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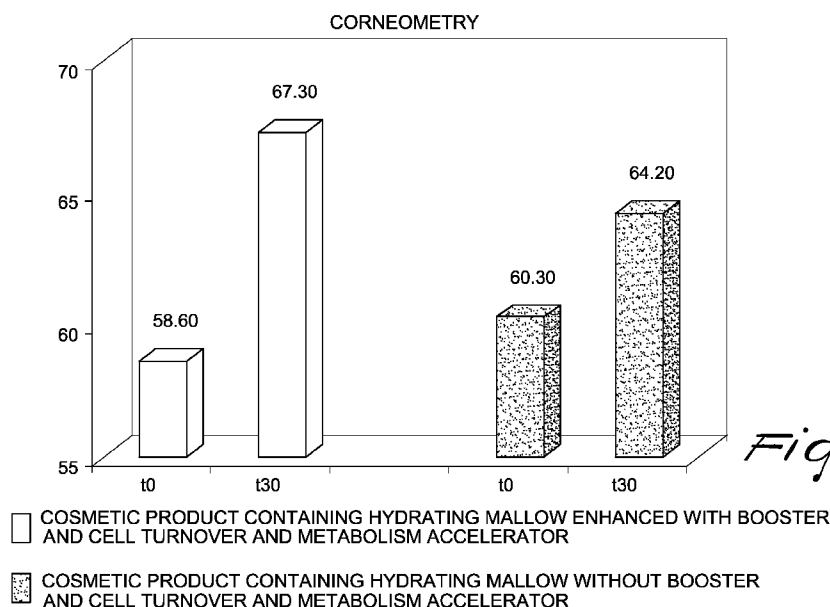
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(54) Title: FORMULATION FOR INCREASING THE ACTIVITY OF A PLANT EXTRACT FOR COSMETIC USE AND COSMETIC PREPARATION WHICH COMPRISES THE SAME



(57) Abstract: A formulation for use in cosmetic preparations which contain plant extracts, which comprises a component for enhancing the activity of plant extracts or booster, cell turnover and metabolism accelerator, and optionally excipients which are acceptable from a cosmetic standpoint, the enhancing component comprising one or more vitamins or fatty acids which are components of vitamins and zinc or zinc salts or biomineral complexes based on zinc or other forms of zinc which are biologically active and cosmetically acceptable.

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FORMULATION FOR INCREASING THE ACTIVITY OF A PLANT EXTRACT FOR COSMETIC USE AND COSMETIC PREPARATION WHICH COMPRISES THE SAME

Technical Field

5 The present invention relates to a formulation for use in cosmetic preparations which contain plant extracts in order to increase the activity of the plant extract for cosmetic use, and to cosmetic preparations which contain plant extracts with improved cosmetic activity which contain said formulation.

10 Background Art

 Several plant extracts, also known as phytoextracts, are known for their beneficial activity in cosmetic preparations. However, known cosmetic preparations based on plant extracts are not free from drawbacks, including the unsatisfactory level of cosmetic activity of the preparation with respect
15 to the potential of said plant extract.

 Plants for cosmetic use can be used in cosmetic preparations in the form of extracts obtained with any extraction technique (glycolic, glycerin, oil-based, et cetera) and include plants of alpine origin.

 The alpine flora consists of 4500 species of plants, approximately
20 40% of the European flora.

 No other region of our continent can boast such a variety of plant species.

 Alpine plants have had to develop "particular methods", which have allowed them to survive in such a hostile environment, characterized by
25 short vegetative cycles, dry air, soil with limited humus and nutrients, considerable temperature variations and strong winds. The higher the altitude, the smaller the plants: nanism is a solution for protection against the wind and the weight of snow. Underground, however, most alpine plants develop an extensive and ramified root system.

30 Cosmetic preparations are also known which contain vitamins which

are known for their beneficial cosmetic effects.

Moreover, zinc is known as a component of cosmetic preparations due to its positive activity on the skin.

The aim of the present invention is to eliminate the drawbacks
5 mentioned above of cosmetic preparations which contain plant extracts, by providing a formulation for use in cosmetic preparations which contain enhanced plant extracts which makes any kind of plant extract for cosmetic use more active.

Disclosure of the Invention

10 This aim and other objects, which will become apparent from the following detailed description of the invention, are achieved by the formulation according to the present invention for use in cosmetic preparations which contain plant extracts, comprising an activity enhancement component or booster for plant extracts and an accelerator for
15 cell turnover and metabolism, and optionally excipients which are acceptable from a cosmetic standpoint, the enhancement component comprising one or more vitamins, or fatty acids which compose vitamins, and zinc or a zinc salt or other biomineral complexes based on zinc or other forms of zinc which are biologically active and cosmetically acceptable.

20 The present invention also provides a cosmetic preparation comprising one or more plant extracts for cosmetic use and a formulation according to the present invention, intended also as all its components.

The inventors of the present invention have found that the formulation according to the present invention allows to enhance the
25 activity of all plant extracts for cosmetic use; in particular, it allows the possibility to enhance the activity of any plant extract for cosmetic use thanks to the enhancement of the activity on the part of the enhancement component or booster and to the increased possibility of the plant extract to interact with a skin tissue which is richer in more active cells, which are
30 rendered such by the cell turnover and metabolism accelerator.

The inventors of the present invention have found that by using the formulation according to the present invention, the plant extract, enhanced by the enhancement component or booster, by virtue of the cell turnover and metabolism accelerator, can be received by more active, more numerous and metabolically more lively cells, which are therefore capable of performing at their best the functions produced by the intervention of such enhanced plant extract, which in this manner can have a higher activity on the skin.

The enhanced plant extract, introduced in a cosmetic preparation, thus provides a higher capacity for benefit.

10 **Brief Description of the Drawings**

Figure 1 shows the corneometry results obtained with a cosmetic product comprising hydrating mallow enhanced with booster and cell turnover and metabolism accelerator and without booster and cell turnover and metabolism accelerator, at the start of the treatment and after 30 days of treatment.

Figure 2 shows the percentage of satisfied volunteers after treatment with hydrating mallow enhanced with booster and cell turnover and metabolism accelerator.

Figure 3 shows the percentage of satisfied volunteers after treatment with hydrating mallow without booster and cell turnover and metabolism accelerator.

Figure 4 shows the corneometry results obtained with a cosmetic product comprising purifying thyme enhanced with booster and cell turnover and metabolism accelerator and without booster and cell turnover and metabolism accelerator, at the start of the treatment and after 30 days of treatment.

Figure 5 shows the sebometry results obtained with a cosmetic product comprising purifying thyme enhanced with booster and cell turnover and metabolism accelerator and without booster and cell turnover and metabolism accelerator, at the start of the treatment and after 30 days of

treatment.

Figure 6 shows the percentage of satisfied volunteers after treatment with purifying thyme enhanced with booster and cell turnover and metabolism accelerator.

5 Figure 7 shows the percentage of satisfied volunteers after treatment with purifying thyme without booster and cell turnover and metabolism accelerator.

Figure 8 shows the corneometry results obtained with a cosmetic product comprising anti-wrinkle plantain enhanced with booster and cell
10 turnover and metabolism accelerator and without booster and cell turnover and metabolism accelerator, at the start of the treatment and after 30 days of treatment.

Figure 9 shows the elastometry results obtained with a cosmetic product comprising anti-wrinkle plantain enhanced with booster and cell
15 turnover and metabolism accelerator and without booster and cell turnover and metabolism accelerator, at the start of the treatment and after 30 days of treatment.

Figure 10 shows the percentage of satisfied volunteers after treatment with anti-wrinkle plantain enhanced with booster and cell turnover and
20 metabolism accelerator.

Figure 11 shows the percentage of satisfied volunteers after treatment with anti-wrinkle plantain without booster and cell turnover and metabolism accelerator.

Ways of carrying out the invention

25 Vitamins for use in the formulation and cosmetic preparation of the present invention are preferably selected among vitamin B3, vitamin F, vitamin A, vitamin C, vitamin E and mixtures thereof, more preferably among vitamin B3, vitamin F and mixtures thereof.

The fatty acids that compose vitamins for use in the present invention
30 are for example fatty acids which are components of vitamin F, such as fatty

acids selected among linoleic acid, linolenic acid and arachidonic acid and mixtures thereof. Zinc salts, for example zinc sulfate or zinc gluconate or zinc stearate or zinc pyrithione or zinc ricinoleate or zinc oxide and mixtures thereof, as well as other biomineral complexes based on zinc or
5 other forms of zinc which are biologically active and cosmetically acceptable, are suitable for use in the present invention.

Preferably, the cell turnover and metabolism accelerator comprises an algae extract, particularly an extract of *Dunaliella salina*.

The cell turnover and metabolism accelerator is present in the
10 formulation and the cosmetic preparation according to the present invention for example at a concentration from 0.05% to 5% by weight with reference to the total weight of the formulation or the cosmetic preparation. When the accelerator comprises an algae extract, particularly an extract of *Dunaliella salina*, such concentration is understood to be expressed by weight of
15 extract.

The enhancement component or booster is present in the formulation and the cosmetic preparation according to the present invention for example at a concentration from 0.01 to 15% by weight based on the total weight of the formulation or the cosmetic preparation.

