes evaluated during the 28 days of treatment. In this analysis time and cluster were considered and their interaction as fixed factors. By Tukey test (p<0.05), significant differences were identified among mean values.

All the analyses were performed with XLStat 2017 (Addinsoft, NY) software.

**Results and discussion**

**Bee venom analysis**

BV mellitin concentration used in the cream was of 36.5±1.4%. BV content of the product to be assessed would be within the values that are used in the market.<sup>2</sup> Melittin content (0.0022%) in the cream, on the other hand, would not be irritant according to HET-CAM test.<sup>8</sup>

**Participants**

The 30 recruited women accomplished the irritation test. They did not present significant cutaneous irritation (erythema, edema, papules or vesicles) on the areas where patch was applied. Only 23 women completed the test of cream efficacy with BV, 7 of them quit the study due to personal issues. Their ages ranged between 35 and 62 years old, mean 51.3 and SD 8.3 years-old.

**Characterization of skin of the volunteers before starting treatment.**

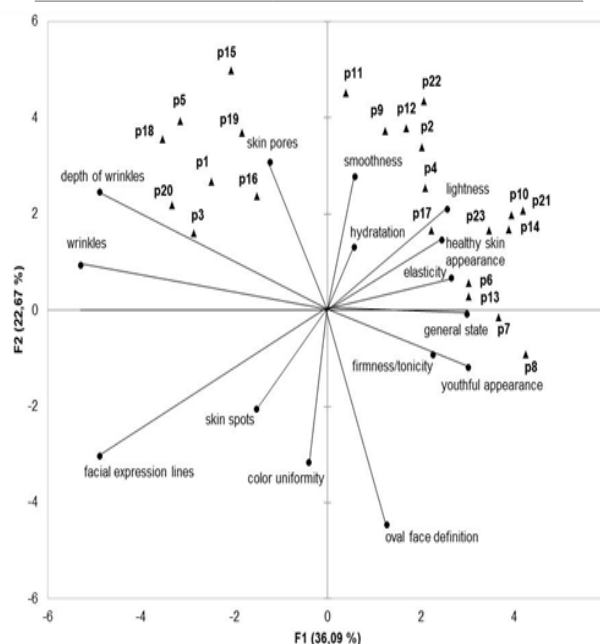
A thoroughly skin characterization of each volunteer was achieved based on Baumann classification, Fitzpatrick phototype classification and Glogau photoaging as well as quantifying the 15 attributes by means of scales. Baumann suggested four parameters to classify the skin phenotypes. Each one of them presents two alternatives (oily vs. dry, sensitive vs. resistant, pigmented vs. non-pigmented, and wrinkle-prone vs. tight), obtaining 16 types. Participants presented six types of the sixteen described by Baumann: 14 participants presented dry skin, 9oily skin (oily), 10 sensitive skin and all of them pigmented skin.<sup>20</sup>

Regarding the phototype: 9 women phototype II, 11 women phototype III and 3 women phototype IV. Related to photoaging: 5, 5 and 13 participants types I, II and III Glogau, respectively. The age is an important factor in intrinsic ageing, whereas photoaging is mainly related to environmental expositions due to life style.<sup>21-23</sup>

Mean values of the 15 attributes evaluated are displayed in Table I and the outcomes of Principal Component Analysis (PCA) in Figure1.

**Table I** Mean values of scores of the attributes which were used to evaluate the skin state of participants before starting treatment. (T<sub>0</sub>)

| Attributes              | Total Mean (9-Points Scale) |
|-------------------------|-----------------------------|
| Wrinkles                | 4.7 ± 2.1                   |
| Depth of wrinkles       | 5.1 ± 2.2                   |
| Facial expression lines | 3.4 ± 1.6                   |
| Skin spots              | 4.4 ± 2.0                   |
| Hydration               | 5.4 ± 1.3                   |
| Smoothness              | 5.8 ± 1.9                   |
| Elasticity              | 5.6 ± 1.4                   |
| Firmness                | 5.1 ± 1.5                   |
| Lightness               | 5.9 ± 1.8                   |
| Color uniformity        | 3.9 ± 2.1                   |
| Oval face definition    | 4.0 ± 1.7                   |
| Skin pores              | 5.9 ± 1.5                   |
| Healthy appearance      | 5.8 ± 1.5                   |
| Youthful appearance     | 5.0 ± 1.7                   |
| General State           | 5.4 ± 1.5                   |



**Figure I** Principal Component Analysis (PCA).

The first and second main components accounted for 36.1 and 22.7 % of the variance and the clinical evaluation data respectively. As Figure 1 shows, the first main component (F1) contrasts positively with lightness, healthy appearance, elasticity, general state, firmness and youthful appearance and negatively with depth of wrinkles, wrinkles and facial expression lines. The second main component (F2) contrasts positively with the presence of pores and smoothness and negatively with color uniformity, oval face definition and facial expression lines.

