A masterful pro-collagen solution: the firming ingredient combination that enhances results while maximizing skin tolerance.

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Abstract

Well-balanced type I type I collagens essential to preserve healthy-looking and firm skin. However, the aging process, accentuated by internal factors like hormonal changes, affects type I collagen levels, its activity and consequently skin firmness.

Our goal was to demonstrate that a combination of retinol and bakuchiol, at the right concentration, produced superior results on collagen production compared to using both ingredients alone. The additive effect of this combination allowed for enhanced collagen production while facilitating a reduction in overall concentration of retinol and guarantying skin tolerance.

A formulation containing the well-known anti-aging gold standard, retinol and a natural ingredient with a demonstrated retinol-like activity, bakuchiol, was studied. For both ingredients an in vitro assay was performed to prove their anti-aging action as type I collagen boosters, alone and combined.

In summary, a stable and well tolerated topical formulation was developed with the aim of having an anti-aging effect through the combination of an optimal concentration of retinol and bakuchiol. Firmness due to intrinsic factors, like hormonal changes in menopause, improved and skin healthy appearance were restored.

Keywords: Retinol, bakuchiol, menopause, type I collagen

Introduction

Skin aging is defined as the degenerative process in which structural and physiological alterations cause an impairment of skin biological functions. Skin aging can be induced by intrinsic factors, such as genetic background and hormonal levels, or by extrinsic factors, such as UV light, pollution, or exposure to environmental stressors [1]. These factors origin irregular epidermal thickness, uneven pigmentation, abnormal elastin deposition and loss of collagen and other extracellular matrix proteins among other defined markers [2], [3].

Type I collagens the main constituent of the skin and provides the major support for skin resistance. Human skin contains 14 types of collagens; among them 80% of the collagen found in skin is type I collagen while 15% is type III. Type I type I collagens more responsible for the strength of the skin; type III is more responsible for the elastic properties. It is a key protein player especially during hormonal aging as menopause and after menopause. The decrease in levels of type I collagen the skin occurs at a rapid rate immediately during menopause and becomes more gradual thereafter. Approximately 30% of skin type I collagens lost in the 5 years after the menopause, with an average decline of 2.1% per postmenopausal year over a period of 20 years [4] . The effect of reduced collagen levels in skin can be dramatic – dryness, dullness, loss of moisture, tone, and radiance. Knowing which ingredients are truly going to help is key to have a glow, healthy, strong skin during and after menopause.

Many compounds and topical treatment options have been used for ameliorating the signs of skin aging like hormonal changes -antioxidants, peptides or alpha-hydroxy acids [4]. Among them, retinoids have proven to be effective as skin aging treatment and are still considered the gold standard for clinically effective topical antiaging products [5]. They act through their binding and activation of retinoid acid receptors (RAR-α, -ß, -γ) and retinoid X receptors (RXR-α, -ß, -γ) increasing the amount of type I procollagen and decreasing the amount of matrix metalloproteinases (MMP's) [6] [7] [8]. This mechanism of action is extrapolated to a skin benefit through clinical studies conducted on volunteers, as less skin wrinkles, improved skin texture and improved firmness/elasticity.

Nevertheless, retinoids have their disadvantages. From a formula development perspective, retinoids have a challenge to those who develop that kind of products because of their proven instability (specially to light), low penetration, and potential for skin irritation. Retinoic acid is the major bioactive form while the tolerance ranking is reversed: retinyl esters> retinol = retinaldehyde >> retinoic acid (Figure 1) [9].

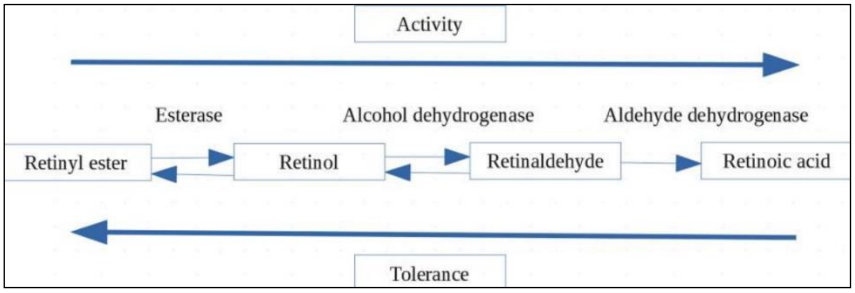

Figure 1: *Representation of retinoid activity and tolerance considering the metabolic pathway.*

Therefore, when topically applied, retinol is believed to be the more efficient method to deliver retinoic acid to the skin cells than direct treatment with retinoic acid [3] as it is metabolized on the skin to retinaldehyde and retinoic acid in two-step oxidation process [3] , [9]. However, topical retinoid therapy using even the newer analogues is still restricted by many undesirable side effects, such as irritation, dryness, peeling, erythema and a sensation of burning on the skin [10], [11].

