Moisturising effect of cosmetic formulations containing different concentrations of aloe vera extract, assessed by means of biodermatological methods

Keywords:
- Improved skin moisture
- Dry skin

Summary:
The study demonstrates that aloe vera is a wonderful natural agent for the improvement of skin moisture levels. The use of aloe vera on dry and chapped skin is therefore highly recommended.

Moisturizing effect of cosmetic formulations containing *Aloe vera* extract in different concentrations assessed by skin bioengineering techniques

Susi Elaine Dal'Belo, Lorena Rigo Gaspar and Patrícia Maria Berardo Gonçalves Maia Campos

Laboratory of Cosmetic Technology, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, University of São Paulo, Brazil

Background/purpose: The polysaccharide-rich composition of *Aloe vera* extracts (*Aloe barbadensis* Miller), often used in cosmetic formulations, may impart moisturizing properties to the product. The aim of this study was to evaluate the effect of cosmetic formulations containing different concentrations of freeze-dried *Aloe vera* extract on skin hydration, after a single and a 1- and 2-week period of application, by using skin bioengineering techniques.

Methods: Stable formulations containing 5% (w/w) of a trilaureth-4 phosphate-based blend were supplemented with 0.10%, 0.25% or 0.50% (w/w) of freeze-dried *Aloe vera* extract and applied to the volar forearm of 20 female subjects. Skin conditions in terms of the water content of the stratum corneum and of transepidermal water loss (TEWL) (Corneometer™ CM 825 and Tewameter™ TM 210) were analysed before and after a single and 1- and 2-week period of daily application.

Results: After a single application, only formulations supplemented with 0.25% and 0.50% (w/w) of *Aloe vera* extract increased the water content of the stratum corneum, while after the 2-week period application, all formulations containing the extract (0.10%, 0.25% and 0.50%) had the same effect, in both cases as compared with the vehicle. TEWL was not modified after a single and after 1- and 2-week period of application, when compared with the vehicle.

Conclusion: Our results show that freeze-dried *Aloe vera* extract is a natural effective ingredient for improving skin hydration, possibly through a humectant mechanism. Consequently, it may be used in moisturizing cosmetic formulations and also as a complement in the treatment of dry skin.

Key words: *Aloe vera* extract – moisturizers – corneometer – TEWL – skin hydration

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THE APPEARANCE and function of the skin are maintained by an important balance between the water content of the stratum corneum and skin surface lipids (1, 2). Exposure to external factors, i.e., air humidity, ultraviolet radiation, temperature, as well as endogenous factors, i.e. hormones (3–5), may disrupt this balance. In addition, frequent use of soaps, detergents and topical irritants such as alcohol and hot water can remove the skin surface lipids (6). When this balance is disrupted, a dermatological condition known as dry skin ensues, a phenomenon that is observed particularly in patients with atopic dermatitis, a chronic and pruritic form of dermatitis (7).

In these cases, effective cosmetic products must be used to improve skin hydration not only for aesthetics purposes but also to maintain the normal conditions of skin and to prevent dry skin alterations. The moisturizing effect of formulations may be influenced by many factors, such as type and concentration of the active substances used, as well as the composition of the vehicle (8, 9).

Medicinal plants of the lily family (Liliaceae), genus Aloe, have been used for the treatment of skin diseases for more than 2000 years (10). Among more than 360 Aloe species, *Aloe vera* (*Aloe barbadensis* Miller) has been the most popular in both folk and officinal medicine (11).

Aloe vera extracts are widely used in a variety of over-the-counter and dermatological products. Many studies report the effective use of this plant when applied topically for the treatment of

burns, sunburns, inflammatory skin disorders and wounds (12, 13). However, the action of *Aloe vera* as a moisturizing agent still mostly remains a popular concept (12), and it has been used in many moisturizing products, in different concentrations, an important factor in the efficacy and cost of a cosmetic product.

Consequently, clinical studies to evaluate the moisturizing effect of *Aloe vera* extracts scientifically are necessary to validate this claimed effect. Objective methodologies are considered appropriate to prove and to clarify the mechanisms of action of substances that improve skin hydration. Among these are non-invasive skin bioengineering techniques, which are often used as they allow evaluation of cosmetic products under actual conditions of use.

In the present study, we used skin bioengineering techniques to evaluate the effects of cosmetic formulations containing different concentrations of freeze-dried *Aloe vera* extract on skin hydration, after a single and a 1- and 2-week period of application

Methods

Formulations

The formulations studied (Table 1), containing 5% (w/w) of a trilaureth-4 phosphate-based blend (Hostacerin SAF™, Clariant, São Paulo, Brazil), were prepared in a Heidolph RZR 2021 shaker at approximately 625 r.p.m., and supplemented or not with 0.10%, 0.25% or 0.50% (w/w) of freeze-dried *Aloe vera* (*Aloe barbadensis* Miller) extract, a commercial 200:1 concentrate (ACTIValoe™ Aloe vera GEL FD200 × , Aloecorp, Washington, DC, USA).

Study protocol

Approval for the study was obtained from the Faculty of Pharmaceutical Sciences of Ribeirão Preto – USP Ethics Committee (CEP/FCFRP 07/2001).

TABLE 1. *Vehicle components*

Components	Percentage of components (w/w)
Trilaureth-4 phosphate-based blend	5.0
Propyleneglycol	2.50
Glycerin 86%	2.50
Phenoxyethanol and parabens	0.80
Hydrogenated and etoxillated castor oil 40 OE	2.00
NaOH (sol 10%)	pH 5.5–6.0
Deionized water	87.2

Twenty healthy female subjects 20–45 years old, having skin Fitzpatrick types II and III, participated in this study after having given their informed consent. The exclusion criteria were as follows presence of, any dermatitis and/or other skin or allergic diseases, smokers and previous treatment of forearms' skin with cosmetic formulations such as moisturizers, sunscreens or anti-ageing cosmetics. During the test period, the subjects were allowed to wash normally, but were instructed not to use any other skin care products on their arms.

Prior to all measurements, subjects remained in the room for at least 30 min in order to allow full skin adaptation to room temperature (20 ± 2 °C) and humidity (45–60%) (14). The forearm skin area of each volunteer was subdivided into two sites (36 cm^2). The formulations studied and the measurement sites were randomized between subjects.

All measurements were carried out according to the relevant guidelines (15, 16).

Effects after a single application

After the baseline measurements, 0.2 g of each formulation containing the three different concentrations of *Aloe vera* extract (0.1%, 0.25% and 0.5%, w/w) and the formulation without extract (vehicle) were applied on the different sites; 1, 2 and 3 h after application, new measurements were carried out.

Effects after 1- and 2-week period of daily applications

After the baseline measurements, the subjects applied 0.2 g of the formulations studied on their forearms, twice daily, in the morning and in the evening; 1 and 2 weeks after application, new measurements were carried out, 10–15 h after the last treatment, i.e., the formulations were applied in the evening and the measurements were taken the following day (8).