20 The zinc or zinc salt or complex with zinc is present in the formulation and the cosmetic preparation according to the invention at a concentration from 0.001 to 5% by weight based on the total weight of the formulation or the cosmetic preparation.

The one or more vitamins, or the fatty acids that compose vitamins or
25 mixtures thereof, are present in the formulation and the cosmetic preparation according to the present invention for example at a total concentration from 0.001 to 5% by weight based on the total weight of the formulation or the cosmetic preparation.

The enhancement component or booster comprises preferably vitamin
30 B3, vitamin F and zinc, preferably from 0.001 to 5% by weight of vitamin

B3, from 0.001 to 5% by weight of vitamin F, and from 0.001 to 5% by weight of zinc or salt or complex based on the total weight of the formulation or the cosmetic preparation.

The present invention further provides a cosmetic preparation which
5 comprises one or more plant extracts for cosmetic use and a formulation according to the present invention, intended also as all the components of such formulation.

Preferably, the formulation is present in the cosmetic preparation according to the present invention at a concentration from 0.1 to 20% by
10 weight based on the total weight of the preparation.

The plant extract can be present in the cosmetic preparation, for example, at a concentration of 0.01 to 10% referred to the total weight of the preparation.

In a preferred embodiment of the present invention, the formulation
15 and the cosmetic preparation comprise an association of vitamin B3 and vitamin F and zinc and a cell turnover and metabolism accelerator, preferably an algae extract, particularly an extract of *Dunaliella salina*.

The present invention relates to the possibility to enhance, by means of the invention, all plant extracts for cosmetic use; in particular, it relates to
20 the possibility to increase the activity of any plant extract for cosmetic use due to the aggregation of such plant extract with a booster which is composed of an exclusive association of vitamin B3, vitamin F and zinc.

Moreover, the inventors have identified a particular cell turnover and metabolism accelerator (extract of *Dunaliella salina*) which allows the plant
25 extract, enhanced by the booster, to interact with a skin tissue which is richer in more active cells.

Thanks to the formulation according to the present invention, the enhanced plant extract, inserted in a cosmetic preparation, provides a superior capacity for cosmetic benefit.

30 As regards plant extracts or phytoextracts for use with the present

invention, the research of the inventors has been aimed particularly at plants for cosmetic use which can be used and obtained by means of any form of extraction (glycolic, glycerin, oil-based, et cetera) and preferably at plants of alpine origin. Preferably, the plants used with the present invention
5 originate from the Swiss Alps.

Preferably, the high quality of the extracts used with the present invention is ensured by the selection of varieties which are rich in active compounds, grown in appropriate soils and at optimum altitudes.

Preferably, fully biologic crops are used which have the following
10 advantages:

- natural selection
- botanically and biochemically defined plants
- renewable and reliable supply source
- preservation of natural sites and rare plants
- 15 – preservation of biodiversity and retention of botanical assets.

By way of non-limiting example of the scope of the present invention, a list of plants with cosmetic functionalities which can be used with the present invention and are examined by the inventors is given:

- Mallow [10, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42]
- 20 Hypericum [10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23]
- Echinacea [1, 2, 3, 4, 5, 6, 7, 8, 9]
- Plantain [24, 25, 26, 27, 28, 29, 30, 31, 32]
- Borage [10, 43]
- Thyme [10, 44, 45, 46, 47]
- 25 Edelweiss [24, 48, 49, 50]
- White genepi
- Butterfly bush
- Calendula [10, 51]
- Hyssop [10]

- Master wort
- Horehound [10]
- Elder [10]
- Arnica [10]
- 5 Mountain pine
- Yarrow [10]
- Eglantine [10]
- Bilberry [10]
- Burdock [10]
- 10 Thistle [10]
- Coltsfoot [10]
- Eyebright [10]
- Hop [10, 51]
- Mullein [10]
- 15 Alpine lady's mantle [10]
- Skullcap
- Sage [10, 51]
- Lemon balm [51]

The inventors of the present invention have found that the
20 formulation according to the present invention is adapted to increase the
activity of any type of plant extract for cosmetic use, since there are
common functionalities between vitamins and zinc, particularly vitamins
B3, F and zinc in a cosmetically acceptable form, and the many plants used
in cosmetics, such functionalities increasing synergistically by using the
25 formulation according to the present invention.

The functionalities of vitamins B3, F and zinc are described hereafter
and then the common cosmetic functionalities of said vitamins and plant
extracts are outlined schematically.

Vitamin B3

The term "vitamin B3" (or "vitamin PP" or niacin) is used to reference two mutually similar molecules: nicotinic acid (niacin in the proper sense of the term) and its amide, nicotinamide (or niacinamide).

5 The invention relates to the use of all forms of vitamin B3.

Nicotinamide is a component and is therefore necessary for the production of nicotinamide adenine dinucleotide (NAD) and of nicotinamide adenine dinucleotide phosphate (NADP), two extremely important coenzymes involved in more than 40 biochemical reactions which
10 require the transfer of hydrogen. Nicotinamide is an essential part of more than 800 enzymes involved in the formation of intermediates of cell metabolism in glycolysis reactions, in pyruvate and citrate metabolism, and in pentose biosynthesis [52].

15 Main functions

It has anti-inflammatory properties [52]. These properties are due to the ability to inhibit poly(ADP-ribose) polymerase (PARP), an enzyme which catalyzes the transfer of chains of poly(ADP-ribose) from its precursor NAD to the carboxylic groups of proteins. DNA damage is a
20 stimulus which is necessary and sufficient to activate PARP. Hyperactivation of PARP would have a "suicidal role", which would end with cell death, since extensive poly ADP-ribosylation of proteins leads to a great consumption of NAD and to great cellular reduction of ATP.

Moreover, PARP would increase the transcription of a nuclear factor
25 which has a key role in the expression of inflammatory cytokines and inflammation intermediaries.

Nicotinamide inhibits tumor necrosis factor alpha (TNF- α), interleukins 1 and 12 (IL-1 and IL-12), the release of histamine by mastocytes and antigen-induced lymphocyte transformation.

30 **It is active against free radicals** [52]. Nicotinamide is capable of

neutralizing oxygen radicals and inhibiting nitric oxide synthase, an enzyme which forms the free radical nitric oxide. In this manner, it protects the cell and particularly its membranes against destruction by free radicals.

It stimulates cell proliferation [57]. In vitro tests have allowed to
5 notice that if hepatocytes are cultured in the presence of nicotinamide, an increase of the replication and of the survival of the cultured cells up to more than one month is observed. Indeed, in the presence of 10 mM of nicotinamide, the number of cells continues to increase (by 2.7 times with respect to day 1), reaching a plateau after 5 days. Moreover, even after 33
10 days approximately 70% of the cells remain alive when cultured with nicotinamide but die after 5-7 days if the active ingredient is not present in the culture. The higher cell vitality and the higher cell replication are due very presumably to the fact that the intracellular levels of NAD in hepatocytes treated with nicotinamide are maintained, but this does not
15 occur with untreated hepatocytes. NAD is in fact the most abundant cell coenzyme and plays a part in many biological reactions within cells. Therefore, intracellular reduction of NAD in untreated hepatocytes might affect cell functions, leading to cell death.

It has an anti-wrinkle action [56]. A study involved 70 female
20 volunteers, who applied a cream containing 5% niacinamide for 12 weeks. After 8 weeks of treatment, a reduction in wrinkles was observed; this reduction is due very probably to the increase in the production of collagen (as observed in a culture of fibroblasts) and to the reduction of the excess of dermal glycosaminoglycans (GAG) which characterizes wrinkly skin aged
25 by sunlight. Measurement with a cutometer showed an increase in elasticity after 12 weeks of treatment.

It increases the barrier functions of the epidermis [52, 53, 54, 55]. Nicotinamide is capable of stabilizing the homeostasis of the epidermal barrier by increasing the levels of ceramides, cholesterol and fatty acids in
30 the corneal layer, which decrease with age, thus reducing Transepidermal

Water Loss (TEWL), consequently increasing the amount of water contained in the skin. The increase in the synthesis of sphingolipids occurs as a consequence of higher activity of the serine palmitoyltransferase enzyme (SPT), which promotes the synthesis.

- 5 **Moisturizing function.** As a consequence of the increase in the barrier function of the skin, nicotinamide therefore acts also as an excellent moisturizer.

It promotes the synthesis of collagen [52, 56]. The synthesis of collagen, protein secretion and the number of cells increase when a culture
10 of fibroblasts from senior donors is cultured with nicotinamide.

It has an anti-acne/skin cleansing action [52, 53, 56]. A study involved 76 patients affected by a moderate acne problem, who applied twice a day for 12 weeks a gel containing 4% nicotinamide. After eight weeks, 82% of the volunteers had improved.