It is observed that the attributes which are more correlated to the general state of the skin are: lightness, healthy appearance, elasticity,

firmness and youthful appearance. On the other hand, the depth of wrinkles and wrinkles also showed a strong positive correlation among themselves as well as mainly negative with: general state, firmness and healthy appearance and youthful appearance. These results are in agreement with those reported in previous studies for diagnosing the state of the skin.<sup>14</sup>

It can be observed that participants were distributed in two groups: one of them is located on the upper left quadrant and the other on the upper and lower right quadrants. This distribution was made according to their state of skin and was also confirmed by the hierarchical cluster analysis. Figure 1 shows the two clusters that were obtained: cluster 1 (C1) with 8 participants and cluster 2 (C2) with 15 participants.

C1 participants were characterized by presenting high scores in depth of wrinkles, wrinkles and pores of skin, whereas C2 participants were characterized by high scores of smoothness, lightness, elasticity, firmness, youthful appearance, healthy appearance and general state of skin before treatment started (Tables 3 & 4).

**Table 2** Type of skin of participants in each cluster before treatment started, according to Baumann

| Baumann Classification                      | C1 (N=8) | C2 (N=15) |
|---|----------|-----------|
| DSPW Dry/Sensitive/Pigmented/Wrinkle prone  | 4        | 1         |
| OSPT Oily/Sensitive/Pigmented/Tight         | 0        | 3         |
| OSPW Oily/Sensitive/Pigmented/Wrinkle prone | 1        | 1         |
| ORPT Oily/Resistant/Pigmented/Tight         | 0        | 1         |
| ORPW Oily/Resistant/Pigmented/Wrinkle prone | 0        | 3         |
| DRPW Dry/Resistant/Pigmented/Wrinkle prone  | 3        | 6         |

**Table 3** Average scores for clinical evaluation of the state of the facial skin of the volunteers throughout treatment. Cluster 1

| Attributes              | T <sub>0</sub>   | T <sub>14</sub>   | T <sub>28</sub>  | p-value |
|-------------------------|------------------|-------------------|------------------|---------|
| Wrinkles                | 6.9 <sup>a</sup> | 6.9 <sup>a</sup>  | 7.0 <sup>a</sup> | 0.9565  |
| Depth of wrinkles       | 7.1 <sup>a</sup> | 6.8 <sup>ab</sup> | 5.8 <sup>b</sup> | 0.0432  |
| Facial Expression lines | 5.1 <sup>a</sup> | 5.0 <sup>a</sup>  | 5.0 <sup>a</sup> | 0.9753  |
| Skin spots              | 4.3 <sup>a</sup> | 4.3 <sup>a</sup>  | 4.3 <sup>a</sup> | 0.9999  |
| Hydration               | 4.9 <sup>b</sup> | 6.4 <sup>a</sup>  | 7.4 <sup>a</sup> | <0.0001 |
| Smoothness              | 5.5 <sup>b</sup> | 7.0 <sup>a</sup>  | 8.1 <sup>a</sup> | 0.0004  |
| Elasticity              | 4.4 <sup>a</sup> | 4.9 <sup>a</sup>  | 5.3 <sup>a</sup> | 0.2560  |
| Firmness                | 4.0 <sup>b</sup> | 4.8 <sup>ab</sup> | 5.8 <sup>a</sup> | 0.0148  |
| Lightness               | 4.4 <sup>c</sup> | 6.1 <sup>b</sup>  | 7.3 <sup>a</sup> | <0.0001 |
| Color uniformity        | 3.6 <sup>a</sup> | 4.3 <sup>a</sup>  | 4.8 <sup>a</sup> | 0.5190  |
| Oval face definition    | 3.0 <sup>a</sup> | 3.4 <sup>a</sup>  | 4.1 <sup>a</sup> | 0.9936  |
| Skin pores              | 6.5 <sup>a</sup> | 6.1 <sup>a</sup>  | 5.9 <sup>a</sup> | 0.6636  |
| Healthy appearance      | 4.6 <sup>b</sup> | 5.4 <sup>ab</sup> | 6.0 <sup>a</sup> | 0.0491  |
| Youthful appearance     | 3.3 <sup>b</sup> | 3.9 <sup>ab</sup> | 4.9 <sup>a</sup> | 0.0185  |
| General state           | 3.9 <sup>b</sup> | 4.3 <sup>ab</sup> | 5.1 <sup>a</sup> | 0.0456  |

Values within a row with different superscript are significantly different according to Tukey Test ( $p \leq 0.05$ )