Instrumentation

The water content of the stratum corneum was measured with a skin capacitance meter (Corneometer™ CM 825, Courage & Khazaka Electronic GmbH, Cologne, Germany) (17, 18). The device determines the water content of the superficial epidermal layers down to a depth of about 0.1 mm and expresses the values in arbitrary

units (19). The average values of 20 measurements/site were used in subsequent calculations.

The transepidermal water loss (TEWL) was measured with an evaporimeter (Tewameter™ TM 210, Courage & Khazaka Electronic GmbH), and registered in g/m^2h during 2 min after probe equilibration on the skin for 30 s (20).

Statistical analysis

Non-parametric tests were selected for statistical analysis of the experimental data points, as they showed a non-Gaussian distribution. The paired Friedman test was used for comparison of multiple measured data points using statistical software, GMC. Differences were considered as statistically significant at $P < 0.05$.

Results

Effects after a single application

Electrical measurements in the short-term study are reported in Figs 1 and 2.

Significant increases in the water content of stratum corneum readings ($P < 0.001$) relative to baseline were observed 1, 2 and 3 h after application of all formulations studied (vehicle and vehicle containing *Aloe vera* extract) (Fig. 1).

However, when compared with the vehicle, only the formulation containing 0.50% of freeze-dried *Aloe vera* extract increased the water content

* Significantly different from the vehicle ($P < 0.01$)
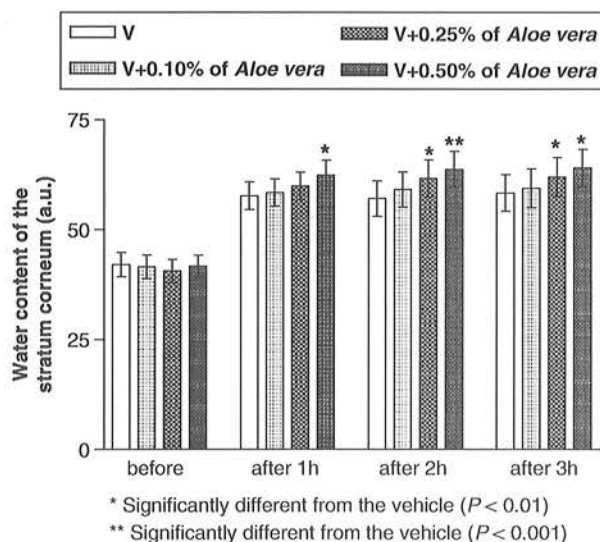** Significantly different from the vehicle ($P < 0.001$)

Fig. 1. Water content of the stratum corneum before (baseline values) and 1, 2 and 3 h after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).
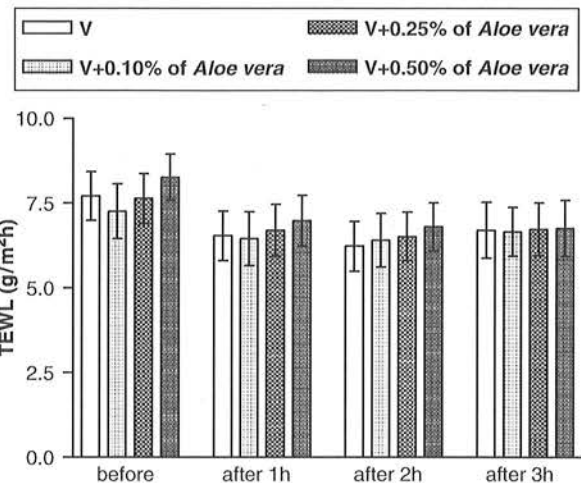
Fig. 2. Transepidermal water loss before (baseline values) and 1, 2 and 3 h after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).

of the stratum corneum ($P < 0.01$) after 1 h (Fig. 1). After 2 and 3 h, formulations containing 0.25% ($P < 0.01$) and 0.50% ($P < 0.001$) of *Aloe vera* extract enhanced the water content of the stratum corneum when compared with the vehicle (Fig. 1).

One, 2 and 3 h after the application, all formulations studied reduced TEWL values significantly ($P < 0.05$) when compared with baseline values (Fig. 2).

Nevertheless, when compared with the vehicle, TEWL values were not modified after a single application of the *Aloe vera* extract-supplemented formulations, which means that the skin barrier function was not altered by this extract (Fig. 2).

Effects after 1- and 2-week period of daily applications

All participants reported strict compliance with the instructions. Electrical measurements in the long-term study are reported in Figs 3 and 4.

Significant increases in the water content of stratum corneum readings ($P < 0.001$) relative to baseline were observed 1 and 2 weeks after the application of all formulations studied (vehicle and formulations containing *Aloe vera* extract) (Fig. 3).

When compared with the vehicle, all formulations containing freeze-dried *Aloe vera* extract (0.10%, 0.25% and 0.50% w/w) increased the water content of the stratum corneum ($P < 0.01$), after a 1-week period of application, but they were not statistically different among themselves (Fig. 3).
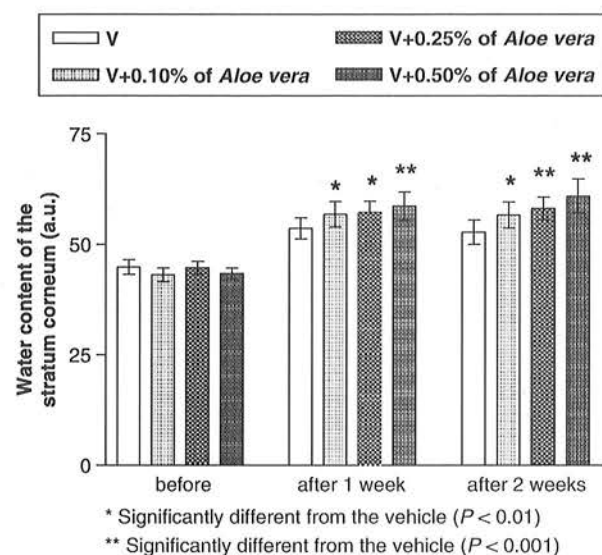
Fig. 3. Water content of the stratum corneum before (baseline values) and 1 and 2 weeks after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).
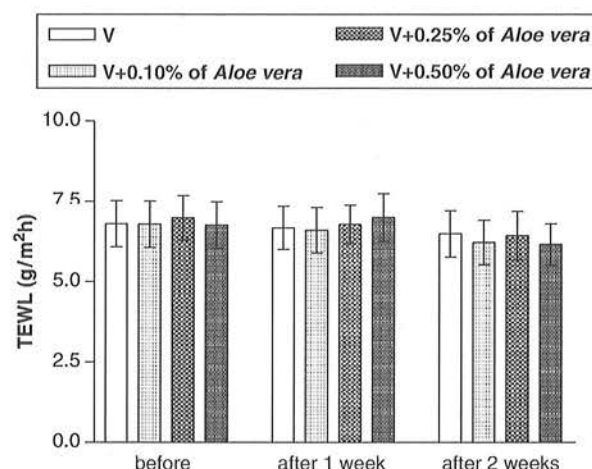

Fig. 4. Transepidermal water loss before (baseline values) and 1 and 2 weeks after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).

