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"Comprehensive Evaluation of a Skincare Formulation Containing Recombinant Type III Collagen and Anti-Wrinkle Peptide for Anti-Aging Efficacy"

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1. Introduction

Aging skin still becomes one of the most popular concerns for consumers. Anti-aging claims in skincare products have increased by 17% during the 2020-2025 period. However, the anti-aging concept has shifted to more preventive and positive notions such as longevity, reflecting the widening consumers from older users to the younger ones during their onset of aging. Consumers also become more familiar with collagen as the main marker of skin aging and its importance in the chronological aging process [1].

Collagen is the most abundant protein in the human body. Collagen can be divided into 29 types, consisting of both fibril-forming and non-fibril-forming proteins, encoded by 45 different genes [2][3]. In the skin, collagen is mainly located in the dermis layer as the principal constituent of the skin structural component, providing tensile strength and firmness of the skin [2]. Collagen formed three-dimensional structures enclosing the skin cell with various macromolecules between it, such as glycosaminoglycans, hyaluronic acid, fibronectin, and others [3]. Collagen is supported by elastin, another fibrous structural protein that provides skin elasticity due to its elastic fiber structure that can stretch and recoil [4]. Both collagen and elastin, as well as the supporting macromolecules, make up the skin extracellular matrix (ECM).

The fibril-forming collagen type I and type III are the main types of collagens that constitute the dermal ECM, comprising 80% and 15% of total collagen content in young skin respectively [5]. These collagen and elastin are produced by fibroblast cells in which their activity will decrease over time from early adulthood. The decline of fibroblast activity will reduce collagen content in the skin by about 1.0%-1.5% a year. Collagen degradation rate can increase with the skin exposure to external factors such as sun exposure and free radical formation due to smoking and pollution [2]. Collagen degradation will change the skin structural strength and can be observed by the appearance of fine lines and wrinkles, the main clinical sign of skin aging [6]. The important role of collagen as one of the main hallmarks of skin aging is making it the focus of anti-aging treatment strategies, either by direct supplementation or by formulating topical regiment that can boost the production of native skin collagen.

The oral ingestion of collagen as treatment of skin aging has been proved to reduce wrinkles and improve skin elasticity in several in-vivo studies [7]. Collagen is also gaining traction as an active ingredient in skincare products, with consumers hoping to gain the benefit of collagen supplementation in topical application. Skincare products with “collagen” claim have increased by 50% in the last 5 years, further strengthening the popularity of collagen as anti-aging ingredients [1]. Currently, most collagen in skincare products is sourced from marine animals, such as tilapia fish, in which collagen constitutes nearly 75% of its body weight [8]. Marine collagen is widely available and relatively cheap, but it has a strong fishy odor, as well as stability issue in odor and color [9]. Other sources of collagen include bovine and porcine animals. However, bovine collagen has a risk of transmitting bovine spongiform encephalopathy, while porcine collagen might not be acceptable in certain markets due to religious reasons [10].

The advent of synthetic biology introduced a new approach in producing collagen for skincare products. Gene recombination technology, along with fermentation technique, can create recombinant collagen that resembles human collagen [11]. Recombinant collagen is synthesized by introducing genes that code collagen production into specific host cells followed by gene expression and protein translation, and subsequent collagen extraction and purification [12].

Besides collagen, peptides have also become the key ingredients in anti-aging skincare products, due to its ability to stimulate skin collagen synthesis. Several peptide derivatives such as acetyl hexapeptide-8 and Pentapeptide-4 are proven to improve skin elasticity and reduce wrinkles [13]. While its main mechanism is reducing muscle contraction in wrinkles by inhibiting neurotransmitter release, acetyl hexapeptide-8 can also increase the production of skin collagen [14]. Peptides are also preferred ingredients in anti-aging skincare products, because of their versatility and can be incorporated into various types of formulations [13]. This study aims to evaluate a moisturizer formulation containing recombinant collagen and acetyl hexapeptide-8, which represent two anti-aging strategies: direct topical collagen supplementation and stimulation of native skin collagen production. The evaluation focuses on the formulation performance, stability, efficacy, and safety.