To improve regimen compliance, development of new retinoid compounds and searching new strategies in formulation are needed. On the one hand, it is well known that encapsulating retinol ensures stability and increases efficacy [12]. On the other hand, another strategy is to search compounds with an action like that of retinol but without producing undesirable side effects.

Bakuchiol- a meroterpene phenol from seeds of the Indian plant *Psoralea corylifolia* [13], [14] was proposed as a potential alternative to retinol. It showed a retinol-like mechanism of action as it has close structural similarities with retinoids, exhibiting a similar gene expression profile, especially on certain key anti-ageing genes related with type I collagen protein, without inducing adverse effects [6], [7].

Besides, bakuchiol has several advantages over retinol, including excellent photochemical and hydrolytic stability, a good safety profile and ease to formulate due to miscibility with a wide variety of emollients and solubilizers. Interestingly, bakuchiol is an excellent stabilizer of retinol under photo oxidative as well as singlet oxygen environments. This property may help reduce oxidative stress caused by retinol when bakuchiol is combined with retinol and used at higher concentrations [15].

The aim of this work was to explore the effects of a formulation containing retinol and bakuchiol at the right concentrations as an alternative approach to prevent skin aging by increasing type I collagen level, improving skin firmness lost due to intrinsic factors like hormonal ageing in menopause while maximizing the skin tolerance.

Materials and methods

Formula development and manufacturing process

Selection of raw materials used in this skincare composition and their content (%) as well, were performed according to a documented developmental process. The formulation contained polyacrylate crosspolymer-6 and xantan gum as the stabilizing system; bakuchiol as a retinol-like ingredient, and propanediol as a key solvent agent. Concerning retinol, several sources of retinol were tested both free retinol and encapsulated retinol. Free retinol sources were embedded in polysorbate-20 or a mixture of alkanes (undecane and tridecane). As for encapsulated retinol several carrier systems were tested like liposomes, polymeric shells, cyclodextrins or agar microspheres. The formulation also included emollients of natural origin, silicones, perfume, and preservatives.

To ensure the correct stability of the formulation, different strategies were developed in the production method, like alternating the order of addition of the raw materials or applying different degrees of shear, with the aim of obtaining the maximum stability of the formulation and its cosmetics ingredients.

Stability tests

Samples of the formulations being studied were put under different thermal conditions and the organoleptic; physicochemical and microbiological stability [16] was checked at different times following the scheme in table I:

Table I: *stability test controls: FQ: phisico-chemical; ACO: Organoleptic; M: microbiologic*

Time		**2 Weeks**	**4 Weeks**	**8 Weeks**	**12 Weeks**	**24 Weeks**
condition	25ºC	ACO FQ	ACO FQ	ACO FQ	ACO FQ	ACO FQ M
	40ºC	ACO FQ	ACO FQ	ACO FQ	ACO FQ	ACO FQ
	50ºC	ACO FQ	ACO FQ	ACO FQ	ACO FQ	
	4ºC	ACO FQ	ACO FQ	ACO FQ	ACO FQ	ACO FQ

At each stability point, the organoleptic (appearance, color and odor), physicochemical (pH measurement with the Mettler Toledo pH meter, SevenCompact TMS 210), viscosity (Viscometer Brookfield BR-RV-DVIP) and microbiological (only initially and then after six months) parameters were checked. Furthermore, different assays were performed with LumSizer to foresee which formulations during development were more stable.

Skin tolerance test

To check the good skin compatibility of the formulation a study was performed under occlusive patch for 48 hours, in the adult subject and after a single application.

10 female or male healthy volunteers between 18 to 70 years old were recruited. The area application was back, between the hips and the shoulders (area without redness or imperfection). The product was applied under occlusive patch (Finn Chambers), single application, for about 48 hours.

Clinical evaluation was performed by the determination of the index of Primary Cutaneous Irritation (I.P.C) about 30 minutes after removal of the patches and comparing to those obtained with the “negative” control [17].

I.P. C is the cutaneous irritation quantification according to a given numerical scale as follows:

- Erythema: factor 1
- Edema, papulae, vesicles, bullae, pustules: factor 2
- Dryness, detergent effect, reflectivity: factor 0.5

Formulas containing different combination of cyclodextrin encapsulated retinol or free retinol in oil solution were mixed with backuchiol and were tested, as seen in table II:

Table II: *composition of the formulas tested by patch test.*

	CYCLODEXTRIN ENCAPSULATED RETINOL (ACTIVE MATTER 9% RETINOL)	**FREE RETINOL OIL SOLUTION (ACTIVE MATTER 47.45% RETINOL)**	**BAKUCHIOL**
Formulation 1	3.3%	X	X
Formulation 2	1.1%	X	0.5%
Formulation 3	X	0.65%	X
Formulation 4	X	0.22%	0.5%
Formulation 5	X	0.65%	0.5%

Determination of type I collagen synthesis modulation in human fibroblasts

Prior to conduct the collagen test in skin human fibroblasts, a preliminary Neutral red uptake (NRU) cytotoxicity test of the products was carried out under the ISO 10993-5 [18]. The study described in this publication concerns with the in vitro evaluation of the capability of the tested products to increase type I Collagen (COL I) protein synthesis in human skin fibroblasts. In addition, a comparison retinol vs bakuchiol as well as the enhancing effect of the retinol-bakuchiol mix on the COL I synthesis modulation was evaluated.