Similarly, the results obtained after a 2-week period application showed that all formulations containing Aloe vera extract produced a significant increase in skin hydration when compared with the vehicle ($P < 0.01$). However, when these formulations were compared with each other, the water content of the stratum corneum values obtained with the formulation containing 0.50% of Aloe vera extract was significantly higher ($P < 0.05$) (Fig. 3).

TEWL did not change when compared with baseline values 1 and 2 weeks after application for all Aloe vera concentrations and for the vehicle (Fig. 4). The different results in relation to the single-application study were probably because of measurements taken shortly after the application of the formulations (1, 2 and 3 h after the application), which were altered by the greasy film formed by the vehicle lipophylic components. In the long-term study, the TEWL measurements carried out 10–15 h after the treatment were not disturbed by the lipophylic components of the vehicle, which had already been removed.

In addition, when compared with the vehicle, formulations supplemented with Aloe vera extract did not change TEWL values as well, which means that the presence of Aloe vera in the formulations did not alter skin barrier function (Fig. 4).

Discussion

Studies of skin hydration have been performed mainly using short-term studies, where the measurements are carried out between 1 and 8 h after the application of the product, as it is possible to attain improved skin moisture shortly after a single application. Nevertheless, long-term studies are important to assess the maintenance and enhancement of this effect.

The moisturizers may act by an occlusive mechanism, impairing evaporation of skin moisture by forming an epicutaneous greasy film that prevents water loss, as is the case with oils and lipids, or as humectants, i.e., glycerin, urea, sodium pyrrolidone carboxylic acid, which act by attracting water from the other layers of the epidermis to the stratum corneum (5, 21). Consequently, studies with moisturizing products should evaluate the increase in the water content of the stratum corneum and also the decrease in TEWL, in order to determine their mechanism of action.

Our results showed that the freeze-dried Aloe vera extract studied improved skin moisture by a humectant mechanism, since when compared with the vehicle, the treatment with supplemented formulations significantly increased the water content of the stratum corneum but did not change the TEWL. This result probably occurred because the freeze-dried Aloe vera extract has a rich composition in hygroscope mono- and polysaccharides (22) and in the amino acids histidine, arginine, threonine, serine, glycine and alanine,

which may improve water retention in the stratum corneum (23).

As similar results of skin hydration were obtained after a single and 1- and 2-week period of application, the long-term results can be predicted by the single-application data. This is in agreement with the report by Li et al. (9), who found a linear correlation between changes in the electrical measurements after 1 h and the change in skin dryness grade after 1-week period of application of a glycerin lotion. As a result, these authors suggested that a single application could accurately predict results of long-term (2-week) studies with multiple applications. However, we concluded that it is very important to undertake both studies, as our findings showed that the concentration of *Aloe vera* extract also influences the improvement of skin hydration. Lower concentrations of this extract, like 0.10%, only lead to a significant increase in the water content of the stratum corneum in the long-term application.

Thus, we suggest that the daily use of moisturizers containing *Aloe vera* extract is important to maintain a humectant effect on the skin, which is usually immediate.

The presence of *Aloe vera* in the formulations did not alter the skin barrier function, as TEWL values were not changed when compared with the vehicle. Consequently, *Aloe vera* does not have an occlusive property. The only effect on TEWL values was because of the formation of a greasy film by the lipophylic components of the vehicle, which was observed only for a few hours (up to 3 h).

This study constitutes an objective evaluation of the moisturizing effect of cosmetic formulations containing *Aloe vera* extract, and contributes to the elucidation of its mechanism of action. In addition, the results showed that the formulations studied caused an immediate hydration effect, which was maintained after 1 and 2 weeks with daily applications.

Conclusion

Formulations containing different concentrations of freeze-dried *Aloe vera* extract showed efficacy in improving skin moisture by a humectant mechanism, when evaluated in short- and long-term application studies. After a single application, only formulations supplemented with concentrations above 0.25% improved the water content of the stratum corneum. After 1- and 2-week period of application, all the concentrations were significantly effective.

Thus, freeze-dried *Aloe vera* extract is a natural effective ingredient for improving skin hydration, which can be used in moisturizing cosmetic formulations and also to complement the treatment of dry skin.

Acknowledgements

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Dal'Belo et al.

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Address:
Dr P.M.B.G. Maia Campos
Av. do Café s/n, Bairro Monte Alegre
Ribeirão Preto 14040-903, SP
Brazil

Tel: +55 16 3602 4197
Fax: +55 16 3625 7202
e-mail: pmcampos@usp.br

ALOECORP

Reevaluation of functionality of the KFDA [Korean Food and Drug Administration] pre-confirmation registered functional health foods; products related to dermatological function

[Redacted: only contains data related to study parameters and Aloe vera products]

Leader of the detail subject:
Seoul National University School of Medicine

Research results of the third detailed research-and-development subject

Manufacturer Information
Test Product: AloeXGold®, an unflavored Aloe vera drink made with ACTIValoe® from Aloecorp, Inc.
Definition of Dosage Units: AloeXGold is a liquid product manufactured by dissolving 10g of Aloecorp's ACTIValoe® gel fillet powder in one liter of purified water. The high dose in the study was achieved by formulating the liquid product with 30 g of the powder in one liter of purified water.

Chapter 1. Objectives of final research and development of the third detail research and development subject

1.1. Objectives of detail research and development subject

1) Domestic and overseas documents, particularly the data regarding dermatological function-related functional foods (4. aloe products) of the KFDA pre-confirmation registered functional health foods were collected. Quality and quantity evaluations were carried out on them using the evaluative system we have developed. Those cases about which we judged the scientific evidence to be insufficient were selected as products for additional experimentation.

2) Of the aforementioned products for additional experiments, animal experiments were done with regard to the currently suggested intake dosage of the functional health foods.

Originally, human experiments were planned for the products whose functionality was acknowledged in the event animal experiments were also required. However, since the above- mentioned KFDA pre-confirmation registered health food products had no related documents or the related documents showed conflicting results, it was decided that animal experiments on all products would be omitted and that the human experiments would be performed with the human subjects divided into low-dosage and high-dosage groups.

3) Human experiments were done at the intake dosage suggested for the functional health foods of the products selected for additional experimentation.

4) The validity of biomarkers suggested in the 2004 result report, entitled "Dermatological function-related functionality evaluation system build-up project of functional health foods," was reviewed and supplemented.