**Table 4** Average scores for clinical evaluation of the state of the facial skin of the volunteers throughout treatment. Cluster 2

| Attributes              | T <sub>0</sub>   | T <sub>14</sub>   | T <sub>28</sub>  | p-value |
|-------------------------|------------------|-------------------|------------------|---------|
| Wrinkles                | 3.5 <sup>a</sup> | 3.5 <sup>a</sup>  | 3.3 <sup>a</sup> | 0.9487  |
| Depth of wrinkles       | 4.0 <sup>a</sup> | 3.9 <sup>a</sup>  | 2.9 <sup>a</sup> | 0.1716  |
| Facial expression lines | 2.5 <sup>a</sup> | 2.4 <sup>a</sup>  | 2.1 <sup>a</sup> | 0.5760  |
| Skin spots              | 4.4 <sup>a</sup> | 4.4 <sup>a</sup>  | 4.2 <sup>a</sup> | 0.9523  |
| Hydration               | 5.6 <sup>b</sup> | 6.9 <sup>ab</sup> | 7.5 <sup>a</sup> | 0.0003  |
| Smoothness              | 6.0 <sup>b</sup> | 6.9 <sup>ab</sup> | 7.9 <sup>a</sup> | 0.0079  |
| Elasticity              | 6.2 <sup>b</sup> | 6.3 <sup>b</sup>  | 7.3 <sup>a</sup> | 0.0222  |
| Firmness                | 5.7 <sup>b</sup> | 6.1 <sup>b</sup>  | 7.5 <sup>a</sup> | 0.0063  |
| Lightness               | 6.7 <sup>b</sup> | 7.2 <sup>b</sup>  | 8.4 <sup>a</sup> | 0.0025  |
| Color uniformity        | 4.0 <sup>a</sup> | 4.3 <sup>a</sup>  | 4.9 <sup>a</sup> | 0.5305  |
| Oval face definition    | 4.5 <sup>b</sup> | 4.9 <sup>b</sup>  | 6.1 <sup>a</sup> | 0.0286  |
| Skin pores              | 5.6 <sup>a</sup> | 5.3 <sup>a</sup>  | 4.7 <sup>a</sup> | 0.2774  |
| Healthy appearance      | 6.5 <sup>b</sup> | 6.9 <sup>ab</sup> | 7.7 <sup>a</sup> | 0.0054  |
| Youthful appearance     | 5.9 <sup>b</sup> | 6.3 <sup>ab</sup> | 7.1 <sup>a</sup> | 0.0245  |
| General state           | 6.3 <sup>b</sup> | 6.4 <sup>b</sup>  | 7.5 <sup>a</sup> | 0.0002  |

According to square chi test, the distribution of participants in both clusters was not significantly affected ( $p > 0.05$ ) by either their marital status, level of education, menopause, smoking, nor for having been a smoker or having had acne, and nor for the type or frequency of use of cosmetic products. There was a significant difference in the age of participants of both clusters ( $p = 0.015$ ), having C1 participants a mean age of  $56.9 \pm 5.0$  years-old and C2 mean age of  $48.3 \pm 8.3$  years-old.

The prototype of the participants of each cluster, presented the following distribution: in C1, 3 volunteer had prototype II, 4 prototype III and 1 prototype IV, whereas in C2, 6, 7 and 2 of the volunteers presented prototypes II, III and IV respectively. No significant differences were found among the prototypes of clusters ( $p > 0.05$ ).

Table 2 shows the classification of the participants of each cluster according to Baumann. According to square chi test, the frequency of Baumann classification did not present significant differences ( $p = 0.133$ ) between C1 and C2. All C1 participants had wrinkles and pigmented skin, whereas in C2 a variety of alternatives of this classification was presented. It was of importance the fact that 11 of participants presented wrinkles.

According to chi-square test, significant differences ( $p = 0.0089$ ) were found in the distribution of Glogau indexes when analyzing the photoaging of the members of each of the clusters. Total of volunteers belonging to C1 had type III Glogau, whereas C2 showed a lower level of photodamage. They were distributed in three groups of 5 participants each one with type I, II and III Glogau, respectively.

This shows that the initial state of the skin of participants was influenced by age and photodamage that was accumulated during life. Between C1 and C2, the participants of C2 presented a better state of skin before treatment started.



## Treatment evaluation

ANOVA that was performed on average scores of the data of each attribute before starting treatment ( $T_0$ ), at averaging ( $T_{14}$ ) and ending ( $T_{28}$ ), showed that in C1, 8 of 15 attributes which were evaluated, improved with treatment application ( $p < 0.05$ ), whereas in C2, 9 of the 15 attributes evaluated improved with treatment application ( $p < 0.05$ ). Tables III and IV show the average values evaluated at each time as well as the outcomes of variance.