2. Materials and Methods

Sample Preparation of Anti-Aging Moisturizer

A formulation of O/W (oil in water) cream was made using Multimix homogenizer. The active ingredients, 0.005% recombinant collagen and 2% acetyl hexapeptide-8, were added into the formulation. Performance evaluation was done including organoleptic evaluation, viscosity measurement using Brookfield Viscometer RV (helipath stand spindle 93, speed: 12 rpm, stop time: 60 seconds). and pH measurement using Mettler Toledo pH meter.

Stability Test

The stability test was carried out with these several conditions: room temperature, 45 ^{0}C, -4^{0}C and sunlight exposure. Parameters that were being checked were the same as those in performance tests. The samples were tested after 1 week, 2 weeks, and 1, 2, and 3 months in each condition.

Patch Test

A single patch test was conducted to evaluate the primary skin irritation potential of the test product. The study involved healthy adult volunteers (24 subjects) who had no history of skin diseases or hypersensitivity. A defined amount of the test product was applied onto a patch

and placed on the back of each subject under occlusive conditions. The patch was left in place for 24 hours.

After removal, the test site was evaluated by a dermatologist at 30 minutes and 24 hours post-removal using a standardized scoring system based on erythema / dryness/ wrinkles and oedema as per the Draize scale for scoring at the treatment site.

HRIPT

The Human Repeat Insult Patch Test (HRIPT) was conducted to evaluate the potential of the test product to cause skin sensitization in human subjects. The study followed standardized protocols and ethical guidelines, and all participants provided informed consent.

A total of 50 healthy adult volunteers with no history of allergic skin conditions were enrolled. The test product was applied under semi-occlusive/occlusive patches to the same site (the upper back) of each subject, three times per week (e.g., Monday, Wednesday, Friday) for three consecutive weeks (induction phase), resulting in a total of 9 applications. Each patch remained in place for 24 hours.

Following a rest period of 1 week, a single challenge application of the test product was applied to another site and left for 24 hours (challenge phase). Skin reactions were evaluated by trained dermatologists at 24, 48, and 72 hours after patch removal using a standardized scoring system for erythema, edema, and other visible skin responses.

Efficacy Test

This clinical study included 36 female subjects aged 20–50 years and was conducted using a double-blind protocol over a 4-week period. Participants applied the test product twice daily—once in the morning and once at night—and were instructed to apply a placebo sunscreen in the morning after the day cream. Redness assessments were carried out using Visia®, a spectrophotometer, and Antera 3D. Skin brightness was evaluated using a colorimeter, Antera 3D imaging, and dermatologist grading. Moisture levels were measured by Tewameter® and Corneometer®. Wrinkle evaluation was performed by dermatologists using the Aging Skin Atlas as a reference tool.

Statictical Analysis

All data collected were analyzed using IBM SPSS Statistics version 29. The results were expressed as mean ± standard deviation (SD). Prior to hypothesis testing, the data were checked for normality using the Shapiro–Wilk test. If the data were normally distributed, paired t-tests were performed to compare pre- and post-treatment values. For non-normally distributed data, the Wilcoxon signed-rank test was used.

A confidence level of 95% was applied, and statistical significance was defined as $p < 0.05$.

3. Results

Cream Formulation and Stability Test

In the experiment, the O/W emulsion cream was developed and give the properties as shown on the Table 1. After 3 months stability test, the cream could maintain its properties without any significant change (Figure 1), and quite stable pH and viscosity (Figure 1 and 2).

Table 1. Specification of the anti-aging cream

Parameter	Specification
Appearance	White, opaque cream
pH	5.5 – 6
Viscosity	25000 - 26000 cPs