15

Vitamin F or essential fatty acids

 In the skin, essential fatty acids (EFA) are mainly located in phospholipids and have a structural function in the lipoproteins of cell membranes and enzymes [58]. The biological function of essential fatty
20 acids includes stimulating growth, maintaining the growth of skin and hair, and preserving the integrity of membranes. They act as precursors of prostaglandins and of biologically associated active compounds. Polyunsaturated fatty acids (PUFA) play an important and vital role in the properties of many biomembranes. The physical properties, such as fluidity,
25 of phospholipids are determined to a large extent by the length of the chain and the degree of unsaturation of their fatty acids. Membrane proteins, such as for example receptors, enzymes, ion channels, et cetera, are highly sensitive to the lipid environment. The physical properties affect the capacity of phospholipids to perform structural functions, such as
30 maintaining the normal activities of membrane enzymes such as adenylate

kinase and Na^+/K^+ ATPase [59]. Some polyunsaturated fatty acids (PUFA) cannot be synthesized by the body and therefore must be introduced with the diet and are therefore termed essential. The essential nature of these polyunsaturated acids is linked to the presence of one or more double bonds within the terminal 7C. In particular, two unsaturated fatty acids are required for life: linoleic acid (an omega 6 acid) and linolenic acid (an omega 3 acid). Arachidonic acid can be synthesized from linoleic acid if it is supplied to the body in sufficient quantity by the diet. Accordingly, it is essential only in case of lack of linoleic acid.

Among the several essential fatty acids, linoleic acid alone is important for maintaining normal barrier functions [57].

It increases the barrier functions of the skin. One of the main functions of linoleic acid is to maintain the integrity of the epidermal barrier in the skin. The physical structure of this water barrier is due to the sheets of stacked bilayers which fill the intercellular voids of the topmost layer of the epidermis. These lipids arranged in a double layer (bilayers) contain large quantities of sphingolipids, which contain large quantities of ceramides which are rich in linoleate. In conditions of lack of essential fatty acids, linoleic acid is replaced with oleic acid, which causes a severe loss of water from the skin [59].

Moisturizing/emollient function. The use of essential fatty acids is of considerable interest in treating skin which is dry due to excessive dehydration and unbalanced sebogenesis and of elderly skin with altered turnover. Topical application for two to four weeks of creams containing percentages of essential fatty acids has revealed the biostimulating effects of such fatty acids, i.e.: reconstitution of the corneal layer, reduced loss of water due to increased membrane integrity, improved elasticity and sebogenesis. Under the action of this vitamin, the skin remains soft and most of all more imbibed due to a considerable reduction in loss of water through so-called "perspiratio insensibilis".

In mice, when the diet is completely deprived of essential fatty acids, the first change, after just one week, is the rapid reduction of linoleic and arachidonic acid in skin lipids. After five weeks, these acids are virtually absent from the skin and the animals begin to accumulate weight more slowly than the control, drink more water and the skin develops flakes. After approximately ten weeks of diet without essential fatty acids, the TEWL (TransEpidermal Water Loss) begins to rise rapidly to values ten times higher than in normal mice. As the quantity of linoleic and arachidonic acid decreases in the skin, the quantity of δ -5,8,11-eicosatrienoic acid (ω -9) accumulates. This acid is normally present only in small amounts in the normal skins of mammals except when the amount of linoleic acid decreases (as in the onset of a deficit of essential fatty acids), where the enzymes which generally convert linoleic acid to arachidonic acid instead convert oleic acid into ω -9 eicosatrienoic acid. The latter is believed to form in order to support the total degree of unsaturation in phospholipidic membranes in the absence of essential fatty acids.

Microscopic changes in the skin of mice in conditions of essential fatty acid deficit are epidermal thickening, particularly the development of the granular layer and the thickening of the corneal layer. The latter becomes thicker and more tightly packed and adherent. Sebaceous glands, which are normally hyperplastic, with numerous large acinose structures, due to a deficit of fatty acids become more atrophied (fatty acids are transformed into triglycerides, promoting the storage of energy or precursors of sebum). The capillaries of the most superficial dermal layer become more dilated and the number of mastocytes and of mononuclear cells increases [58].

It stimulates cell proliferation. Specific epidermal enzymes metabolize arachidonic acid into a variety of oxygenated metabolites, which have a variety of functions in the skin. One of the main functions is that metabolites of arachidonic acid regulate proliferation and/or differentiation

processes in the epidermis [60].

It has an anti-inflammatory function. Essential fatty acids are the precursors of prostaglandins. Prostaglandins are acknowledged widely as important in the inflammatory process as mediators of the slowing of vasoactivity; they are believed to be involved in the regulation of the different functions of leukocytes, such as the release of histamines on the part of basophils and the release of lysosomal enzymes from neutrophils. It has been found that prostaglandins might improve the formation of flakes on the skin in addition to being closely involved in the control of mitosis and differentiation as a consequence of their interaction with cyclic AMP and GTP.

Arachidonic acid is the origin of many messengers with pro- or anti-inflammatory effects. An increasing amount of experimental and clinical evidence suggests that PUFAs n-3 can alleviate a series of inflammatory responses. Eicosanoids derived from EPA (eicosapentaenoic acid) act as *antagonists* of inflammation. Studies on healthy volunteers show that supplements based on PUFA n-3 inhibited the production of IL-1, IL6, TNF and IL-2 on the part of white blood cells [61].

It has an anti-acne action. The major pathogenetic factor of acne is altered keratinization of the follicular infundibulum. It has been hypothesized that a relative decrease in linoleic acid in sebum is partly responsible for this. A study has been carried out on patients affected by average acne who applied linoleic acid topically. A 25% reduction in the size of follicles and of microcomedones after one month of treatment was observed [62].

Acne patients have shown low levels of linoleic acid in the composition of surface lipids. It has been hypothesized that low concentrations of linoleate in sebum lead to a deficit of essential fatty acids in the cells of the follicular epithelium and to the characteristic hyperkeratosis [63].

It facilitates skin healing. It has been pointed out that linolenic acid (n-3), linoleic acid (n-6) and oleic acid (n-9) can modulate the healing of surgically induced skin wounds. Oleic acid induces the closure of the wound more rapidly than linoleic and linolenic acid. Moreover, oleic acid
5 inhibits greatly the production of nitric oxide at the level of the wound [64].

Zinc

The ubiquity of zinc in organs, tissues and fluids of the body (2.5 g per person) bears witness to its critical role in human development and
10 health. Approximately 99% of the zinc that is present in the human body is located inside cells. Zinc is particularly predominant in the epidermis: the epidermis contains 20% of the zinc that is present in the entire body (71 µg/g dry weight) [65].

Zinc binds to a certain number of biological molecules, affecting their
15 shape, stability and activity. It acts as a catalyst for enzymes responsible for DNA replication, gene transcription and protein synthesis. It has a critical role in the survival of cells, it regulates signal transduction, and DNA replication and transcription.

More than 300 enzymes require zinc in order to be active; among the
20 main ones, the following are mentioned: superoxide dismutase (SOD), which is very important for skin health due to its antioxidant properties, metallothionein (MT), another enzyme which has an antioxidant activity although its main role is to store and transport zinc and alkaline phosphatase (AP), an enzyme involved in the metabolism and suppression of
25 inflammatory processes [65].

Main functions

It performs an anti-free radicals and antioxidant activity. Zinc has the ability to inhibit the production of nitric oxide (NO). Nitric oxide can interact with the superoxide anion (O_2^-), generating a cytotoxic agent which
30 causes tissue damage which leads to skin inflammation. Moreover, zinc

protects the skin against other oxygen radicals in view of its antioxidant properties. The mechanism of action by means of which zinc performs its antioxidant action is unknown. However, two options are suggested: the stable redox status of zinc (Zn^{2+}) might replace reactive redox metals such as iron and copper in critical cellular and extracellular sites, or zinc might induce synthesis of metallothionein, forming a zinc-thiolate portion which would act as a "sacrificial" site for oxidant attack, thus preserving the skin and its components. Therefore, the antioxidant protective effect provided by zinc relates to the stabilization of membrane lipids and to the prevention of peroxidation of lipids by free radicals [65,68].

It has an anti-inflammatory function. The effect of zinc on the different cell types involved in inflammation, such as mastocytes, platelets, macrophages, neutrophils and lymphocytes, is cell-specific. Zinc can also affect cytokines, cell messengers which intervene and facilitate communication with these cells. Zinc inhibits the degranulation of mastocytes, thus reducing the secretion of histamine, which is an important mediator of inflammation and pruritus. An example of an enzyme involved in the regulation of inflammation is AP. AP is released by the surface of epithelial cells and dephosphorylates AMP to generate adenosine. Adenosine is a potent anti-inflammatory and is involved in the reduction of the inflammatory step of the healing of a wound [65].

It increases the synthesis of collagen [66, 67].

It stimulates cell proliferation [66, 67] of keratinocytes and fibroblasts.

It facilitates the reepithelization and healing of the skin. Zinc and proteins which contain zinc are involved in almost any stage of skin repair. Zinc has a significant role in modifying the extracellular matrix (ECM), in cell migration, in protein synthesis and in inflammation reduction [66, 67].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteins involved actively in wound repair. Matrix degradation is developed

by MMPs, which specifically digest matrix proteins such as collagen, elastin, laminin, vitronectin and fibronectin. This degradation is the driving force behind tissue remodeling and facilitates the migration of new cells toward the wound.

5 Zinc finger transcription factors cooperate with DNA polymerase and RNA polymerase (both of which are zinc-dependent enzymes) to begin the transcription of the key genes involved in wound healing, such as for example genes involved in cell replication and in genes which code for proteins of the extracellular matrix [65].

10 Following wounds, zinc stimulates the proliferation of cells of the epidermis, keratinocytes and fibroblasts, and increases the synthesis of collagen. Zinc is involved in the migration of keratinocytes during the first steps of healing and in epidermis-dermis cohesion during the final step of healing [66, 67].