1.2. Achievement progress of detailed research-and-development subject

Classification / Descriptions of research	Achievement progress	Level of contribution to technical development of related fields
Collection and evaluation of documents	Completed	
Selection of products for additional experiment subjects	Completed	Established the scope of application of the given functional health foods
Setting of intake dosage for additional experiment subjects	Completed	Verified the intake scope of the products currently for sale
Performance of animal experiments regarding the intake dosage	Omitted	Not applicable
Performance of human experiments regarding the animal-experiment intake dosage	Completed	Established the deliberation guidelines of reevaluation of functionality of the given functional health foods
Review and analysis of the validity of biomarkers for reevaluation of functionality	Completed	Established the deliberation guidelines of reevaluation of functionality of the given functional health foods
Suggestion of allowable range of functionality indication	Completed	Contributed to development of the industry of functional health foods and the promotion of people's health
Completion of the final report	Completed	

1.3. Status of domestic and overseas technical development

(1) Status, problems and prospects of domestic and overseas technical development

(A) Status of domestic and overseas technical development

• Research on dermatological functions has primarily focused on **skin-moisturizing function, wrinkle reduction and whitening.** The skin health-related functional health foods generally used in Korea are very diverse, including Korean medicinal materials such as the fruit of the Chinese matrimony vine, Korean angelica, licorice root, dodder seed, stiff silkworm, moxa leaves, chestnut skin, seaweed, chrysanthemum indicum linne, peppermint, etc.; nutritional supplements such as vitamins and minerals, as well as functional health foods such as aloe; and folk therapies including mung beans, brown rice, red beans, adlay, green tea, honey, plum, gorosoe sap (acer mono max.), rice water, shigoka, elvan, etc. Outside Korea, nutrients including vitamin C, herbal products such as lavender and tea-tree oil, and plant materials such as rooibos, oatmeal, etc., have been used for the sake of skin beauty.

• It is expected that sales of functional health foods will be more active in the future, via the Internet and home shopping.

• Recently the domestic market for functional health foods has shown a consistent increase. In addition to existing food product manufacturers such as Namyang Aloe, Semo, AloeMime and PulmuoneTech, over 20 pharmaceutical companies such as Ilyang Pharmaceutical, Chongkeundang Health, Daewoong Pharmaceutical, Kwangdong Pharmaceutical, Hanmi Pharmaceutical and Hyundai Pharmaceutical have either newly set up or strengthened existing food divisions. Domestic and overseas dermatological function-related health products consist mostly of sales of extracts or raw materials. The domestic market for functional health foods has demonstrated record high sales in the product categories of chitosan, aloe, enzymes and squalene.

• Among the 32 types of KFDA pre-confirmation registered products in the Functional Health Food Code, the following can be classified as functional in terms of skin health: 4) **aloe products.** In terms of indications and advertisements, it is possible to indicate the functional descriptions within the scope suggested in Table 1.

• **Aloe products** are the leaves of those edible aloe species (vera, arborescens and saponaria) that are processed to a degree adequate for consumption, or are processed for easy intake in liquid, paste, powder, granule, tablet and capsule forms. They are known to be effective in antibacterial, anti-inflammation, cell restoration, immune enhancing purposes, and also for atopic skin, skin burn and skin disorders.

(B) Problems

• Given the fact that South Korea has become an aging society with its aged population of persons 65 years old or more amounting to 7.1% of the entire population (as of the year 2000), there has been an increased demand to keep healthy or to prevent adult diseases through foods. However, **there has been a significant lack of information to support the efficacy of such foods.** Moreover, there are very limited research results that can support the "skin function enhancement" of the KFDA pre-confirmation registered functional health foods that are currently for sale. The reality is that the functional description called "skin health maintenance" has been allowed and sold despite the absence of any systematic, objective system of evaluation.

Table 3-1 KFDA pre-confirmation registered products related to skin health

Product Group	Types	Descriptions of function
3) Aloe products	· Aloe gel · Aloe gel concentrate · Aloe gel powder · Pressed aloe juice · Aloe powder · Aloe gel products · Pressed aloe juice products · Aloe gel powder products · Aloe powder products	· Assists intestinal movement · Functions for enhancing immune system · Assists stomach and intestinal health · Assists skin health (aloe vera) · Assists bowel movement (aloe essence)

- Numerous functional health foods related to skin function have been used worldwide. However, their effects are mostly dependent on old documents or simple in-vitro experiments or partial animal experiments. The reality is that there has been no product that has successfully passed the process of scientific verification through systematic human subject experiments.

(C) Prospects

- Because the twenty-first century is expected to see great advancements in biology and biotechnology, the functional foods directly related to them are expected to rise dramatically owing to their high-value-added characteristics. Considering that currently just 2% - 3% of the world's food market is occupied by functional foods, there is enormous promise in terms of further development.

- Recently, as the market related to skin beauty-related functional health foods has grown at a very high rate in the name of "edible cosmetics," the objective biomarker-based data of scientific evidence regarding food materials is expected to accumulate rapidly as the "beauty-related functionality evaluation system" of functional health foods is implemented.

- Dermatological function-related evaluation will be focused on **skin wrinkle reduction, whitening and moisturizing effects.**

(2) Need for and importance of domestic research and development

(A) Economic aspect
- The global market for functional health foods, whose size was calculated at US$65 billion in 1997, is expected to reach US$206 billion in 2005. Moreover, the domestic market is expected to reach the scale of 1.9 trillion won in 2005 from the scale of 980 billion won calculated for 1997. In other words, the industrial market scale of functional health foods is expected to soar dramatically, owing to the increased desire for health and the international opening of the market.

(B) Social aspect
- Humans have the natural desire to pursue beauty and youth, as well as the desire to live a healthful life. The elements of beauty and youth include beautiful skin and a beautiful figure, and all are directly related to physical health. Recently the scope of research regarding skin beauty has expanded to include the efficacies of foods, not to mention the field of cosmetics.

- Recently, the market for functional health foods in relation to skin beauty has used the term "edible cosmetics." However, the efficacies of most such products have not yet been proved, and it is urgent to accumulate scientific research data in order to limit the risk of damage to consumers. Particularly since numerous functional health foods are expected to be available in the market in the future, all with claims of skin beauty functions, there is a need for a set of verified standards that reasonably evaluate such efficacies.

(C) Technical aspect
- Definition of "dermatological function enhancement": Based on the objective and scientific definitions about skin beauty in terms of wrinkles, moisturizing and whitening.

First, definition of a skin wrinkle: This is a phenomenon where folds of skin overlap each other due to the decrease in tension and elasticity. Early wrinkles are thin but gradually grow thicker and deeper. Depending on the characteristics of protein and changes in its amount, as well as the dermal cells, the physical properties of skin include changes in elasticity. Wrinkles can also form due to the decrease of moisture and subcutaneous fat. They appear around the age of 20 and continue to appear as a person ages. Areas more subject to use because of movement throughout life may get wrinkles earlier, as in the facial wrinkles that appear due to the movement of facial expressions.