This evaluation was carried out by determination of COL I amount after cell treatment with products under study for 24 hours.

Experimental protocol provided:

- untreated cell culture (CTR-).
- cell culture treated with tested product, retinol.
- cell culture treated with tested product, bakuchiol.
- cell culture treated with retinol + bakuchiol.

Cells culture supernatant was used for the quantification of COL I by means of ELISA method. Commercial kits were used for the determination. The microplate had been pre-coated with an antibody specific to COL I. Standards or samples were then added to the appropriate microplate wells with a biotin-conjugated antibody specific to COL I. Next, Avidin conjugates to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solutionwas added, only those wells that contain COL I, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of COL I in the samples was then determined by comparing the OD (optical density) of the samples to the standard curve.

For the statistical analysis, the data obtained for each parameter were compared to the negative control (CTR-), as baseline condition, to evaluate the percentage variations versus the reference conditions.

Obtained results were subjected to statistical analysis by means of Student test. The variations versus negative control and positive control are considered significant for $p^* < 0.05$.

As a first step, a stock solution of both substances to be tested were prepared (Table III).

Table III: *Type I collagen test – concentrations tested*

	RETINOL	BAKUCHIOL
Sample 1	0.05%	---
Sample 2	---	0.25%
Sample 3	0.05%	0.25%

The combination of both active ingredients was evaluated following the same range of concentrations as the ingredients tested individually, based on a ratio of 1:5, the same proportions as in the chosen formulation (Table III).

Clinical evaluation

The study aimed to evaluate the efficacy in improving skin barrier conditions, anti-wrinkle, and firming efficacy of the retinol-bakuchiol formula for facial application (night treatment). To achieve this goal, a study was conducted on 30 healthy Caucasian women between 40 and 60 years of age of which 10 women under menopause, with normal to dry skin, clinically showing crow's feet wrinkles. They applied the product every evening for 28 days. An informed consent was obtained by the volunteers to start the topical experimental study [19]Assessments were carried out at baseline (T0) and after 14 days. Furthermore, the skin firmness and skin wrinkledness were measured by clinical evaluation at baseline (T0), after 14 (T14) and 27 days (T28). The instrumental and clinical analysis were further complemented with self-assessment questionnaires filled out by the subjects who completed the study.

The evaluations were conducted initially (T0) and at the end (T14 and T28) of the treatment in a temperature and humidity-controlled environment (respectively T = 18–26°C and RH = 50±10%). Digital photographs of the face were taken using VISIA-CR® (Canfield Scientific).

The barrier function measurement was based in the measurement of the trans epidermal water loss (TEWL) by the recognized TEWAMETER ® method. The instrument used was a Tewameter 300® (Courage+Khazaka, electronic GmbH). Physical basis for the measurement was the Diffusion law discovered by Adolf Fick in 1855.

Meanwhile, skin firmness and skin wrinkledness were assessed by clinical evaluation. The experimenter evaluates the crow's feet wrinkledness and skin firmness according to the following clinical scores scales:

- **Crow's feet wrinkles**:
    - Skin Aging Atlas Vol 1-Caucasian type-Bazin Roland (from 0, no wrinkle→ to 6, remarkable wrinkle)
- **Skin firmness**:

Clinical classification of skin firmness at T0/T14/T28	Score
NOT FIRM SKIN Unelastic skin and characterized by a strong loss of tone. The skin appears completely thinned like emptied, not dense and the tissues appear clearly relaxed. The skin has bad resistance to pinching and pulling, as well as a poor elastic recovery after traction.	**1**
NOT VERY FIRM SKIN Poorly elastic skin and characterized by an evident loss of tone. The skin appears thinned and less dense in some areas; the tissues start to relax. The skin has poor resistance to pinching and pulling, as well as a poor elastic recovery after traction.	**2**
SUFFICIENTLY FIRM SKIN Sufficiently elastic skin and characterized by a medium tone. The skin appears sufficiently full, plump and dense and the tissues appear slightly relaxed. The skin has sufficient resistance to pinching and pulling; the elastic recovery after traction is quite good.	**3**
FIRM SKIN Elastic skin and characterized by a good tone. The skin appears full, plump and the tissues don't appear relaxed. The skin has good resistance to pinching and pulling; the elastic recovery after traction is good.	**4**
VERY FIRM SKIN Elastic skin and characterized by an excellent tone. The skin appears full, plump and the tissues don't appear relaxed. The skin has an excellent resistance to pinching and pulling; the elastic recovery after traction is excellent.	**5**

Statistical analysis of instrumental measures was subjected to paired Student t-test (within-group analysis vs T0). Variations are considered statistically significant when the p value is <0.05.

Results

Formula development and stability tests

The final goal of this work was to obtain a low viscosity stable gel in oil emulsion with good sensory performance.