15 **It has a photoprotective function** [68]. This function was observed after an in vitro test, in which fibroblasts were exposed to UVA and UVB rays. Zinc reduces both the quantity of DNA damaged following exposure to UVB rays and the number of observed nucleosomes, which are considered as a true marker of cell apoptosis or cell death.

20 **It has beneficial effects on acne** [65]. It is in fact capable of inhibiting the 5-alpha reductase enzyme. This enzyme is responsible for conversion of testosterone into the biologically active metabolite dihydrotestosterone, which mediates many of the actions typically assigned to androgenetic hormones, including stimulation of the activity of sebaceous
25 glands. Moreover, zinc may be involved directly in the inhibition of bacterial lipase, which converts the triglycerides of sebum into fatty acids, which are the most potent mediators of acne. Many studies have demonstrated the existence of benefits following topical application of zinc as acne treatment, very probably due to these anti-inflammatory effects
30 thereof.

The following diagram highlights the functionalities that are common among the plants tested, which represent a sample of the botanical-cosmetic universe, and the above described vitamins B3, F and zinc:

Anti-inflammatory function	Vitamin F Vitamin B3 Zinc	Edelweiss Mallow Hypericum Echinacea Plantain Borage Thyme White genepi Butterfly bush Calendula Hyssop Mullein Alpine lady's mantle Master wort Horehound Elder Eyebright Skullcap Lemon balm
Antioxidant function	Vitamin F Zinc	Edelweiss Hypericum Echinacea Plantain Thyme

		White genepi Butterfly bush Calendula Hyssop Master wort Horehound Elder Sage
Antiacne / dermopurifying action	Vitamin B3 Vitamin F Zinc	Echinacea Yarrow Bilberry Burdock Thistle Coltsfoot Gentian Sage Thyme
Antiradical action	Vitamin B3 Zinc	Edelweiss Hypericum Butterfly bush Calendula Hyssop Master wort Elder
Antiwrinkle action	Vitamin B3	Echinacea Borage
Stimulates cell proliferation	Vitamin B3	Mallow

	Vitamin F Zinc	
Stimulates collagen synthesis	Zinc Vitamin B3	Mallow
Healing / reepithelizing action	Vitamin F Zinc	Mallow Hypericum Echinacea Plantain Borage Butterfly bush Calendula Master wort Arnica Yarrow Eglantine Hop Lemon balm
Moisturizing / emollient action	Vitamin F Vitamin B3	Mallow Plantain Borage Calendula Master wort Eglantine Bilberry Coltsfoot Hop Mullein

		Alpine lady's mantle
Increases the barrier function of the skin	Vitamin B3	Mallow
	Vitamin F	Borage
Photoprotective function	Zinc	Hypericum
		Echinacea

5

Cell turnover and metabolism accelerator

To complete the enhancement of plant extracts on the skin, the inventors have associated with the booster a cell turnover and metabolism accelerator which allows the plant extract, enhanced by the booster, to provide a higher capacity for benefit to the skin tissues with which it interacts.

This accelerator has been identified preferably in an extract which is produced biotechnologically starting from the microalgae *Dunaliella salina*. This extract consists of an effective mixture of different molecules, such as amino acids and carbohydrates, which have the ability to replenish the skin with new energy. The composition of the extract is the following: polysaccharides, trisaccharides, disaccharides, glucose, fructose, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, valine, phenylalanine, isoleucine, leucine, lysine.

The extract contains a titer of active ingredient (as described above) from 1 to 5% by weight.

Dunaliella salina is a red unicellular microalgae: extremely rich in beta-carotene, it is useful for the skin due to its antioxidant and anti-wrinkle properties.

Its dimensions are as follows: length 16-24 μm , width 10-13 μm . Its natural habitat is constituted by salt lakes with 30% salinity (nine times higher than seawater). This small microalgae is exposed permanently to extreme living conditions, such as high osmotic pressures, high oxygen

levels and intense UV irradiation. To deal with these conditions, the algae requires a large amount of energy, which is obtained by photosynthesis. The photosynthesis energy of the algae is obtained by sunlight.

Culture. The microalgae is selected to be converted into the metabolically active extract after being cultured in optimum growth conditions and after undergoing a special fermentation process in photobioreactors. The following are the production passages to obtain the final algae extract.

1. *Dunaliella salina* is selected during cell screening thanks to its capacity to energize cell metabolism, increasing levels of ATP and increasing cell multiplication in fibroblasts.
2. *Dunaliella salina* is cultured in photobioreactors in optimum growth conditions.
3. Cell harvesting.
4. Extraction with hot water and obtainment of the raw extract.
5. Centrifugation.
6. Ultrafiltration with concentration of the algae extract.
7. The concentrate is then diluted and preserved in an aqueous solution so as to obtain the finished product used for the invention.

The *Dunaliella salina* algae can be used advantageously in cosmetic treatments thanks to its ability to:

1. protect and stimulate mitochondria, energy centers of epidermal cells which are responsible for the production of ATP, which in turn is necessary in order to maintain cell functionality. By doing so, it stimulates cell metabolism.
2. Stimulate cell turnover.

Studies of in vitro and in vivo effectiveness on *Dunaliella salina* algae are described in order to demonstrate the above described properties.

Mitochondrial stabilization test. Human epidermal keratinocytes of the HaCaT line are cultured and incubated with different concentrations of

Dunaliella salina extract (0.25%, 0.5%, 1%, 2%). The cells are marked with a JC-1 fluorescent dye and then washed. Polarization of mitochondrial membranes is indicated by the emission of fluorescence, detected with a fluorimeter. Hydrogen peroxide (2 nM) is then added and fluorescence is
5 detected again. Under the microscope, polarized mitochondria appear colored in orange-red, depolarized ones are colored in yellow-green.

Mitochondrial polarization with respect to the control increases in a dose-dependent manner (extract of *Dunaliella salina* 2% -> +55% approximately), demonstrating an action which protects against oxidation
10 stresses caused by free radicals.

ATP synthesis test. Human keratinocytes of the HaCaT line are cultured and incubated with different concentrations of *Dunaliella salina* extract (0.125%, 0.5%, 1%, 2%). The content of ATP is quantified by Cell Titer-Glo Luminescent cell viability assay.

15 The extract of *Dunaliella salina* increases the content of ATP in keratinocytes in a dose-dependent manner (extract of *Dunaliella salina* 2% -> +43% approximately) with respect to the control.

Test for collagen synthesis assessment. Fibroblasts obtained from prepuce are cultured and incubated with different concentrations of extract
20 of *Dunaliella salina* (0.5% and 1%). The amount of type I collagen is determined by using antibodies with a semiquantitative method (ELISA test).

The extract of *Dunaliella salina* increases the content of collagen I in a dose-dependent manner (extract of *Dunaliella salina* 1% -> +30%
25 approximately) with respect to the control.

Evaluation of cell proliferation. Two cultures were organized: one with fibroblasts obtained from prepuce by enzyme digestion with collagenase, and one with a line of human keratinocytes having a high multiplication rate (HaCaT). The cells, after culture, were incubated with
30 different concentrations of extract of *Dunaliella salina* (0.0625%, 0.125%,

0.25%, 0.5%, 1%) for 24 hours. After marking with 5'-bromo-2'-deoxyuridine (BrdU) for 4 hours, the cells were fixed and the BrdU inserted in the DNA was quantified by means of the "ELISA Cell Proliferation" kit by Roche. In the fibroblast culture, an increase in cell proliferation of the dose-dependent type is observed (1% *Dunaliella salina* extract: approximately +220% with respect to the control).

With the keratinocytes, a maximum increase in cell proliferation (+170% with respect to the control) is observed at 0.25% of *Dunaliella salina* extract.

10 **In vivo testing for cosmetic effectiveness.** 30 subjects applied to half of their face, for 28 days, twice a day, a cream containing 3% of *Dunaliella salina* extract. Before (T0) and after (T28) the treatment, facial macrophotographs were taken to assess complexion color variation linked to cell turnover (an assessment which is based on a scale of four colors, red /
15 beige / olive / pink) and to assess brightness (the ability of the skin to reflect light more or less intensely), brilliance (uniformity of skin color and texture) and transparency (the possibility to see blood vessels indicates that the skin is particularly thin). With the application of the product, a significant reduction in olive complexion (-2.8%), an increase in pink complexion
20 (+2.4%), of brightness, of brilliance and of transparency of the skin were observed.

In vivo testing to assess cell turnover. 20 subjects applied to their forearm a cream containing an extract of 1% and 3% *Dunaliella salina* for four weeks. To determine the cell renewal rate after two weeks of
25 application of the active ingredient, a preparation containing DHA was applied to the skin and colored the skin brown. The variation of the coloring over time was assessed by using the Minolta CR200 Chromameter. With 1% of *Dunaliella salina* extract, the reduction in pigmentation between the beginning and the end of the study was 82%.

30 With 2% *Dunaliella salina* extract, the reduction in pigmentation

between the beginning and the end of the study was 105%.

The booster and the accelerator are then joined and added to the product being prepared in such a quantity as to maintain the above described quantity ranges.

- 5 The following example of formulation of a cosmetic preparation is provided merely by way of illustration of the present invention, which must not be understood in a sense which limits the scope of the protection as provided by the appended claims.