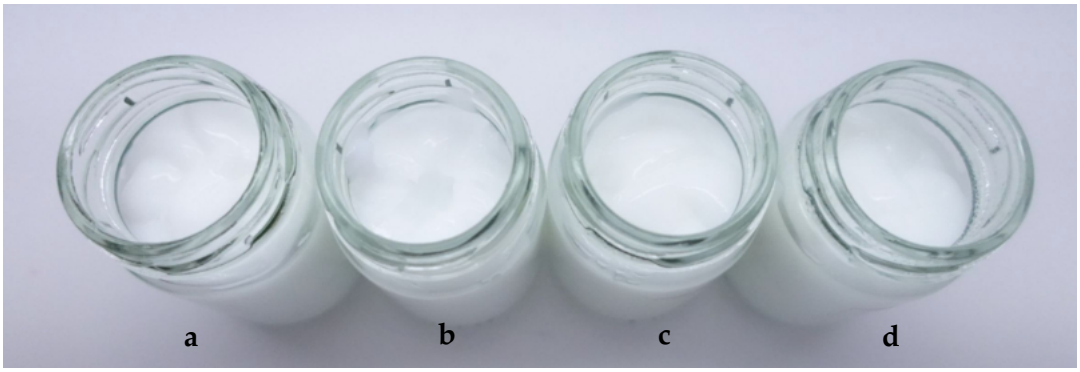

Figure 1. Appearance of the cream after 3 months stability test under several conditions: (a) Room temperature; (b) Temperature -4 ^{0}C (c) Under sunlight; (d) Temperature 45 ^{0}C

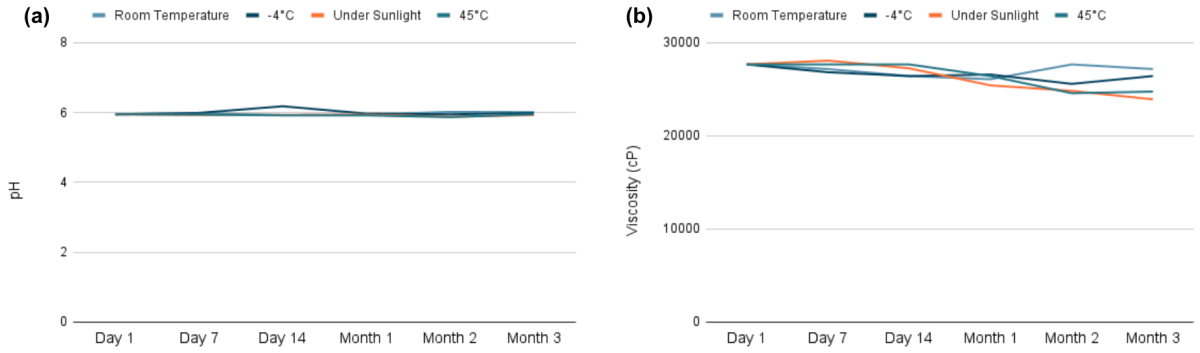

Figure 2. Stability profile of the cream during 3 months stability test for parameter (a) pH; (b) viscosity

Safety Testing

The safety of the cream formulation was evaluated using a single patch test and Human Repeat Insult Patch Test (HRIPT), following the protocol previously described in the methodology section. The results from the single patch test showed that the cream can be classified as non-irritant, with M.I.I. values of 0.21 at 0 hours (20-30 minutes), 0.00 at 24 hours, and 0.00 at 7 days post-patch removal. Furthermore, during the induction phase of the HRIPT,

the cream exhibited non-irritant properties when applied in its pure form under occlusion, with M.I.I values consistently recorded at 0.00 across all observation points. Moreover, no sensitization reactions were observed during the challenge phase, further supporting the product's excellent skin compatibility. These HRIPT results confirmed that the cream can be classified as hypoallergenic. Moreover, safety evaluation under dermatological monitoring showed a 4.45% (ns) reduction in non-inflammatory acne lesions and a 70.37% ($p<0.05$) reduction in inflammatory acne lesions. These findings demonstrated that the cream formulation is safe for daily use.

Efficacy Testing

The anti-aging efficacy of the cream was evaluated in 36 subjects during 28-day use of the product. A dermatological evaluation showed a reduction in wrinkles, as presented in Table 2 and visualized with Visia photography as Figure 3. Furthermore, skin firmness significantly improved by 26.91%, and folds in the eye area were significantly reduced by 14.12% compared to baseline, as measured by a Cutometer and Antera 3D, respectively.

Table 2. Dermatologist evaluation of skin wrinkles compared to baseline (Day 0) (N=36).