Table IV summarizes the stability results and sensory perception of the different formulations tested.

Table IV: *stability result and sensory perception of tested formulation.*

	CYCLODEXTRIN ENCAPSULATED RETINOL (ACTIVE MATTER 9% RETINOL)	FREE RETINOL OIL SOLUTION (ACTIVE MATTER 47.45% RETINOL)	BAKUCHIOL	SUMMARY OF STABILITY RESULTS	SENSORY PERCEPTION
Formulation 1	3.3%	X	X	Good	Sticky with low extensibility and greasy residue
Formulation 2	1.1%	X	0.5%	Good	Nice and light application, soft and fresh residue
Formulation 3	X	0.65%	X	Unstable	Not tested
Formulation 4	X	0.22%	0.5%	Unstable	Not tested
Formulation 5	X	0.65%	0.5%	Unstable	Not tested

Real time and accelerated stability testing revealed no changes in pH and viscosity parameters over time and temperature in formulation 2 (table IV). Figure 2 and Figure 3 show the good stability evolution of the selected composition, formulation 2.

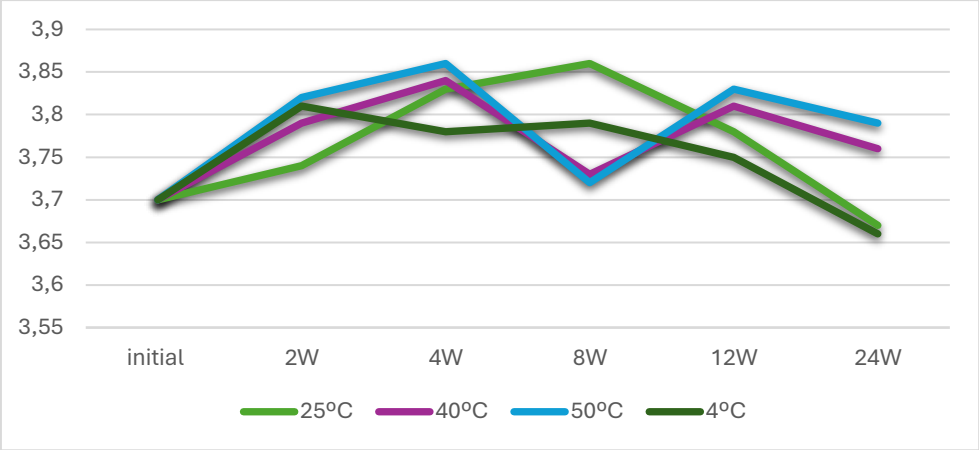

***Figure 2**: pH stability evolution*

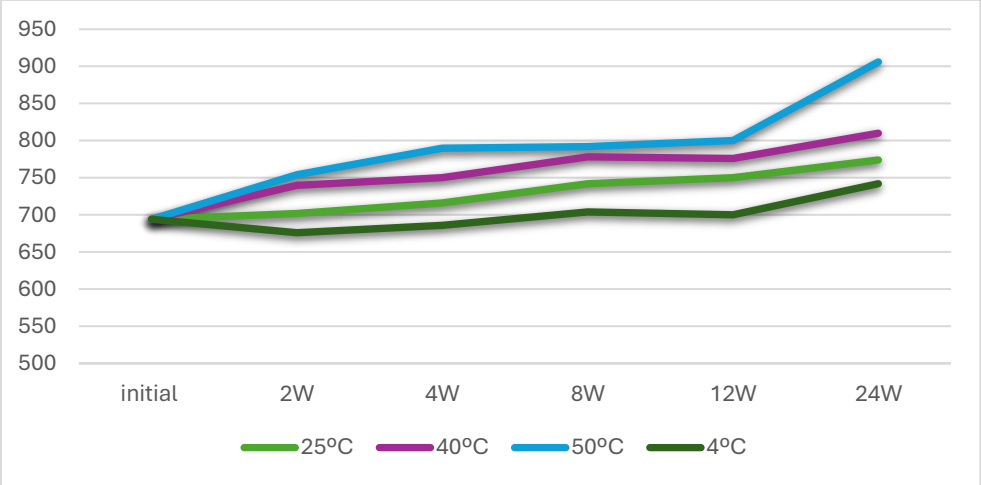

***Figure 3**: viscosity stability evolution (cP)*

In addition, intense shear rate at the end of the formulation changes stability prediction profile. The graphs offered by LumSizer after a program at 30ºC, 15h, interval 175, 3700 rpm, 300 profiles show (Figure 4) different results depending on the final shear stir that is given to the formulation once the retinol encapsulated in cyclodextrins is included. Formulation with strong shear rate (Figure 4A) showed lower stability profile compared with the formulation with gentle agitation at the end of the process (Figure 4B).