EXAMPLE 1

10	Glyceryl stearate	1-5%
	Cetyl stearyl(12)OE	1-5%
	Polysorbate 80	0.5-1%
	Cetyl alcohol	1-3%
	Octyldodecanol	1-3%
15	Glycerin	0.5-1%
	Cetyl stearyl isononanoate	1-3%
	C ₈₋₁₀ triglyceride	10-20%
	Dimethyl polysiloxane	0.01-0.1%
	Karite butter	0.1-0.5%
20	Sweet almond oil	0.1-0.5%
	Vitamin F	0.3%
	Vitamin B3	0.1%
	Zinc gluconate	0.1%
	Dunaliella salina algae extract	1.0%
25	Antioxidants and preservatives	q.s.
	Water	q.s. to 100
	Fragrance	

In order to demonstrate that, in normal conditions of use, the

effectiveness of a cosmetic product based on Swiss plant extracts enhanced by a booster and a cell turnover and metabolism accelerator is enhanced with respect to the same product with the same plant extracts but without a booster and a cell turnover and metabolism accelerator, three in vivo tests, 5 i.e., home half-face usage tests, are described during which volunteers simultaneously used two products, one for each half of the face, applying them to the skin of the face for 30 consecutive days, as follows:

Right side: product containing 0.5% by wt of plant extract, 0.5% by wt of a 10 booster and 1% by wt of a cell turnover and metabolism accelerator.

Left side: same product with same plant extract, without booster and cell turnover and metabolism accelerator

The booster contained :

15	Niacinamide (Vit. B3)	0.10%
	Zinc gluconate (zinc)	0.10%
	Glyceryl linoleate (Vit.F)	0.20%
	Glyceryl linolenate (Vit.F)	0.05%
	Glyceryl arachinodate (Vit.F)	0.05%

20 expressed as percentage by weight of the total product.

The cell turnover and metabolism accelerator is *Dunaliella salina* extract.

A. First test with product based on hydrating mallow enhanced by a booster and a cell turnover and metabolism accelerator with a composition 25 as shown in Table 1 and, the same product based on hydrating mallow without booster and cell turnover and metabolism accelerator.

Table 1

Composition of a product containing mallow enhanced by a booster and a cell turnover and metabolism accelerator

- 30 Aqua
- Cyclopentasiloxane
- Glycerin
- HDI/Trimethylol hexyllactone crosspolymer
- Polysilicone-11
- 35 C30-45 alkyl cetearyl dimethicone crosspolymer
- Dimethicone
- Steareth-2
- Ammonium acryloyldimethyltaurate / VP copolymer
- Butylene glycol
- 40 Steareth-21
- Stearic acid
- Xylitol
- Limnanthes alba seed oil
- Boron nitride
- 45 Phenoxyethanol
- Parfum
- Tocopheryl acetate
- Sodium chloride
- Diazolidinyl urea
- 50 Glyceryl linoleate
- Allantoin
- Histidine
- Niacinamide
- Zinc gluconate
- 55 Xanthan gum
- Butyrospermum parkii butter

- Silica
BHT
Disodium EDTA
Sodium hyaluronate
5 Glyceryl linolenate
Glyceryl arachinodate
Algae extract
Hexyl cinnamal
Leontopodium alpinum extract
10 Benzyl salicylate
Hydroxyisohexyl 3-cyclohexene carboxaldehyde
Butylphenyl methylpropional
Malva sylvestris extract
Alpha-isomethyl ionone
15 Sodium hydroxide
Citronellol

The effectiveness of the two products was assessed:

- 20 – by instrument-based corneometry measurement in order to assess skin hydration.
- with a subjective assessment of the volunteers who took part in the test and who after 30 days of treatment expressed their assessment of the products being tested as regards the following statements:
- 25 ● the product makes the skin more hydrated
- the product makes the skin smoother
- the product makes the skin more radiant

Assessment of cosmetic effectiveness (instrument-based measurements)

Corneometry: in order to assess skin hydration, the amount of water

present in the horny layer was measured on the right and left sides of the face (cheekbones) of the volunteers who took part in the test by using the CM 825 Corneometer (Courage + Khazaka).

- 5 This instrument provides values which are directly proportional to the the degree of skin hydration and can be interpreted as shown in Table 2:

Table 2

	Corneometric values (referred to the face skin)
Very dry skin	<50
Dry skin	50-60
Sufficiently hydrated skin	>60

10

Cosmetic product based on hydrating mallow enhanced by a booster and a cell turnover and metabolism accelerator

- 15 The results obtained in the instrument-based corneometry measurement performed on the right side of the face showed that after 30 days of treatment the product being tested induced a **14.85%** increase in the amount of water present in the horny layer, indicating an increase in skin hydration.

- 20 Cosmetic product based on hydrating mallow without booster and a cell turnover and metabolism accelerator

- 25 The results obtained in the instrument-based corneometry measurement performed on the left side of the face showed that after 30 days of treatment the product being tested induced a **6.47%** increase in the amount of water present in the horny layer, indicating an increase in skin

hydration.

Comparison between the two products

The comparison between the results obtained with the two products
5 revealed the following:

- the cosmetic product based on hydrating mallow enhanced by a booster and a cell turnover and metabolism accelerator induced a percentage increase in the amount of water present in the horny layer which was 129.52% higher than the percentage increase induced by the cosmetic
10 product based on hydrating mallow without booster and a cell turnover and metabolism accelerator.

These results are displayed in Figure 1.

15 **Assessment of cosmetic effectiveness (subjective assessment)**

Cosmetic product based on hydrating mallow enhanced by a booster and a cell turnover and metabolism accelerator

The results obtained in the subjective assessment performed after 30 days of treatment revealed the following:

- 20 – **80%** of volunteers reported that the product makes the skin more hydrated;
- **90%** of volunteers reported that the product makes the skin smoother;
- **40%** of volunteers reported that the product makes the skin more
25 radiant.

The percentage of satisfied volunteers is shown in Figure 2, wherein

1 is the percentage of volunteers that consider that the product makes the

skin more hydrated

2 is the percentage of volunteers that consider that the product makes the skin smoother

3 is the percentage of volunteers that consider that the product makes the

5 skin more radiant

Cosmetic product based on hydrating mallow without booster and a cell turnover and metabolism accelerator

The results obtained in the subjective assessment performed after 30
10 days of treatment revealed the following:

- 70% of volunteers reported that the product makes the skin more hydrated;
- 90% of volunteers reported that the product makes the skin smoother;
- 15 – 30% of volunteers reported that the product makes the skin more radiant.

The percentage of satisfied volunteers is shown in Figure 3, wherein

20 1 is the percentage of volunteers that consider that the product makes the skin more hydrated

2 is the percentage of volunteers that consider that the product makes the skin smoother

3 is the percentage of volunteers that consider that the product makes the

25 skin more radiant

On the basis of the instrument-based corneometry results obtained during the study, we can say that although both products tested induced an increase in skin hydration, a significantly higher activity of the product with

Swiss plant extracts enhanced by booster and cell turnover and metabolism accelerator is observed with respect to the same product without booster and cell turnover and metabolism accelerator.

It has in fact been pointed out that the cosmetic product based on hydrating mallow enhanced by booster and cell turnover and metabolism accelerator induced a 129.52% higher percentage increase in the amount of water present in the horny layer with respect to the percentage increase induced by the same product based on hydrating mallow without booster and cell turnover and metabolism accelerator.

The higher activity of the product with Swiss plant extracts enhanced by booster and cell turnover and metabolism accelerator was confirmed also by the assessments of the participants in the test.

B. Second test with product based on purifying thyme enhanced by booster and cell turnover and metabolism accelerator with a composition as shown in Table 3 and the same product based on purifying thyme without booster and cell turnover and metabolism accelerator.

Table 3

20

Composition of a product containing thyme enhanced by booster and cell turnover and metabolism accelerator
--

Aqua

Cyclopentasiloxane

Glycerin

25 HDI/trimethylol hexyllactone crosspolymer

Polysilicone-11

C30-45 alkyl cetearyl dimethicone crosspolymer

Dimethicone

- Steareth-2
Ammonium acryloyldimethyltaurate / VP copolymer
Butylene glycol
Steareth-21
- 5 Stearic acid
Xylitol
Limnanthes alba seed oil
Boron nitride
Phenoxyethanol
- 10 Tocopheryl acetate
Parfum
Sodium chloride
Diazolidinyl urea
Glyceryl linoleate
- 15 Allantoin
Histidine
Niacinamide
Zinc gluconate
Xanthan gum
- 20 Linalool
Butyrospermum parkii butter
Silica
BHT
Disodium EDTA
- 25 Sodium hyaluronate
Glyceryl linolenate
Glyceryl arachinodate
Algae extract

Leontopodium alpinum extract

Thymus vulgaris extract

Limonene

Citronellol

5 Sodium hydroxide

The effectiveness of the two products was assessed with the following instrument-based measurements:

- corneometry: to assess skin hydration
- 10 – sebometry: to assess the amount of sebum present on the skin surface
- with a subjective assessment of the volunteers who took part in the test and who after 30 days of treatment expressed their assessment of the products being considered as regards the following
- 15 statements:
 - the product reduces the greasiness of the skin
 - the product reduces skin impurities
 - the product does not dry the skin

20 **Assessment of cosmetic effectiveness (instrument-based measurements)**

Corneometry: in order to assess skin hydration, the amount of water present in the horny layer was measured on the right and left sides of the face (cheekbones) of the volunteers who took part in the test by using the CM 825 Corneometer (Courage + Khazaka).