Second, definition of skin whitening: This is a function that corrects or recovers the chloasmas or freckles that appear due to pigmentation of the skin's surface. Chloasmas and freckles are created as melanin increases. Melanin cells increase when skin is continuously exposed to UV rays. As a result, pigmentation increases. Lately, those materials the de-colorizing effect of which has been verified such as hydroquinone, arbutin, kojic acid, ellagic acid, and vitamin C inducers have been used for whitening cosmetics for the purpose of lessening chloasmas and freckles. However, there is little material or product that satisfies the customer's desire for whitening effects via oral administration.

Third, definition of skin moisture: This is the level of moisture that exists in the epidermal keratinous layers. Skin moisture is absolutely essential in order to maintain the elasticity or the soft and moisturized feel of skin. Healthy epidermal keratinous layers contain 15% to 20% moisture. If due to environmental factors such as the climate or the season, and due to endogenous factors such as genetics, aging, hormones, stress and nutrition condition, the skin's sebum layers are lost or their generation decreases, transepidermal water loss (TEWL) is caused and the abnormal reproduction of exposed epidermal cells increases. This makes the skin dry and rough, resulting in keratin. The rate of water contained in keratinous layers is determined by the sebum membrane, which is the mixture of skin lipids generated on the epidermis, and by natural moisturizing factors (NMF) or the soluble substance present in the keratinous layers.

- The leader of the specific assignment has established "Evaluation system of beauty-related functionality of functional health foods" as part of the 2003 research service project. Therefore, objective and strict evaluation standards of functionality evaluation are to be applied for individual confirmation type of materials to be newly acknowledged or KFDA pre-confirmation registered materials. Therefore, it is an urgent task to re-evaluate the efficacy of dermatological functions of existing KFDA pre-confirmation registered categories of products based on systematic biomarkers.

- Research on functionality of KFDA pre-confirmation registered materials related to dermatological function. To date, the descriptions of functionality of KFDA pre-confirmation registered materials related to dermatological function acknowledged by the Functional Health Food Codes can be summarized as shown in Table 1. However, the reality is that there is lack of objective research that supports allowed physiological activation. **It is necessary to reevaluate the efficacy of dermatological function enhancement in the existing KFDA pre-confirmation registered products at least for the purpose of ensuring fairness between the skin beauty-related individual-confirmation types of functional health foods to be newly evaluated and acknowledged based on scientific evaluation system in the future, and the pre-confirmation registered types of functional health foods.**

Chapter 2. Description and method of the final research and development of the third specific research-and-development assignment

2.1. Literature research

(1) Collection of literature

The present researcher of this assignment has experience in research and comparative analysis regarding the status of dermatological functionality research based on domestic and overseas related literature at the time of carrying out the 2003 ["Project of establishing evaluation system of beauty-related functionality of functional health foods"]. That is, the present researcher has searched and obtained related literature with the key terms "collagen," "elasticity" and "MMP" in the literature reported between 1990 and 2004 in the search database of PubMed (http://www.ncbi.nim.nih.gov). Along with the previously obtained research papers, other literature that was added later will be supplemented, and related literature will be searched and collected.

According to the search database, the literature related to the five categories of products their dermatological functions were acknowledged of the pre-confirmation registered functional health foods will be collected and its research status will be evaluated, based on know-how accumulated during the previous year's research. Furthermore, literature related to the items that have been used as folk therapies in Korea since ancient times will be collected using the Korean studies Information Service System (KISS). Additionally, domestic and overseas Oriental medicine-related books and physiological activation research reports will be included for research subjects.

(2) Analysis and organization of the data

The related data is analyzed and organized with the emphasis on the following.

1) By food material: aloe products.
2) By animal test: Species of animals tested, test planning, method of causing wrinkles, method of inducing pigmentation, duration of tests, amount of the test substances used, biomarkers, wrinkle enhancement, whitening, and level of contribution to skin moisture.
3) By human test: Human test subjects, basic requirements, plan of human tests (double-blind, placebo-controlled study, etc.), method of causing wrinkles, method of inducing pigmentation, duration and dosage of oral administration of the test substances, biomarkers, wrinkle enhancement, whitening, and level of contribution to skin moisture.

(3) Quantitative and qualitative evaluation of literature and data

The evaluation of searched literature will be quantified into scores based on the level of academic journals in which the articles appeared, the degree of faithfulness to the basic criteria for evaluation test demonstrated, and the confidentiality level of measuring biomarkers. In the event the number of articles of upper percentile in the score distribution reaches 10 or more, the functionality of the pre-confirmation registered items in question will be judged as possible through study of the literature alone. On the other hand, if the number of the collected related articles is small, or even if the number of the articles suffices but the articles obtained have low objective scores, the functionality of the pre-confirmation registered items will be judged to need additional review through additional tests. Evidentiary documents will be included in determining the need of additional tests for the evaluation of functionality.

* Basic criteria for animal tests and human tests for dermatological functionality evaluation

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- Basic criteria for animal tests

1. Skin beauty-related factors: Wrinkles, whitening, moisturization
2. Species, strain, age and number/group of animal test subjects
3. Period and method of raising the animals, and the groups of the study
4. Protocols of methods of causing wrinkles and pigmentation
5. Biomarkers related to skin beauty
   - Wrinkles, skin color, skin elasticity, moisturization level of skin surface
   - Biochemical biomarkers: Collagen, MMPs, elastin

- Basic criteria for human tests

1. Skin beauty-related factors: Wrinkles, whitening, moisturization
2. Age, and number/group of test subject humans
3. Whether or not to set double-blind, baseline characteristics study groups (placebo included)
4. Protocols of methods of causing wrinkles and pigmentation
5. Biomarkers related to skin beauty
   - Wrinkles, skin color, skin elasticity, moisturization level of skin surface
   - Biochemical biomarkers: Collagen, MMPs, elastin

2.2. Reevaluation of functionality related to dermatological functions through animal tests

Because the time given to this research was comparatively short, the products for reevaluation were as many as four. Therefore, it took a lot of time to determine the material suppliers of these substances and the adequate dosages for human tests, and also to complete an application to Institutional Review Board (IRB) for deliberation and to have deliberation, and human tests are more efficient, and give more direct and convincing conclusion, this research was designed to carry out the human tests, which was the second year research and development plan, by dividing the subjects into low-dosage group and high-dosage group for adequate dosage setting for oral administration, so that it can elicit the approximate adequate dosages. Of the five substances, pollen products have no single related article. Therefore, it was decided not to reevaluate pollen products through human tests, since there is no evidence of functionality related to dermatological function.

2.3. Reevaluation of dermatological function-related functionality with human subjects

(1) Subjects of research

Healthy adult men and women 50 years old or more

(2) Dosages and oral administration of test foods

Based on the study of literature on raw materials for each functional food, low dosages and high dosages were determined. After collecting 30 volunteers per food, the group of 30 people was randomly divided into the low-dosage group and the high-dosage group. After having the people receive the test sample for three months, comparison was made between the conditions before and after the test.