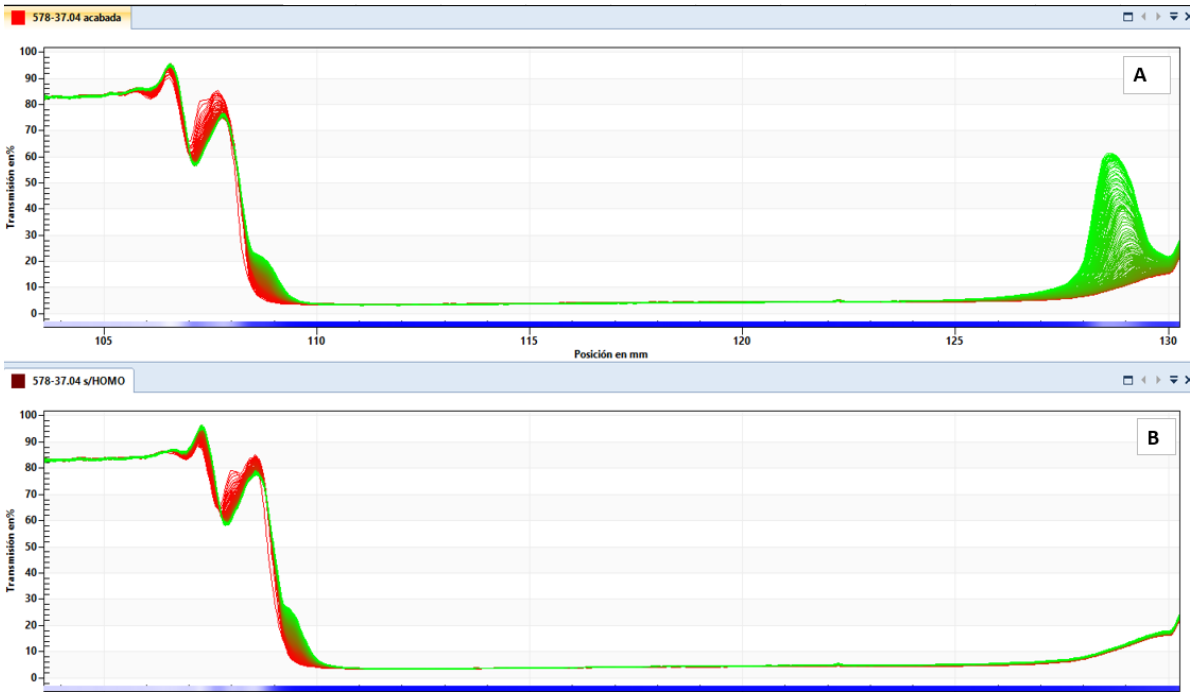

Figure 4: *profiles obtained after a trial in LumSizer (in a 30ºC test, 15 hours, 175 range, 3700 rpm and 300 profiles). A: formulation with strong shear rate at the end of the process. B: formulation with gentle agitation at the end of the process.*

Skin Tolerance Test results

The weighted total results obtained from the patch tests performed on the formulas containing different sources of retinol combined with bakuchiol are shown in Table V. (For more details on composition, see Table IV).

Table V: *skin tolerance results of tested formulations.*

	I.P.C Mean Value	**RESULTS**
Formulation 1	0	Very good skin tolerance
Formulation 2	0	Very good skin tolerance
Formulation 3	0.2	Moderate tolerance
Formulation 4	0.1	Good tolerance
Formulation 5	0	Very good skin tolerance

Determination of type I collagen synthesis

Prior to the evaluation of the in vitro determination of COL I synthesis by the active ingredients, the cytotoxic profile of the active ingredients under study was evaluated as described above in the materials and methods section. Concentrations of retinol and bakuchiol equal or inferior to 0.05% and 0.25%, respectively, did not affect the viability of the cell culture at the exposure time according to the ISO 10993-5.

Using the COL I determination method described also above in the materials and methods section, the COL I production of each active ingredient and their combination were evaluated. The results expressed as COL I (mean value ± dev.st) and as mean % variation compared to negative control are shown in Table VI and Figure 5**.** Significant values vs negative control is referred with asterisk (*).

Table VI: Results of type I collagen production on cell culture at 24h.

Type I collagen (ng/mL) 24h				
SAMPLE	CONCENTRATION IN %	AVERAGE	SD	VAR % vs C-
Control -	-	0.78	0.02	-
Retinol	0.05	1.02	0.05	31.12%*
Bakuchiol	0.25	1.00	0.09	29.44%*
Combination	0.05+0.25 (1/5)	1.06	0.15	36.17%*