- 25 This instrument provides values which are directly proportional to the the degree of skin hydration and can be interpreted as shown in Table 4:

Table 4

35

	Corneometric values (referred to the face skin)
Very dry skin	<50
Dry skin	50-60
Sufficiently hydrated skin	>60

30

Sebometry: the amount of sebum present on the surface of the skin was measured on the right and left sides of the face (cheekbones) of the volunteers who took part in the test by using the SM 810 Sebumeter (Courage + Khazaka).

35 The amount of sebum is expressed in $\mu\text{g}/\text{cm}^2$ and the resulting values can be interpreted as shown in Table 5:

Table 5

	Sebometric values (referred to the face skin)
Hypo-lipidic skin	<70
Normal lipidic skin	70-180
Hyper-lipidic skin	>180

40

Product based on purifying thyme enhanced by booster and cell turnover and metabolism accelerator

The results obtained after 30 days of treatment, in instrument-based measurements performed on the right side of the face, pointed out the
45 following:

- corneometry: the product being tested induced an **8.40%** increase in the amount of water present in the horny layer, indicating an increase in skin hydration;
- sebometry: the product being tested induced a **24.08%** decrease in
50 the amount of sebum present on the skin surface, indicating a

normalization of sebaceous secretion.

Product based on purifying thyme without booster and cell turnover and metabolism accelerator

5 The results obtained after 30 days of treatment, in instrument-based measurements performed on the left side of the face, pointed out the following:

- 10 – corneometry: the product being tested induced a **4.82%** increase in the amount of water present in the horny layer, indicating an increase in skin hydration;
- sebometry: the product being considered induced a **17.33%** decrease in the amount of sebum present on the skin surface, indicating a normalization of sebaceous secretion.

15 Comparison between the two products

 The comparison between the results obtained with the two products pointed out the following:

- 20 – the product based on purifying thyme enhanced by booster and cell turnover and metabolism accelerator induced a percentage increase in the amount of water present in the horny layer which was **74.27%** higher than the percentage increase induced by the product based on purifying thyme without booster and cell turnover and metabolism accelerator.
- 25 – the product based on purifying thyme enhanced by booster and cell turnover and metabolism accelerator induced a percentage decrease in the amount of sebum present on the surface of the skin which was **38.90%** higher than the percentage decrease induced by the product based on purifying thyme without booster and cell turnover and metabolism accelerator.

30 These results are displayed in Figures 4 and 5.

Product based on purifying thyme enhanced with booster and cell turnover and metabolism accelerator

The results obtained in the subjective assessment performed after 30
5 days of treatment pointed out the following:

- **90%** of volunteers reported that the product reduces skin greasiness;
- **70%** of volunteers reported that the product reduces skin impurities;
- 10 – **90%** of volunteers reported that the product does not dry the skin.

The percentage of satisfied volunteers is shown in Figure 6, wherein :

- 1 is the percentage of volunteers that consider that the product reduces skin
15 greasiness
- 2 is the percentage of volunteers that consider that the product reduces skin impurities
- 3 is the percentage of volunteers that consider that the product does not dry the skin

20

Product based on purifying thyme without booster and cell turnover and metabolism accelerator

The results obtained in the subjective assessment performed after 30
days of treatment revealed the following:

- 25 – **70%** of volunteers reported that the product reduces skin greasiness;
- **50%** of volunteers reported that the product reduces skin impurities;
- **100%** of volunteers reported that the product does not dry the skin.

The percentage of satisfied volunteers is shown in Figure 7, wherein:

1 is the percentage of volunteers that consider that the product reduces skin

5 greasiness

2 is the percentage of volunteers that consider that the product reduces skin impurities

3 is the percentage of volunteers that consider that the product does not dry the skin

10

CONCLUSIONS

On the basis of the results obtained in instrument-based measurements, we can say that although both products tested induced an increase in skin hydration and a normalization of sebaceous secretion, a
15 significantly higher activity of the product with Swiss plant extracts enhanced by a booster and a cell turnover and metabolism accelerator is observed with respect to the same product without booster and cell turnover and metabolism accelerator.

It has in fact been pointed out that the cosmetic product based on
20 purifying thyme enhanced by a booster and a cell turnover and metabolism accelerator induced a 74.27% higher percentage increase in the amount of water present in the horny layer and a 38.90% higher percentage decrease in the amount of sebum present on the surface of the skin, with respect to what is induced by the cosmetic product based on purifying thyme without
25 booster and cell turnover and metabolism accelerator.

The higher activity of the product with Swiss plant extracts enhanced by a booster and a cell turnover and metabolism accelerator was also confirmed by the assessments of the participants in the test.

C. Third test with product based on anti-wrinkle plantain enhanced by a booster and a cell turnover and metabolism accelerator with a composition as shown in Table 6 and the same product based on anti-wrinkle plantain without booster and cell turnover and metabolism accelerator.

Table 6

Composition of a product containing plantain enhanced by a booster and a cell turnover and metabolism accelerator

- Aqua
- Paraffinum liquidum
- 5 Polyisoprene
- Polysorbate 60
- Glyceryl isostearate
- Propylene glycol
- Isostearyl alcohol
- 10 Potassium cocoyl hydrolyzed wheat protein
- Potassium palmytoil hydrolyzed wheat protein
- Lanolin oil
- Panthenol
- Ceteareth-20
- 15 Parfum
- Carbomer
- Titanium dioxide
- Glycerin
- Dimethicone
- 20 Imidazolidinyl urea
- Tocopheryl acetate
- Glyceryl linoleate (Vit.F)
- Mica
- Sodium hydroxymethylglycinate
- 25 Phenoxyethanol
- Ethylhexyl methoxycinnamate
- Histidine

- Niacinamide (Vit. B3)
- Zinc gluconate (zinc)
- Sodium hydroxide
- Disodium EDTA
- 5 Methylparaben
- Glyceryl oleate
- Benzophenone-3
- Benzophenone-4
- Ethylparaben
- 10 Glyceryl linolenate (Vit.F)
- Glycyrrhetic acid
- Glyceryl arachinodate (Vit.F)
- Propylparaben
- Aluminum hydroxide
- 15 Stearic acid
- Algae extract (accelerator)
- Allantoin
- Leontopodium alpinum extract
- Sodium lauroyl lactylate
- 20 BHT
- BHA
- Plantago lanceolata leaf extract (plant extract)
- Hydrolyzed soy protein
- Tetrahexyldecyl ascorbate
- 25 Diethylene glycol
- Hexylene glycol
- Butylparaben
- Citral

Limonene

Ceramide 3

Ceramide 6-II

Cholesterol

5 Phytosphingosine

Xanthan gum

Citric acid

Ascorbyl palmitate

Ceramide 1

10

The effectiveness of the two products was assessed with the following instrument-based measurements:

- corneometry: to assess skin hydration
- elastometry: to assess skin elasticity
- 15 – with a subjective assessment of the volunteers who took part in the test and who after 30 days of treatment expressed their assessment of the products being tested as regards the following statements:
 - the product makes the skin more hydrated
 - the product attenuates wrinkles
 - 20 ● the product makes the skin more radiant

Assessment of cosmetic effectiveness (instrument-based measurements)

The cosmetic activity of the two products was assessed with the following instrument-based measurement, performed initially and after 30
25 days of treatment:

- **Corneometry**: in order to assess skin hydration, the amount of water present in the horny layer was measured on the right and left sides of the face (cheekbones) of the volunteers who took part in the test by

using the CM 825 Corneometer (Courage + Khazaka).

This instrument provides values which are directly proportional to the the degree of skin hydration and can be interpreted as shown in Table 7:

5

Table 7

	Corneometric values (referred to the face skin)
Very dry skin	<50
Dry skin	50-60
Sufficiently hydrated skin	>60

- **elastometry**: to assess skin elasticity, the maximum distensibility (R0) and the residual deformation (R1) of the skin were measured by using the Sem 474 Cutometer (Courage + Khazaka) on the right side and the left side of the face (cheekbones) of the volunteers who took part in the test. The values of ultimate elasticity (R2) obtained with the formula $R2 = (R0 - R1)/R0$ were then considered.
- To interpret the results, one must bear in mind that the values of R2 are comprised between 0 and 1, where 0 is minimum elasticity and 1 is maximum elasticity.

Cosmetic product based on anti-wrinkle plantain enhanced by a booster and a cell turnover and metabolism accelerator

The results obtained after 30 days of treatment, in instrument-based measurements performed on the right side of the face, pointed out the following:

- corneometry: the product being tested induced a **12.74%** increase in the amount of water present in the horny layer, indicating an increase in skin hydration;

- elastometry: the product being tested induced a **5.96%** increase in the values of ultimate elasticity (R2), indicating an increase in skin elasticity.