Table 3-2. Dosage amounts of foods to be reevaluated on their dermatological function-related functionality, orally administered to human subjects

Functional food	High-dosage group	Low-dosage group
Aloe products	3,600 mg/day	1,200 mg/day

- As for aloe products, a liquid-type product (1000 g/bottle) made from the **products manufactured and distributed by Univera, Inc.** (excluding other functional substances besides aloe vera gel powder for the purpose of human experiments) was used. Its primary substance was aloe vera gel powder (low-dosage group: 10 g/bottle, high-dosage group: 30 g/bottle), and other additives such as caragel (carrageenan 65%, xanthan gum 35%), potassium sorbate, sodium benzoate, citric acid (anhydrous), and purified water. The dosage for human experiments was determined based on the company's daily recommendation dose. Using aloe vera gel, low dosage was set as 1,200 mg/day, and high dosage as 3,600 mg/day.

(3) Method of evaluation
This research verified the following effects of each functional food through clinical tests:

1) Wrinkle reduction effects: Skin replica and visiometer equipment were used.
2) Skin elasticity enhancement effects: Measured by cutometer
3) Erythema reduction and whitening effects: Erythemas and pigmentations were measured by DermaSpectrometer
4) Skin protection effect from damage caused by UV: Minimal erythema dosage (MED) was measured to see if the reaction to the UV is suppressed. Additionally, research was done to figure out if DNA damage caused by UV can be prevented.
5) Effects on increasing collagen fibers and on skin protection from damage caused by UV: By carrying out biopsy 24 hours after the exposure to UV both before and after the oral administration, observation was made regarding the change in expression of collagen fibers, the effect on prevention of MMP expression increased by UV and the suppression of collagen before and after the oral administration.

(A) Before oral administration (first visit)
1) Determine skin type, measure facial wrinkles, measure skin elasticity
2) Measure the level of facial pigmentation (measure facultative skin color and change in color of pigmented spot)
3) Measure skin type and MED
4) Biopsy (of the volunteers, approximately 10 people per dosage group): One spot respectively in 6 mm punch 24 hours after 2 MED irradiation to normal skin
   - Observe the level of expression of collagen, MMP on normal skin and that of after ultraviolet ray exposure (real time RT-PCR)
   - Observe histological changes (skin depth, damage level caused by UV, melanin, DNA damage, cell deaths, cell density of epidermal Langerhans islets) (H & E stain, immune tissue chemical dyeing)
5) Blood (general blood test/ liver function examination)/ urine test

(B) 1.5 months after oral administration (second visit)
1) Determine abnormality in reaction

(C) 3 months after oral administration (third visit)
1) Measure facial wrinkles, measure skin elasticity
2) Measure level of facial pigmentation (measure facultative skin color and color change of pigmented spot)
3) Measure MED
4) Biopsy (of the volunteers, approximately 10 people per dosage group): One spot respectively in 6 mm punch 24 hours after 2 MED irradiation to normal skin
   - Observe the level of expression of collagen, MMP on normal skin and that of after ultraviolet ray exposure (real time RT-PCR)
   - Observe histological changes (skin depth, damage level caused by UV, melanin, DNA damage, cell deaths, cell density of epidermal Langerhans islets) (H & E stain, immune tissue chemical dyeing)
5) Determine abnormality in reaction
6) Blood (general blood test / liver function examination) / urine test

(4) Analysis of results
A) Measuring of the need of improvement of clinical biomarkers
Of the three total visits, the following method was used during the first and last visiting periods, and their measured areas were taken pictures of and the records were stored. Any measurement of all clinical biomarkers is performed at a constant temperature & humidity chamber at Clinical Research Institute of Seoul National University.

(1) Objective evaluation by equipment

(A) Wrinkles

A replica was made in the week 0 and week 12 of using the product, and the image profile was obtained via skin Visiometer SV 600 (C&K Co., Germany). As wrinkles have directions of multi levels, in order to compensate this, the area in question was cut in a circular shape and the wrinkles were evaluated. R1-R5 were used as the parameter which analyzes the depth of wrinkles. R1 and R3 are parameters that apply in the analysis of wrinkle depth. And R4 and R5 are the parameters that indicate the smoothness and depth of wrinkles. They mean that the smaller each parameter is, the more reduced the wrinkles.
- R1 (skin roughness): The distance between the peak and the valley on the profile.
- R2 (average roughness): The value of each R1 is calculated and its average was taken after dividing the selected profile into five consecutive segments of the same size. Because the average was used, unlike in R1, it is not affected by artifacts.
- R3 (maximum roughness): The highest value of the R1 values of the five segments
- R4 (smoothness depth): Average smoothness of the profile line
- R5 (arithmetic average roughness): Average depth of the profile line

(B) Skin elasticity

Skin elasticity was evaluated by cutometer in the week 0 and week 12 of using the product.

(C) Whether or not the level of skin erythemata and hyper-pigmentation was improved

Skin erythemata and hyper-pigmentation levels were measured using DermaSpectrometer, and compared between the time before and after intake to see if there was improvement. Three areas were taken for measurement: first, the area which does not have hyper-pigmentation adjacent to the tragus of the patient's both ears; second, a spot with hyper-pigmentation was randomly chosen of the patient's two cheeks and marked on its photograph; last, a spot without hyper-pigmentation was chosen which displays the experiment subject's own skin color. In this way, the measurements were performed in the week 0 and week 12.

(2) Subjective evaluation by the experiment subjects based on surveys

The experiment subjects were instructed to evaluate the results on a five phase scale of (1) got worse, (2) got somewhat worse, (3) is acceptable, (4) shows okay improvement, and (5) shows good improvement.

(3) Evaluation of effects by researcher's naked eye

In the periods of the first and last visits (three month point after intake), the researcher evaluates the condition of wrinkles, elasticity, and pigment of the tested areas on a five phase scale of (1) got worse, (2) got somewhat worse, (3) is okay, (4) shows acceptable improvement, and (5) shows good improvement.

(4) The level of increase in minimal erythema dose (MED) caused by UV

Before the intake of the test sample food and after the completion of intake, black cloth which has 10 square-shape windows in the size of 1 cm x 1 cm was placed on the back of the patient before broad band, to which ultraviolet B (hereinafter, BB UVB) was applied at 1 mW/cm² of the intensity of illumination. The minimum

erythema dosage was measured 24 hours after the application of BB UVB. The intensity of radiation was set at the minimal intensity of radiation for detecting an erythema with the four clear corners of the square.