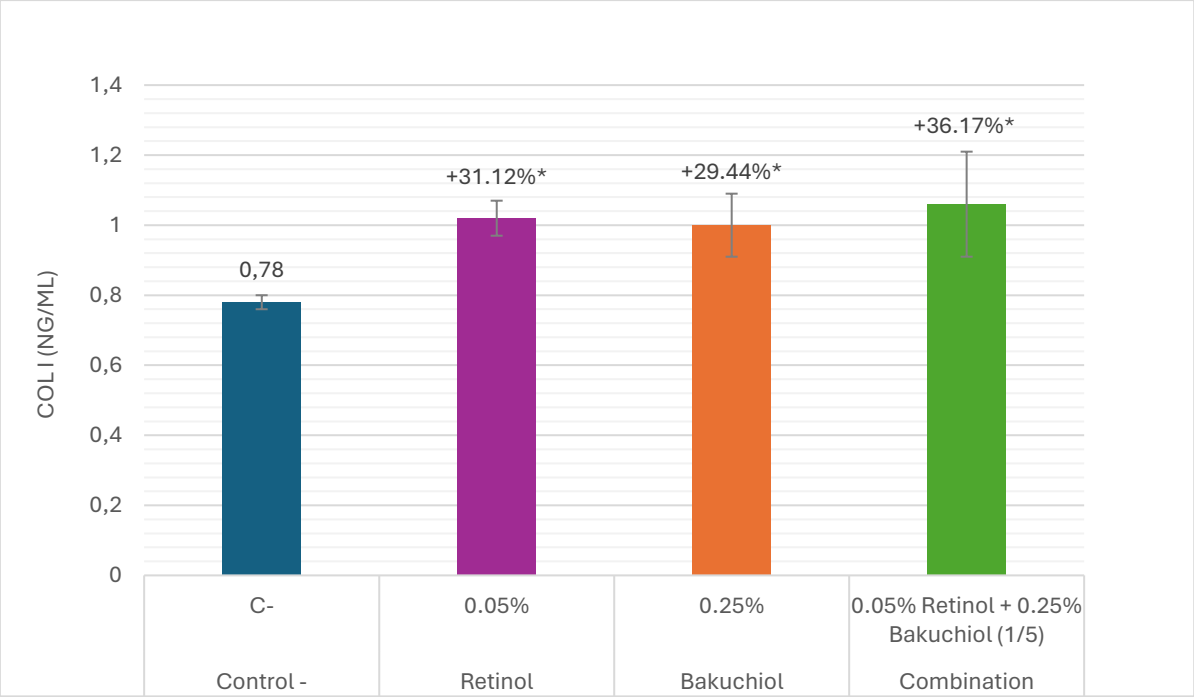

Figure 5*: Type I Collagen I synthesis on cell culture treatment at 24 h. The variations versus negative control are considered significant for *p-value <0.05. The error bars represent the average standard deviation in three independent assays (N=3).*

Clinical evaluation

The efficacy of the emulsion containing retinol and bakuchiol was evaluated on the volunteers clinically showing crow's feet wrinkles and poor firmness. The product was applied to their entire face, every evening for 28 days.

The results in Figure 6 show the transepidermal water loss (TEWL) before (T=0) and after (T=14) application of the formula. A significant decrease of the transepidermal water loss parameter by an average of -8.8% can be seen. A decrease of TEWL parameter indicates an improvement of skin barrier function.

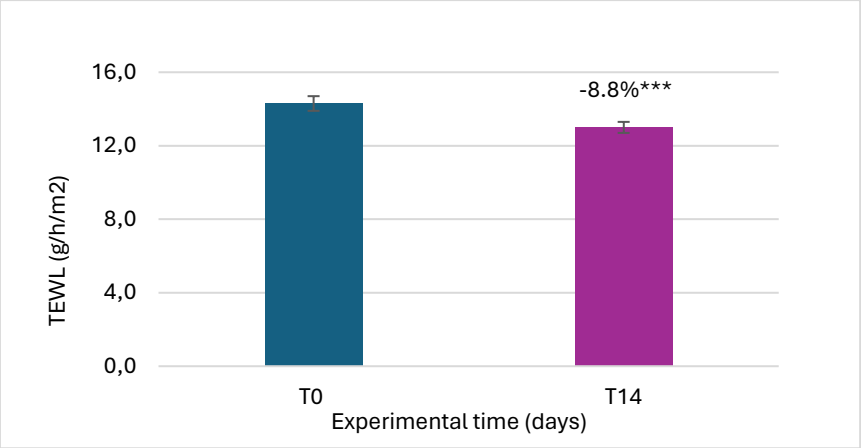

Figure 6: *The graphic shows data obtained at each experimental time for the transepidermal water loss. Data are expressed as a mean ± SE. Above the error bar the inter-group statistical analysis vs. T0 is reported as follow: * p<0.05, ** p<0.01, *** p<0.001.*