- 5 Cosmetic product based on anti-wrinkle plantain without a booster and a cell turnover and metabolism accelerator

The results obtained after 30 days of treatment, in instrument-based measurements performed on the left side of the face, pointed out the following:

- 10 – corneometry: the product being tested induced a **5.34%** increase in the amount of water present in the horny layer, indicating an increase in skin hydration;
- elastometry: the product being tested induced a **3.43%** increase in the values of ultimate elasticity (R2), indicating an increase in skin elasticity.
- 15

Comparison between the two products

The comparison between the results obtained with the two products pointed out the following:

- 20 – the cosmetic product based on anti-wrinkle plantain enhanced by a booster and a cell turnover and metabolism accelerator induced a percentage increase in the amount of water present in the upper horny layer which was **138.50%** higher than the percentage increase induced by the cosmetic product based on anti-wrinkle plantain without booster and
- 25 cell turnover and metabolism accelerator.
- the cosmetic product based on anti-wrinkle plantain enhanced by a booster and a cell turnover and metabolism accelerator induced a percentage increase in the ultimate elasticity values (R) which was **73.76%** higher than the percentage increase induced by the cosmetic
- 30 product based on anti-wrinkle plantain without booster and cell turnover

and metabolism accelerator.

These results are displayed in Figures 8 and 9.

5 **Assessment of cosmetic effectiveness (subjective assessment)**

Cosmetic product based on anti-wrinkle plantain enhanced with a booster and a cell turnover and metabolism accelerator

The results obtained in the subjective assessment performed after 30 days of treatment revealed the following:

- **100%** of volunteers reported that the product makes the skin more hydrated;
- **50%** of volunteers reported that the product attenuates wrinkles;
- **80%** of volunteers reported that the product makes the skin more radiant.

The percentage of satisfied volunteers is shown in Figure 10, wherein:

- 1 is the percentage of volunteers that consider that the product makes the skin more hydrated
- 2 is the percentage of volunteers that consider that the product attenuate wrinkles
- 3 is the percentage of volunteers that consider that the product makes the skin more radiant

Cosmetic product based on anti-wrinkle plantain without booster and cell turnover and metabolism accelerator

The results obtained in the subjective assessment performed after 30

days of treatment revealed the following:

- **100%** of volunteers reported that the product makes the skin more hydrated;
- **40%** of volunteers reported that the product attenuates wrinkles;
- 5 – **70%** of volunteers reported that the product makes the skin more radiant.

The percentage of satisfied volunteers is shown in Figure 11, wherein:

10

1 is the percentage of volunteers that consider that the product makes the skin more hydrated

2 is the percentage of volunteers that consider that the product attenuate wrinkles

15 3 is the percentage of volunteers that consider that the product makes the skin more radiant

CONCLUSIONS

20 On the basis of the results obtained in instrument-based measurements, we can say that although both products tested induced an increase in skin hydration and elasticity, a significantly higher activity of the product with Swiss plant extracts enhanced by a booster and a cell turnover and metabolism accelerator is observed with respect to the same product
25 without booster and a cell turnover and metabolism accelerator.

It has in fact been pointed out that the cosmetic product based on anti-wrinkle plantain enhanced by a booster and a cell turnover and metabolism accelerator induced a 138.50% higher percentage increase in the amount of water present in the horny layer and a 73.76% higher percentage increase in

the ultimate elasticity values (R2) with respect to what is induced by the cosmetic product based on anti-wrinkle plantain without booster and cell turnover and metabolism accelerator.

The higher activity of the product with Swiss plant extracts enhanced
5 by a booster and a cell turnover and metabolism accelerator was also confirmed by the assessments of the participants in the test.

The inventors of the present invention have found that the activity of a plant extract for cosmetic use in the cosmetic preparation according to the
10 present invention has increased synergistically due to the action of the enhancing or booster and cell turnover and metabolism accelerator component, which comprises one or more vitamins or fatty acids which compose vitamins, and zinc or zinc salts, biomineral complexes based on zinc or other forms of zinc which are biologically active and cosmetically
15 acceptable, and the cell turnover and metabolism accelerator. For example, the increased activity of any plant extract in a cosmetic preparation by using a formulation according to the present invention, which comprises a booster for vitamin B3, vitamin F and zinc and an accelerator based on *Dunaliella salina* extract, is surprisingly considerably higher than the effect expected
20 on the basis of the individual activity of plant extracts which are not enhanced by the components of the formulation.

The disclosures in Swiss Patent Application no. 00332/07, from which this application claims priority, are incorporated herein by reference.

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CLAIMS

1. A formulation for use in cosmetic preparations which contain plant extracts, said formulation comprising a component for enhancing the activity of plant extracts or booster, a cell turnover and metabolism accelerator, and optionally excipients which are acceptable from a cosmetic standpoint, said enhancing component comprising one or more vitamins or fatty acids which are components of vitamins and zinc or zinc salts or biomineral complexes based on zinc or other forms of zinc which are biologically active and cosmetically acceptable.
2. The formulation according to claim 1, wherein said one or more vitamins are selected among vitamin B3, vitamin F, vitamin A, vitamin C, vitamin E and mixtures thereof, preferably among vitamin B3, vitamin F and mixtures thereof.
3. The formulation according to claims 1 or 2, wherein said fatty acids which compose vitamins are fatty acids which compose vitamin F, in particular fatty acids selected among linoleic acid, linolenic acid and arachidonic acid and mixtures thereof.
4. The formulation according to one of the preceding claims, wherein said zinc salt is selected among zinc sulfate, zinc gluconate, zinc stearate, zinc oxide, zinc pyrithione and zinc ricinoleate, and mixtures thereof.
5. The formulation according to one of the preceding claims, wherein said cell turnover and metabolism accelerator comprises an algae extract.
6. The formulation according to claim 5, wherein said algae extract is a *Dunaliella salina* extract.
7. A cosmetic preparation comprising one or more plant extracts and a formulation according to one or more of claims 1 to 6, and optionally excipients which are acceptable from a cosmetic standpoint.
8. The preparation according to claim 7, wherein said formulation is present at a concentration from 0.1 to 20% by weight based on the total weight of the preparation.

9. The cosmetic preparation according to claim 7 or 8, wherein said enhancement component is present at a concentration from 0.01 to 15% by weight based on the total weight of the cosmetic preparation.

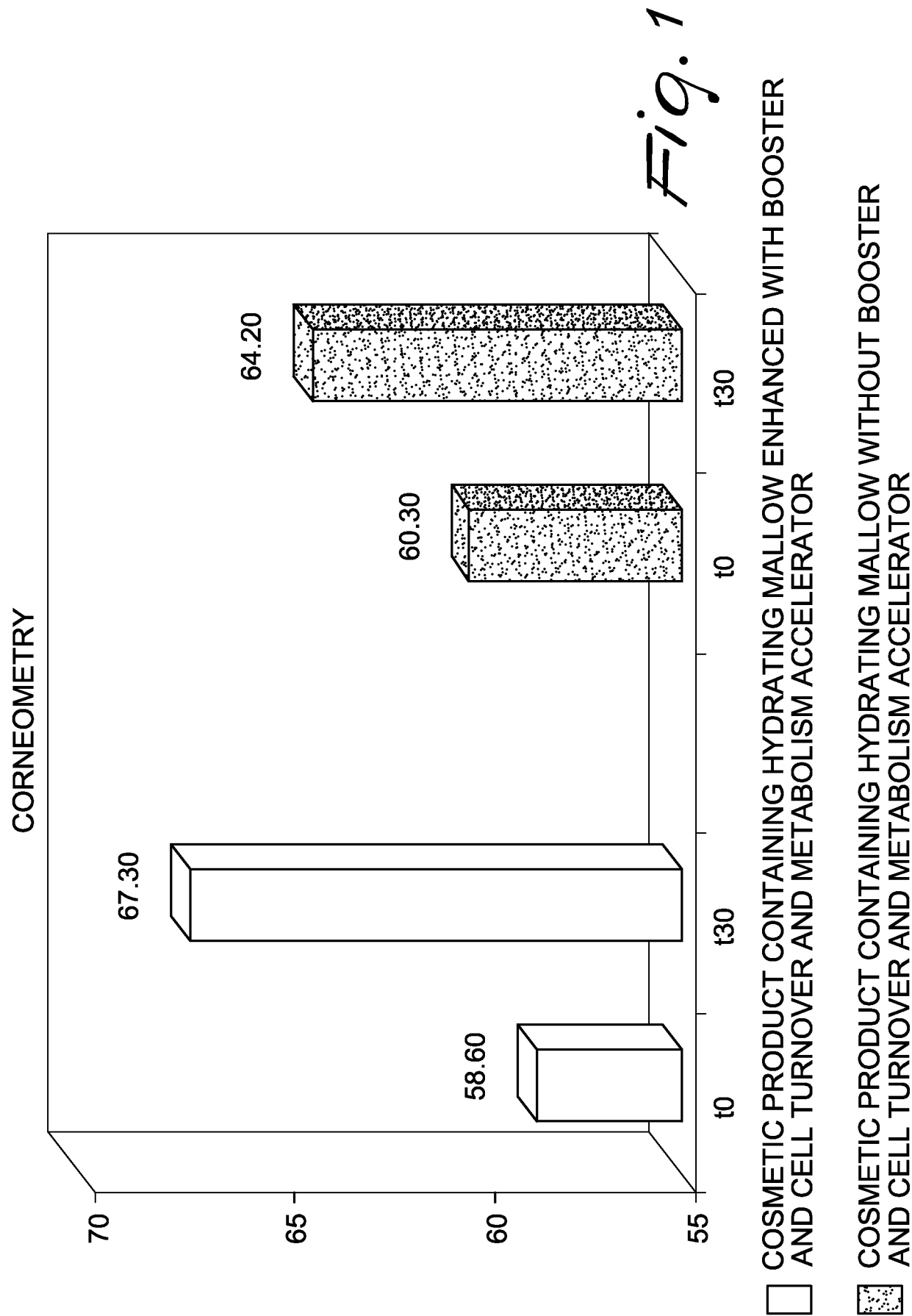
10. The cosmetic preparation according to one or more of claims 7 to 9, wherein said zinc, zinc salt, biomineral complexes based on zinc or other forms of zinc which are biologically active and cosmetically acceptable are present at a concentration from 0.001 to 5% by weight based on the total weight of the cosmetic preparation.