Parameter	**% Improvement compared to baseline (Day 0)**					
	Day 7		**Day 14**		**Day 28**	
	%	***p* value***	**%**	***p* value***	**%**	***p* value***
Fine Lines on Forehead	-7.14%	0.083	-14.29%	0.014	-26.19%	0.001
Eye (Crow's Feet)	-7.41%	0.157	-14.81%	0.046	-22.22%	0.014
Eye (Underneath Eye)	0.00%	1.000	-7.55%	0.046	-13.21%	0.020
Nasolabial Fold	-1.92%	0.317	-25.00%	0.001	-23.08%	0.001
Small Folds on Nasolabial Zone	0.00%	1.000	-36.36%	0.358	-90.91%	0.002
Cheek Sebaceous Pores	-5.80%	0.046	-21.74%	0.001	-28.99%	0.000
Density of Pigmentary Spots	-5.45%	0.083	-25.45%	0.001	-27.27%	0.000
Localized pigmentary	-5.66%	0.083	-18.87%	0.004	-24.53%	0.000

spots on the cheek						
Contrast of isolated pigmentary spot of the face	-1.85%	0.317	-16.67%	0.003	-20.37%	0.001
Wrinkle of the corner of the lips	-24.00%	0.014	-40.00%	0.004	-48.00%	0.001
Eye Bags	-100.00%	0.000	-100.00%	0.000	-100.00%	0.000
Forehead Wrinkles	-100.00%	0.000	-100.00%	0.000	-100.00%	0.000

* p value < 0.05 indicates statistical significance.

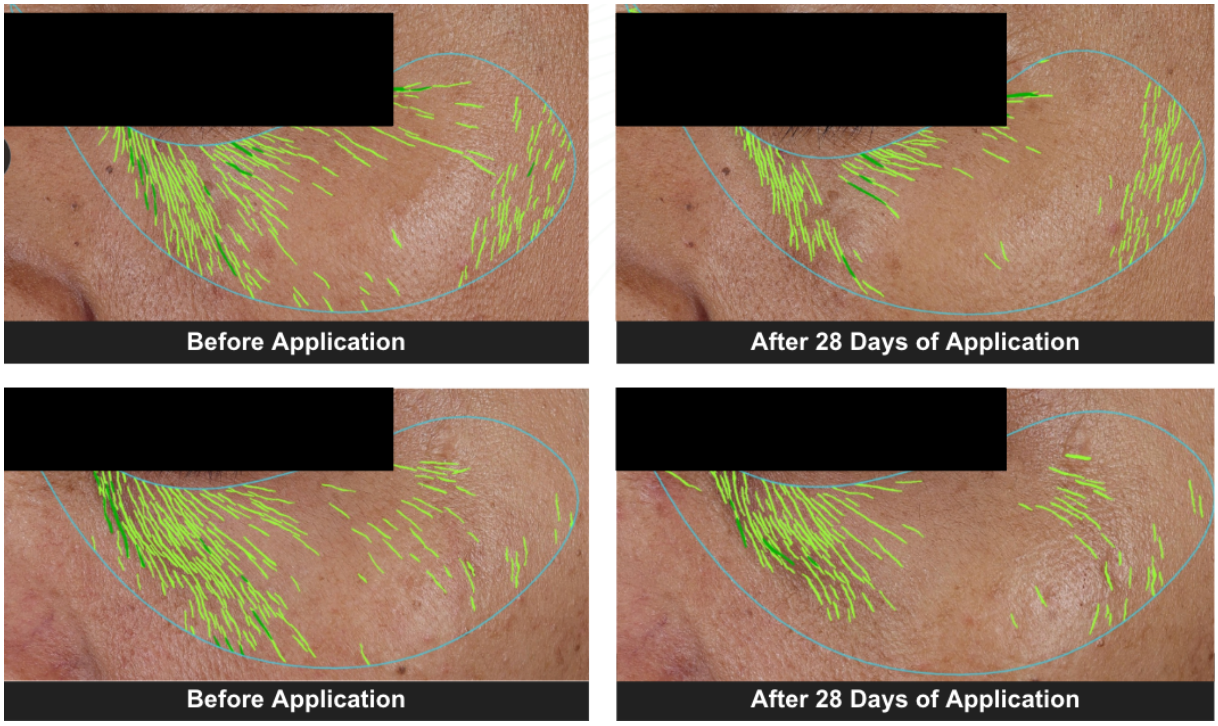

Figure 3. Wrinkle reduction after 28 days of product usage.