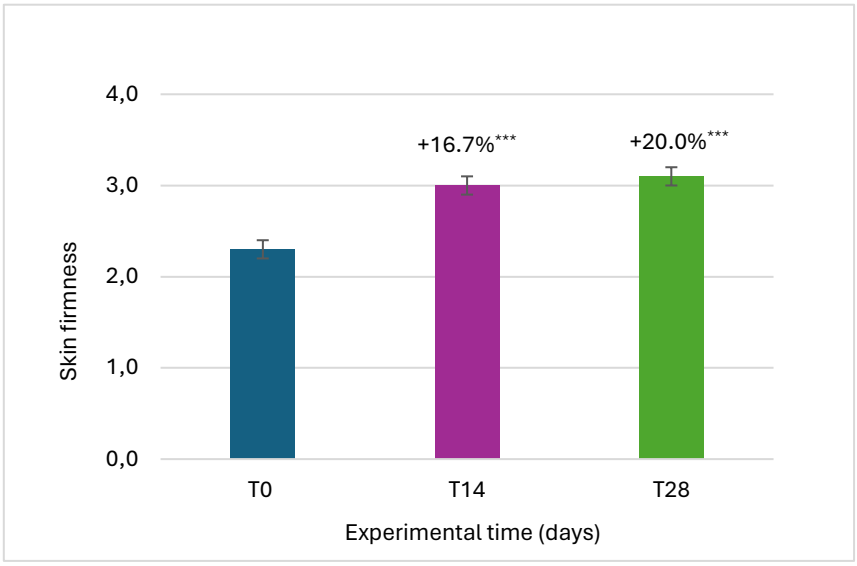

Figure 7: *The graphics show the data obtained at each experimental time for firmness. Data are expressed as a mean ± SE. Above the error bar the inter-group statistical analysis vs. T0 is reported as follow: * p<0.05, ** p<0.01, *** p<0.001.*

The results in Figure 7 show the skin firmness improvement before (T=0) and after (T=14 and T=28) application of the formula. A significant increase of firmness (clinical evaluation) of +16.7% and +20.0%, respectively, was observed.

The results in Figure 8 show the crow's feet wrinkle improvement before (T=0) and after (T=14 and T=28) application of the formula. After applying the solution for 14 and 28 days, a significant decrease of crow's feet wrinkle (clinical evaluation) of -9.1% and -11.3%, respectively, was observed.

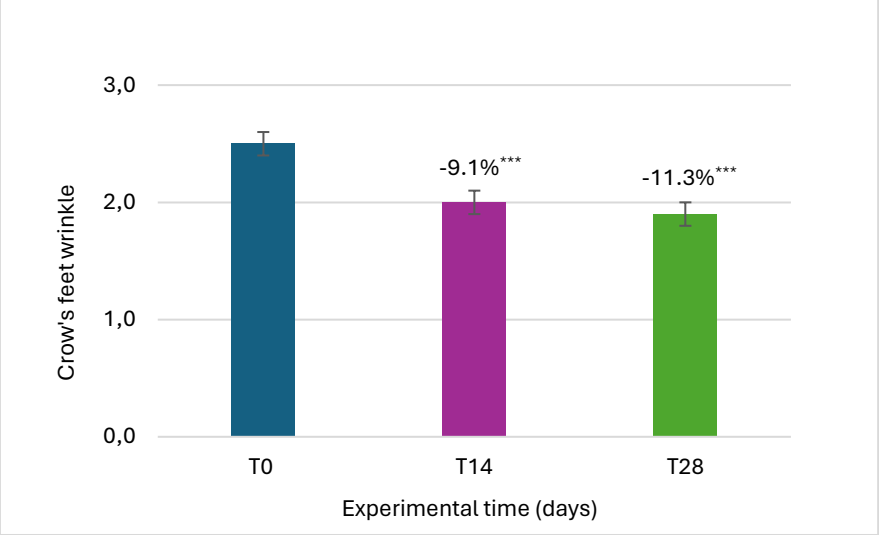

Figure 8: *The graphic shows the data obtained at each experimental time for skin wrinkledness. Data are expressed as a mean ± SE. Above the error bar the inter-group statistical analysis vs. T0 is reported as follow: * p<0.05, ** p<0.01, *** p<0.001.*

Discussion

Topical retinoids, the collective term for retinoic acid and other vitamin A compounds that also includes retinol and its derivatives, are still considered the gold standard for clinically effective topical antiaging products. However, they can be irritating to a significant percentage of population, and further development of ways to reduce their irritation potential is needed [20]. Therefore, there is a need to develop improved retinoid compounds. Such compounds should have similar but not identical mechanism of action as compared with retinol, ideally, resulting in retinol-like beneficial effects, without having retinol-like undesirable side effects. At this point we found that bakuchiol exhibits such retinol-like functionality. Thus, keeping retinol at low doses in topical formulations combined with bakuchiol has been proposed as a promising anti-aging

approach in terms of the restoration of the type I collagen lose and consequently firmness due to intrinsic factors like hormonal ageing in menopause guarantying skin tolerance.

Undertaking this study also revealed the need to find a way to keep retinol unaltered over time as it is well known that its instability is formulation dependent. Retinol preparations must be protected from light and protected with an antioxidant preservative, as exposure to light and oxygen leads to degradation into a variety of potentially harmful compounds, including reactive oxygen species [21], [22], [23]. Also, a substantial amount of research has focused on development of delivery systems for retinol to increase its stability [24] . Besides, bakuchiol has demonstrated its ability to stabilize retinol against oxidation (singlet oxygen environment) [25] and photo-oxidation (UVA and UVB). The results demonstrated that use of only two-fold excess of bakuchiol provided a complete stabilization of retinol [25]. Considering these results and as explained in the results section, the combination of retinol and bakuchiol was based on a ratio five-fold to increase even more retinol stability in the formulation.