Values within a row with different superscript are significantly different according to Tukey Test ( $p < 0.05$ ). If both C1 and C2 are compared at the beginning as well as along the treatment, C1 participants presented values significantly higher ( $p < 0.01$ ) for: wrinkles, depth of wrinkles and facial expression lines and significantly lower ( $p < 0.05$ ) for: elasticity, firmness, lightness, oval face definition, healthy appearance, youthful appearance and general state (Table 3 & 4). This might be due to this group had a higher mean age and its participants had level III photoaging.

At 14 days of treatment, C1 participants already showed significant improvements ( $p < 0.05$ ) in hydration, lightness and smoothness. These attributes depend on the superficial layers of the skin and commonly respond more quickly to the use of suitable products.<sup>24</sup> Moreover, at 28 days of treatment significant improvements were found ( $p < 0.05$ ) in depth of wrinkles, firmness, healthy appearance, youthful appearance and general state. Wrinkles are more related to deep layers of epidermis and dermis, and their improvement commonly responds slowly to treatments. The same happens with oval face definition which in C1 did not present improvements.<sup>14,20,22,24</sup>

At the end of the treatment, C2 participants showed significant improvements ( $p < 0.05$ ) related to: hydration, smoothness, elasticity, firmness, lightness, facial oval definition, healthy appearance, youthful appearance and general state. Participants of this cluster started treatment with a good skin condition, therefore, they had high scores for these attributes and would justify a longer time to obtain significant improvements. In C2 the average scores of wrinkles, depth of wrinkles and facial expression lines were: 3.5, 4.0 and 2.5, respectively and for C1 were notoriously lower (6.9, 7.1 y 5.1). These attributes can be associated with signs of ageing, both chronological and photoaging.<sup>21</sup>

C1 participants significantly improved in depth of wrinkles, whereas C2 did not improve this attribute. It is important to highlight that C1 had a high score in this attribute at the beginning of the treatment. In C2 the situation was different due to the participants were younger and had just a few cases of photodamage. Also, their treatment started with a low score in this attribute.

It is of importance to highlight that, in none of the clusters wrinkle improvements were detected neither in facial expression lines, skin spots, color uniformity or skin porosity. In general, these properties require longer treatments for improvements to be sensorially and consciously noticed. In a study by Han et al. performed with BV serum, a period of 8 to 12 weeks was needed to enhance the state of wrinkles of participants. In this study, skin was evaluated by both, visual observation of a dermatologist and image analysis of the skin replicas.<sup>11</sup>

All volunteers showed an excellent tolerance to treatment:  $8.6 \pm 0.9$  (9-point scale), which deserves to be highlighted considering that ten of them were classified to have sensitive skin according to Baumann. Also, volunteers satisfaction related to treatment received very high scores as well:  $8.5 \pm 0.6$  (9-point scale).

Analyzing the photographs of the participants that were taken before and after treatment, it is of importance the improvements in the state of skin for both, C1 and C2 (examples in Figures 2 & 3). Among the changes registered: glabellar region, generally in facial expression lines, eyelids, oval face definition, upper eyelid (slightly raised), less depth of wrinkles in facial expression lines.



Figure 2 Volunteer of cluster 1, (left) before treatment, (right) after treatment.



Figure 3 Volunteer of cluster 2, (left) before treatment, (right) after treatment.

The outcomes of the present study join others which were obtained in different studies held by other authors by means of either clinical evaluations, studies on cellular cultures. Also, the antioxidant effect shown for BV which is considered to be a prevention factor of ageing. Also, Han et al. 2015b reported antiageing effects during a 12-weeks treatment with BV serum for analyzing periorbital wrinkles.<sup>7,8,11,24,25,26</sup>

## Conclusion

The clinical evaluation used in this study resulted suitable to classify the skin of the volunteers and also to evaluate the treatment outcomes using a cream with a BV incorporation.

During the 28 days of treatment, improvements were observed that depended on the most superficial layers of the skin, which corresponds with the evaluation done by the volunteers regarding tolerance and satisfaction.

The good tolerance manifested by the participants all along the treatment as well as the high score given regarding satisfaction, allows us to have some expectations in reference to continue with their participation in the treatment during a longer period of time, which might mean to contribute to improve the outcomes in regards to reduce ageing signs.

From the outcomes collected in this study, it is possible to affirm that BV is an active cosmetics which, by a 14 days use, it offered improvements to the state of skin of participants and also that their skins continued improving until the end of treatment. Some of the improvements might be associated with anti-ageing effects, in which more research would be required through a longer treatment considering that the attributes associated with deep layers of the skin need longer time to improve.

### Limitation of the study

Sample size of the study was small and may be extended in subsequent researches.

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### Conflicts of interest

We declare no conflict of interest.

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