Signs of skin aging, such as moisture loss, skin dullnes, and redness, were also evaluated in this study. Measurements using Corneometer and Tewameter demonstrated that the cream improved hydration of the skin and reduced transpeidermal water loss (TEWL) from Day 7 to Day 28 of application, as shown in Figure 4. In addition, improvements in skin brightness and reductionss in skin redness were observed after 28 days of use, as presented in Table 3 and Table 4.

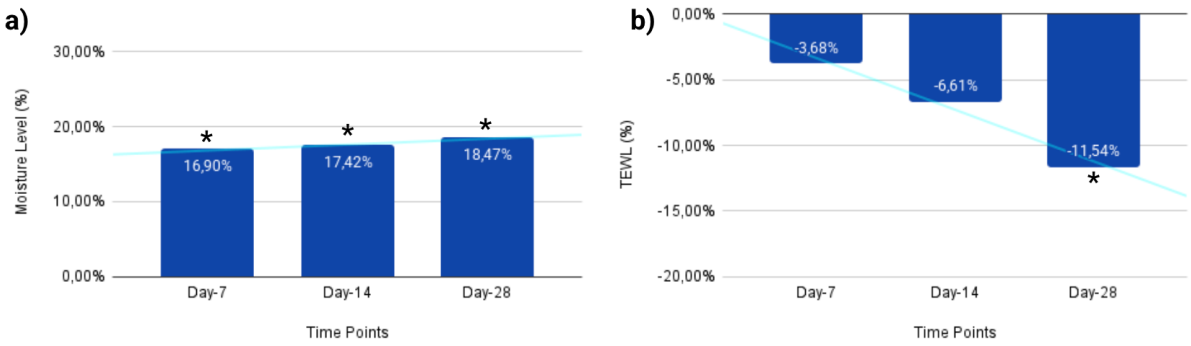

Figure 4. Skin moisture levels increased at D7, D14, and D28 compared to baseline, based on evaluations using: a) Corneometer and b) Tewameter (**p<0.05; statistical significance compared to baseline).*

Table 3. Evaluation of improvement of skin brightening at Day 28 of application compared to baseline (N=36).

Assessment Type	% Improvement at Day 28 compared to baseline (Day 0)	
	%	*p* value**
Colorimeter (b*)	6.61%	0.000
Antera 3D (b*)	1.86%	0.004
Dermatologist Grading	-10.45%	0.000

** *p* value < 0.05 indicates statistical significance.

Table 4. Evaluation of reduction of skin redness at Day 28 of application compared to baseline (N=36).

Assessment Type	% Improvement at Day 28 compared to baseline (Day 0)	
	%	*p* value**
Visia (Score Redness)	-11.28%	0.003
Spectrophotometer CM600D (a*)	-4.40%	0.018
Antera 3D (a*)	-1.83%	0.046
Antera 3D (redness)	-3.84%	0.006

** *p* value < 0.05 indicates statistical significance.

4. Discussion

Combining recombinant type III collagen and acetyl hexapeptide-8 was proposed to get excellent anti-aging efficacy in a moisturizer cream formulation. Recombinant collagen in this study is produced using *Pichia pastoris* yeast as the host cells for the fermentation. The utilization of yeast has several advantages, such as the absence of endotoxins, uncomplicated genetic modification procedures, and relatively low cost of production due to simplicity of operation and the possibility of large-scale high-density culture [11] [12]. The use of eucaryotic yeast host cell is also preferred, compared to procaryotic cells that do not have post-translational modification steps that are needed to create an identical human collagen [15]. The recombinant collagen has 55 kDa molecular weight with identical sequence to human type III collagen α chain. While the molecular size of this recombinant collagen is higher than other recombinant collagen and hydrolyzed collagen used in personal care products, it exhibits collagen synthesis stimulation activity in human dermal fibroblast [16].