The stability studies conducted with the formulas showed satisfactory results in the formulation 2, containing a 0.1% retinol (in cyclodextrin encapsulation) and 0.5% bakuchiol (table IV) both organoleptic and physicochemical. Microbiological stability also remained stable.

In formulation 2, the gelling agent was a key ingredient to obtain stable fluid emulsions in mild acidic pH and retinol containing emulsion. The best performance was achieved by the combination of polyacrylate crosspolymer-6 together with a vegetable origin gums (xanthan gum, sclerotium gum and pullulan) to obtain a gel-in-emulsion texture. Others were discarded due to problems of instability or low sensory profile on the skin.

As far as retinol is concerned to avoid incompatibilities with the formulation and to improve the release profile of the active ingredient, several oily mixes solutions containing retinol as well as different types of encapsulated retinol were tested. The mixture containing retinol and alkanes was a promising source of retinol, but it did not pass stability test. Given this destabilization, the selection of an encapsulated retinol was a must; an encapsulated retinol should enable the slow release of the active molecule along with superior stability. Finally, retinol encapsulated in

hydroxypropyl cyclodextrin was the source of choice because it performed reliable stability results without color and appearance change in contrast with other carrier systems as liposomes, polymeric shells, or agar microspheres. Considering all the results, formulations prepared with beta-cyclodextrin inclusion complex were considered the most stable way to deliver retinol.

Although propylene glycol was the best solvent to disperse the encapsulated retinol it was considered a potential irritative of the skin, so propanediol was selected as a solvent of natural origin and skin-friendly performance.

The selection of the retinol-like ingredient was made through a bibliographic search among those which perform similarly on the skin than retinol or ingredients that increases of endogenous levels of retinoic acid in the skin. Among them, the selected compound was bakuchiol [26], [27] a molecule obtained from *Psoralea corylifolia* that showed no negative side effect and stimulate key anti-aging genes similarly as retinol, for instance retinoid binding and metabolizing genes. Furthermore, it has been reported to be very effective as a retinol photo stabilizer and under singlet oxygen environment [25] using a four-fold or five-fold excess of bakuchiol.

Not only was the selection of excipient ingredients important, but it was also observed that the strong stirring of the emulsion could destabilize the formulation, as observed in stability prediction trials that were done with LumSizer. This was surely due to a release of the molecule of retinol from its encapsulation system, a fact that destabilized the formula as well as the unencapsulated retinol had done in the formulations.

Formulations containing encapsulated retinol are better tolerated than those containing free retinol. However, the formulation with the highest concentration of encapsulated retinol (formula 1, table IV) is discarded due to technical problems in the dispersion of the raw material and due to its lack of a good sensory profile.

After evaluating the irritant potential of formulas containing free retinol, it is observed that the inclusion of bakuchiol in the formulation could act as a protective agent against skin irritation, since formulations in which bakuchiol is included regardless of retinol concentration (formula 4 and formula 5, table IV) have better tolerance.

This could be explained by the protective role of retinol oxidation [25] by bakuchiol and the inhibiting capacity of multiple inflammatory molecules. [28]

Both key active ingredients were the objects of an in vitro efficacy study to prove their anti-aging action as type I collagen boosters alone and even better when combined following the same proportion as the one present in the formula (table VI). The study clearly demonstrated that retinol and bakuchiol increases significatively the type I collagen levels. The combination of both showed and additive effect than the single compounds alone, although it is not a sum of the antioxidant potential of all of them.

These results for in vitro efficacy were validated with an efficacy study in volunteers. Efficacy studies in volunteers have the advantage that they reflect the real conditions of use (skin benefit) of the product. A non-invasive efficacy study was designed on 30 volunteers, between 40-60 years of age of which 10 women under menopause and with normal to dry skin, clinically showing crow's feet wrinkles, in order to evaluate the efficacy of the formulation. The results revealed a statistically significant increase in the parameters of skin barrier function, wrinkles and firmness following topical application of this formulation for 28 days. These improvements were also perceived subjectively among the panelists and may be due to the topical action of retinol and bakuchiol, which augments the type I collagen levels, thus and consequently firmness and anti-wrinkle efficacy due to intrinsic factors like hormonal aging in menopause.

Conclusion

We have studied a formulation that contains two main active ingredients: the well-known anti-aging gold standard retinol; and bakuchiol, a natural ingredient with a demonstrated retinol-like activity as it can function as a functional analogue of retinol. A topical formulation with good stability, tolerance and sensory perception containing retinol and bakuchiol has been developed for the restoration of the type I collagen lose and consequently firmness due to intrinsic factors like hormonal ageing in menopause—an excellent solution to help restore the skin healthy appearance.

Conflict of Interest Statement

The authors state no conflict of interest. All authors declare they are employees of Natura Bisse Int. S.A.

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