(5) Change in the expression of MMP genes before and after intake, and whether or not the expression of MMP by UV was suppressed after intake

Of the volunteers, UV rays were applied to their unexposed areas (buttocks) before and after intake of the experimental substances. A biopsy (6 mm punch) was given to the tissues of 2 MED areas, and the expression of MMP-1 on the epidermal and dermal layers was observed (real-time RT-PCR). At this time skin tissues of the same size were taken from nearby normal skin to which UV rays had not been applied, and were given a real-time RT-PCR to compare with the expression of MMP-1 on epidermal and dermal layers in order to compare the level of expression of MMP genes before and after intake.

(6) Change in the expression of collagen on the skin before and after intake, and whether or not the decrease in collagen caused by UV was suppressed after intake

Of the volunteers, UV rays were applied to their unexposed areas (buttocks) before and after intake of the experimental substances. A biopsy (6 mm punch) was given to the unexposed areas to observe the change in the expression of collagen type I (immunohisto-chemical stain, real-time PT-PCR). At this time, skin tissues in the same size were taken of nearby normal skin to which UV rays were not applied, and were compared with the expression of collagen on epidermal and dermal layers (immunohistochemical stain, real-time PT-PCR), in order to compare (immunohistochemical stain, real-time PT-PCR) the level of expression of procollagen type I before and after intake.

(7) Histological change of the skin and observation of changes

To conduct observation of histological change in the epidermal and dermal layers of the skin before and after intake, H & E staining and chemical staining of immune tissues were performed. This experiment was done in order to observe any changes in the thickness of the epidermal and dermal layers, the number of cells, the index molecules of division and growth, collagen, elastin, and collagen-degrading enzymes. Furthermore, immunohistochemical staining was carried out in order to observe any changes in thymine dimmer, type-I collagen, fibrillin-1 and tropoelastin, using the following method: The tissue to which biopsy was given was placed on the slide, and was incubated for 30 minutes at 95-100°C in the target retrieval solution before it was washed for five minutes, being simultaneously shaken with distilled water. Reaction was made in 0.3% hydrogen peroxide for 30 minutes or 3% hydrogen peroxide for 6 minutes before it was washed with distilled water. Following the removal of moisture, normal blocking serum was dropped and reaction was allowed in a humid chamber for 30 minutes. The first antibodies were diluted in an adequate amount of PBS, and reaction was allowed at room temperature for an hour, before it was washed with PBS for five minutes for a total of three times. An adequate amount of the second antibodies were diluted, and reaction was allowed in the tissue for 30 minutes before it was washed with PBS for five minutes three times. Peroxidase-conjugated biotin-actin complex was reacted

for 30 minutes and was washed with PBS three times before the ACE detection solution was dropped in. The coloration was done while being looked at through a microscope, and counter-staining was made with hematoxylin.

(8) Real-time RT-PCR (observation of the mode of the expression of procollagen type I and MMP-1)

While the tissues subjected to biopsy were being frozen by nitrogen, finely broken tissues were given 1ml of TRIzol reagent and placed at room temperature for five minutes. Then, 0.2 ml (1/ml TRIzol) of chloroform was added and was shaken strongly for 45 seconds. After it was placed at room temperature for two to three minutes, it was centrifuged at 12,000rpm for 15 minutes. After placing the upper-layer liquid into a new tube, the same amount of isopropyl alcohol as the upper-layer liquid was added and stirred well up and down before letting it sit at room temperature for 30 minutes. After being centrifuged at 12,000rpm for 10 minutes, the upper layer of liquid was thrown away, and the sample was given 1ml of the 75%EtOH where RNA pellet was DEPC processed before it was tapped. Subsequently it was centrifuged at 12,000rpm for five minutes. After the upper-layer liquid was disposed of, the remaining RNA pellet was dried at room temperature for 10 minutes. The RNA pellet was dissolved in 0.1% DEPC water and was quantified with UV. After it was compounded into cDNA, the ABI PRISM 7500 Sequence Detection System was used to carry out real-time PCR (the TaqMan probe method was selected).

(9) Statistical process

Regarding the measurement results by equipment of skin wrinkles and elasticity, the difference between before intake of pollens (week 0) and after its intake (week 12) was represented by mean value ± standard error. After a statistical process was done (an independent T-test), in the case of $p<0.05$, it was judged to have significant effects. Regarding the slides used to observe the histological changes, images were caught at the constant magnifications and light, and photographs of them were taken. Additionally, the changes in depth of actual tissues, number of cells were quantified into data and represented by mean value ± standard error. In the case of $p<0.05$, once the statistical analysis had been carried out it was judged to have significant effects. The change in actual protein was shown in the Western blot data of skin tissues. This change was then converted via densitometer into the value of the expressional change compared to the time before intake, subsequently being represented by mean value ± standard error before being statistically processed by Wilcoxon Signed Ranks Test, which pairs mean values before and after intake. In the case of $p<0.05$, judgment was made that it has significant effects. Other than the information about test subjects, written survey results were analyzed by descriptive statistics.

Chapter 3. Final Research-and-Development Result of Third Detail R&D Subject

3.1 Literature search result

(3) In the case of "aloe products," a total of four cases have been reported in the literature (four cases of animal experiments). However, the literatures, which were related to the skin function

Substance	Intake amount	Intake period	Subject	Experiment result	Journal name	Function	T	QL
Aloe extracts	30 mg, twice/d		Rat	Increased collagen content in granulation tissue	Mol Cell Biochem	Skin beauty	T4	QL3
Aloe vera	1% aloe vera filet	Lifetime	Rat	Prevention efficacy of disease occurrence related to aging	Phytotherapy research		T4	QL3
Aloe	30 mg twice/d	16 days	Rat	Increase of collagen content, wound healing efficacy	J. Ethnopharmacol I.	Skin beauty	T4	QL3
Aloe	100 mg/kg	30 days	Mouse	Radioprotective effect	J. Radiat Res (Tokyo)	Skin beauty	T4	QL3

after its intake into the human body, were rarely available. Therefore, it was decided that the efficacy verification through clinical trials was necessary.

3.2 Clinical trial results

We completed the writing of a research grant between August and October of 2005, and passed the review by the Institutional Review Board (IRB) of the Medical School at Seoul National University between October and November of that year. Soon afterward, we started to select candidates and completed clinical trials on aloe.

(3) Aloe Products

(A) Demographics
- Total 30 people (low-dosage group, 15 people; high-dosage group, 15 people)
- No dropouts
  - Average age (yrs.): 56.2 6.8 (49 -74)
  - Weight (kg): 58.8 7.9 (47 -75)
  - Low-dosage group: 15 people, 57.8 7.1 yr, 55.9 5.8 kg
  - High-dosage group: 15 people, 54.5 6.3 yr, 61.8 8.8 kg
- There was no statistically significant difference in terms of ages, heights or weights among the groups (Mann-Whitney test).