Meanwhile, acetyl hexapeptide-8 is a synthetic peptide with N-terminal acetylated six amino acids chain (N-acetyl-L-alpha-glutamyl-L-alpha-glutamyl-L-methionyl-L-glutaminyl-L-arginyl-L-argininamide) and has a molecular weight of 875 Da [17]. This sequence mimics the N-terminal of end of the synaptosomal-associated protein (SNAP25) which is essential in inducing the Acetylcholine neurotransmitter release at the surface of muscle cells, leading to muscle contraction. Acetyl hexapeptide-8 will compete with SNAP25 and prevent the release of Acetylcholine, thus relaxing the muscle that contracted around face wrinkles and fine lines [18]. This mechanism, similar to Botolinum Toxin A, is possibly one of the reasons for a fast anti-wrinkle efficacy (7 days), observed from the moisturizer tested in this study.

In the moisturizer formulation, the two active ingredients—recombinant type III collagen and acetyl hexapeptide-8—can be successfully combined, demonstrating remarkable stability as shown in accelerated stability testing under various storage conditions. After three months of heat and light exposure, the formulation maintained its homogeneity, odor, and color, with no significant changes observed. Moreover, the inclusion of recombinant collagen and peptide did not significantly alter the pH or viscosity of the cream base, indicating their compatibility with the formulation. This stability is essential for commercial scalability and consumer satisfaction, as it directly impacts product shelf-life, safety, and overall effectiveness. These findings highlight the substantial potential of this combination for use in a wide range of anti-aging skincare products.

Additionally, the moisturizer has also been proven to be safe for the skin. Based on the patch test, there were no significant signs of irritation on the panelists. Similarly, the HRIPT indicated no significant adverse reaction and could be considered hypoallergenic. These findings are further supported by the use test on the face of the panelists, which showed no irritation and allergic reaction based on dermatology assessment.

The recombinant collagen will help maintain skin strength, elasticity, and resilience, meanwhile acetyl hexapeptide-8 will relax facial muscles to reduce expression lines [13, 16]. These properties were confirmed by an efficacy test on 36 panelists who used the product twice a day for 28 days. Skin strength was evaluated by improvement in skin hydration. Hydration leads to changes in the molecular arrangement of the peptides in the keratin filaments as well as dynamics of C-H bond reorientation of amino acids in the protruding terminals of keratin protein

within the stratum corneum, thus it can affect the skin strength and elasticity [19]. The hydration of the stratum corneum also plays a role in maintaining the skin's biomechanical properties [20]. The decrease of collagen production due to aging process leads to structural changes in the skin, including a reduction in the thickness and resilience of the skin, thereby increasing the skin's susceptibility to water loss [21]. In this study, hydration improvement 18.47% ($p<0.05$) was observed by Corneometer, and reduction in trans epidermal water loss (TEWL) 11.54% ($p<0.05$) by Tewameter. Those indicate the product can improve skin hydration as well as strengthen skin barrier [22]

Furthermore, moisturizer with the combination of recombinant type III collagen and acetyl hexapeptide-8 showed a significant reduction of fine lines and wrinkles in various parts of the face. The most noticeable result is the –100% reduction of forehead wrinkles and –90.91% reduction of small folds on nasolabial zones by dermatologist evaluation. The wrinkle reduction also starts to be observed since the day 7 of observation and further improved until the efficacy test finished on day 28, indicating fast and prolonged efficacy of the product. Additionally, skin firmness was increasing by 26.91% ($p<0.05$) and skin brightness by 6.61% ($p<0.05$). While this study did not investigate the efficacy of recombinant collagen and acetyl hexapeptide-8 as its own, the significant efficacy result of moisturizer with those to ingredients showed a possible synergistic effect of combining direct topical collagen supplementation and increasing skin collagen production.

This study was limited by its short duration, as it was conducted for only 28 days. A longer study period may be beneficial to observe more significant improvements in skin condition, particularly in reducing wrinkles, which typically require more time to show noticeable changes. Furthermore, incorporating additional studies using complementary instruments such as-resolution ultrasound, 3D skin imaging, or profilometry, could provide valuable insights to further confirm the product’s efficacy.

5. Conclusion

This study showed a formulation containing recombinant type-III collagen and acetyl hexapeptide-8 exhibit significant anti-aging benefit in vivo, while showing good stability profile including homogenity, odor, and color of the formula.

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