(B) Comparison of skin wrinkles before and after intake (smaller R values mean the absence of wrinkles)

GROUP		BEFORE INTAKE	3 MONTHS AFTER INTAKE	P
Low-dosage group	R1	1.37±0.30	1.14±0.22	0.007*
	R2	1.11±0.29	0.91±0.22	0.036*
	R3	0.69±0.15	0.56±0.11	0.012*
	R4	0.38±0.08	0.32±0.07	0.041*
	R5	0.16±0.05	0.14±0.04	0.299
High-dosage group	R1	1.39±0.90	1.15±0.48	0.024*
	R2	1.12±0.72	0.97±0.48	0.078
	R3	0.68±0.34	0.60±0.31	0.033*
	R4	0.44±0.30	0.34±0.24	0.004*
	R5	0.21±0.23	0.16±0.15	0.015*

* $P<0.05$ by Wilcoxon signed rank test

- Before intake there was no significant difference of R1-R5 between the low- and high-dosage groups (Mann-Whitney test).
- In the low-dosage group, R1-R4 decreased significantly after intake.
- In the high-dosage group, R1 and R3-R5 decreased significantly after intake.

(C) Comparison of skin elasticity before intake and after intake (the higher CR values mean higher elasticity)

GROUP		BEFORE INTAKE	3 MONTHS AFTER INTAKE	P
Low-dosage group	CR2	0.7251±0.0460	0.7547±0.0620	0.140
	CR5	0.7370±0.1654	0.9583±0.2282	0.004*
	CR7	0.3986±0.0572	0.4706±0.0734	0.011*
High-dosage group	CR2	0.7290±0.0541	0.7509±0.0527	0.394
	CR5	0.8540±0.1636	0.9002±0.1771	0.363
	CR7	0.4514±0.0542	0.4743±0.0569	0.211

* $P<0.05$ by Wilcoxon signed rank test

- Before intake, there was no significant difference of cutometer values CR0-CR9 between the low- and high-dosage groups (Mann-Whitney test).

- In both the low- and high-dosage groups, CR2, CR5 and CR7 increased significantly after intake, and in the low-dosage group the CR5 increase was statistically significant.

(D) Comparison of facial erythema before and after intake

GROUP		BEFORE INTAKE	3 MONTHS AFTER INTAKE	P
Low-dosage group	EI1	11.3±1.8	11.9±2.8 (increase)	0.712
	EI2	9.8±2.0	11.9±3.2 (increase)	0.005*
	EI3	11.7±2.6	13.1±3.6 (increase)	0.050
High-dosage group	EI1	11.4±2.1	11.4±2.6 (no change)	0.776
	EI2	13.2±4.0	12.2±3.1 (decrease)	0.691
	EI3	13.6±4.6	11.3±3.3 (decrease)	0.007*

* $P<0.05$ by Wilcoxon signed rank test

- In the low-dosage group all of EI1-EI3 increased, while EI2 increased significantly.
- In the high-dosage group EI1 increased but EI2 and EI3 decreased. EI3 decreased significantly.
- Therefore, the partial effect of suppressed erythema was shown only in the high-dosage group.

(E) Comparison of facial pigmentation before and after intake

GROUP		BEFORE INTAKE	3 MONTHS AFTER INTAKE	P
Low-dosage group	MI1	34.8±2.7	34.7±3.1 (decrease)	0.887
	MI2	40.7±2.4	40.8±2.5 (increase)	0.842
	MI3	35.8±1.9	35.6±2.7 (decrease)	0.712
High-dosage group	MI1	33.8±3.9	34.5±3.1 (increase)	0.932
	MI2	40.0±3.3	40.1±2.7 (increase)	0.865
	MI3	34.8±1.9	35.3±1.6 (increase)	0.023*

Statistical test by Wilcoxon signed rank test

- In the low-dosage group, even though it increased or decreased depending on the measured areas, there was no significant change.
- In the high-dosage group, in all three of the measured areas pigment increased, and the increase on the cheek was significant.
- Therefore, aloe indicated no skin-whitening function.

(F) Comparison of minimal erythema dosage (MED) induced by UV before and after Minimal erythema dose (MED): by BB UVB (mJ/cm2)

GROUP	BEFORE INTAKE	3 MONTHS AFTER INTAKE	P
Low-dosage group	178.0±23.4	168.7±31.4	0.087
High-dosage group	178.7±31.1	178.7±37.8	0.954

- Before intake there was no significant MED difference between the two groups (Mann-Whitney test).
- In both the low- and high-dosage groups there was no significant MED change after intake (Wilcoxon signed rank test).

Chapter 4. Research Result Discussion and Conclusion of Third Detail R&D Subject

4.3 Functionality reevaluation of products containing aloe

- There is no available literature on skin function related functionality after taking aloe into the human body. Therefore, we decided to determine the function and effective dosages through clinical trials.
- The functional health foods containing aloe showed no side effects in either the low- or high-dosage groups.
- Facial wrinkles decreased significantly in the two dosage groups after taking aloe.
- Facial elasticity increased significantly and partially in the low dosage group after taking aloe.

- Before and after taking aloe there was no significant change in the degree of erythema or pigmentation. Therefore, aloe is considered as having no function in terms of the suppression of erythema and whitening in human skin.
- Before and after the intake of aloe, there was no significant difference in minimal erythema dosage, so aloe has no effect on skin sensitivity to UV irradiation.
- The functional health foods containing aloe have significantly clinical indexes of skin aging in terms of factors such as wrinkles and elasticity. Thus they are confirmed to have skin function related functionality. The above indexes did not indicate dosage-dependent differences. Therefore, taking the low dosage (1,200mg/day) can be both economical and effective.

Chapter 5. Research Achievements of Third Detail R&D Subject

5.1 Practical achievement

Detail subject name   Re-evaluation of efficacy regarding skin function
Detail subject primary investigator   Medical School, Seoul National University / Dermatology

A. Research paper(s)

- None

B. Conference presentation(s)

- None

C. Intellectual property rights

- None

D. Policy practice

- It has been applied in the official notices as fundamental data for the re-evaluation of functional health foods related to skin function.

E. Other/further research applications

- It is possible to use the data as evaluation data for reviews of the material ingredients of individually tailored functional health foods.

F. Press advertisement and pan-national education

- Press advertisements and the contents and dates of pan-national education were briefly described.

G. Others

- A national and international literature database was established for the evaluation of functional health foods related to skin function.

5.2 Practice plans

- It can be applied in regard to skin-related functional health food and product development research.

Chapter 6. Other Important Modified Items

1) Animal experiments were, in the research proposal, originally scheduled to be performed during the first year. However, we decided to perform clinical trials directly instead of doing animal experiments based upon our investigation of the literature. In the case of functional evaluation using human subjects, extra time was needed for material supply company selection for different materials and the decision on optimal intake dosage, as well as the IRB review application form preparations and the review process. The experimental periods were also delayed longer than expected because a period of three months of clinical experimental period was spent for each product, and because there were problems in the recruitment of subjects for the experimentation.