11. The cosmetic preparation according to one or more of claims 7 to 10, wherein said one or more vitamins or said fatty acids which compose vitamins are present at a total concentration from 0.001 to 5% by weight based on the total weight of the cosmetic preparation.

12. The cosmetic preparation according to one or more of claims 7 to 11, comprising vitamin B3, vitamin F and zinc, preferably from 0.001 to 5% by weight of vitamin B3, from 0.001 to 5% by weight of vitamin F and from 0.001 to 5% by weight of zinc based on the total weight of the cosmetic preparation.

13. The cosmetic preparation according to one of claims 7 to 12, wherein said cell turnover and metabolism accelerator is present at a concentration from 0.05 to 5% by weight with reference to the total weight of the cosmetic preparation.

14. The preparation according to one of claims 7 to 13, wherein said one or more plant extracts are selected among extracts of alpine plants, particularly plants of the Swiss Alps.



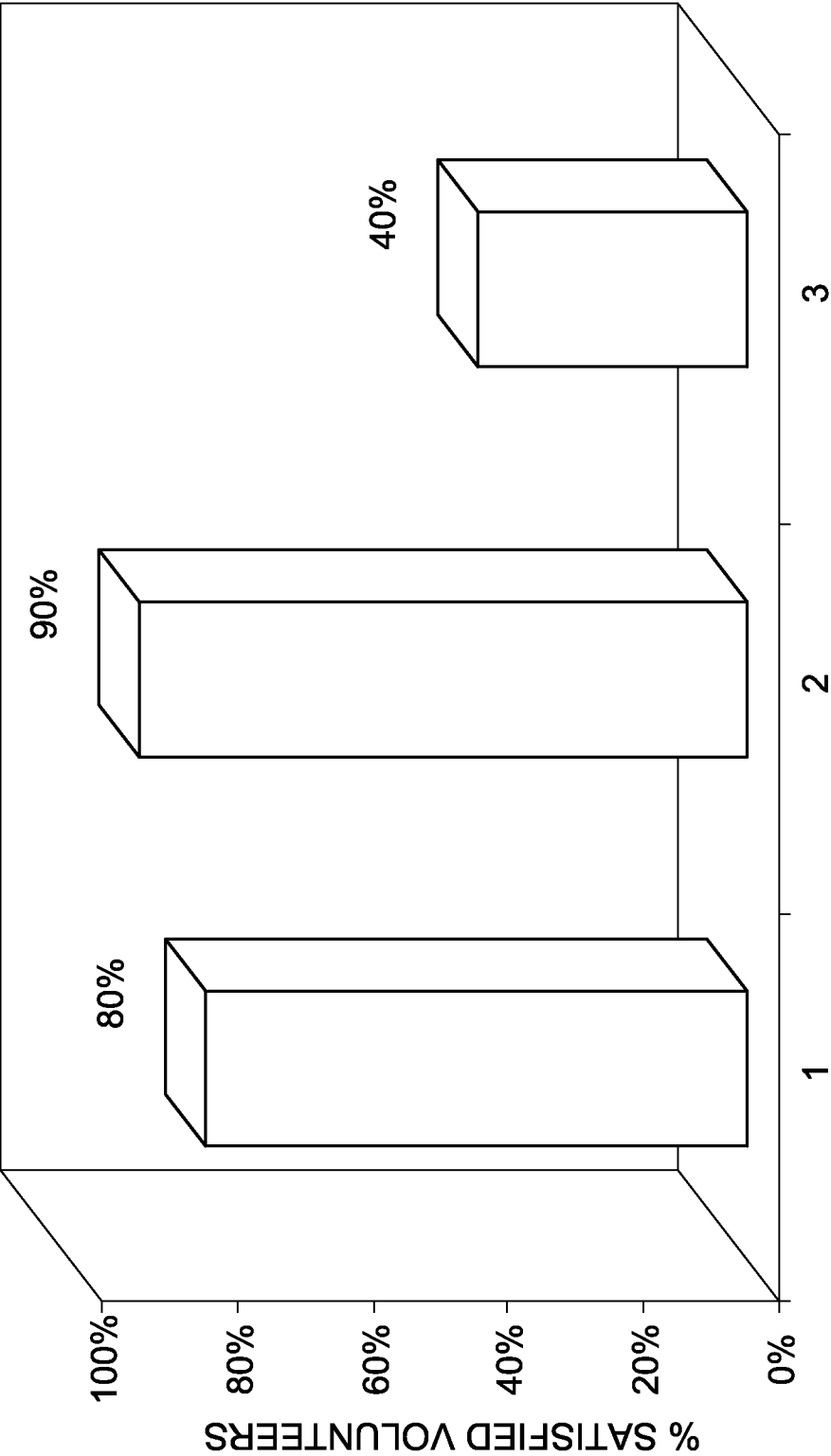


Fig. 2

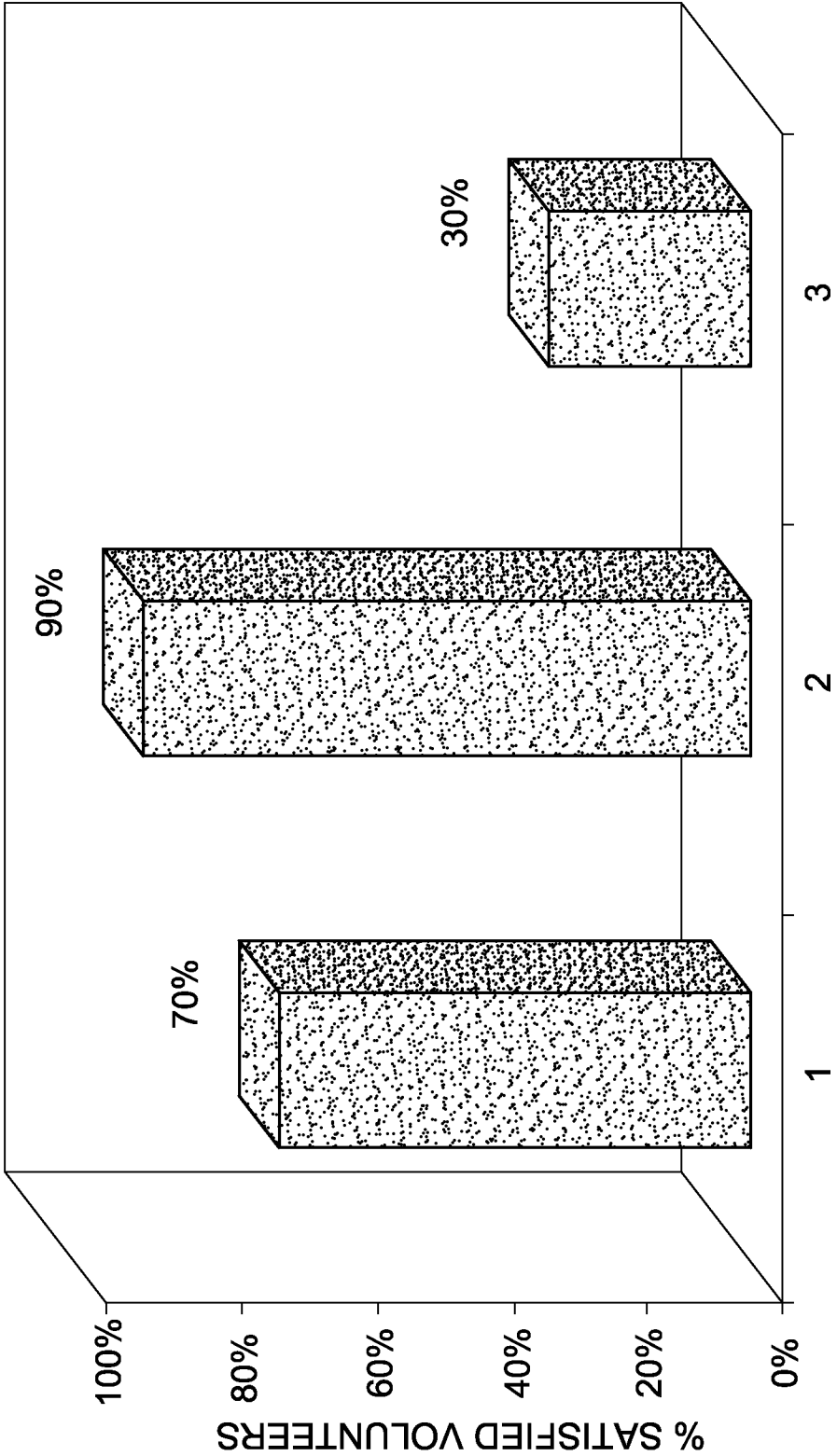


Fig. 3

CORNEOMETRY

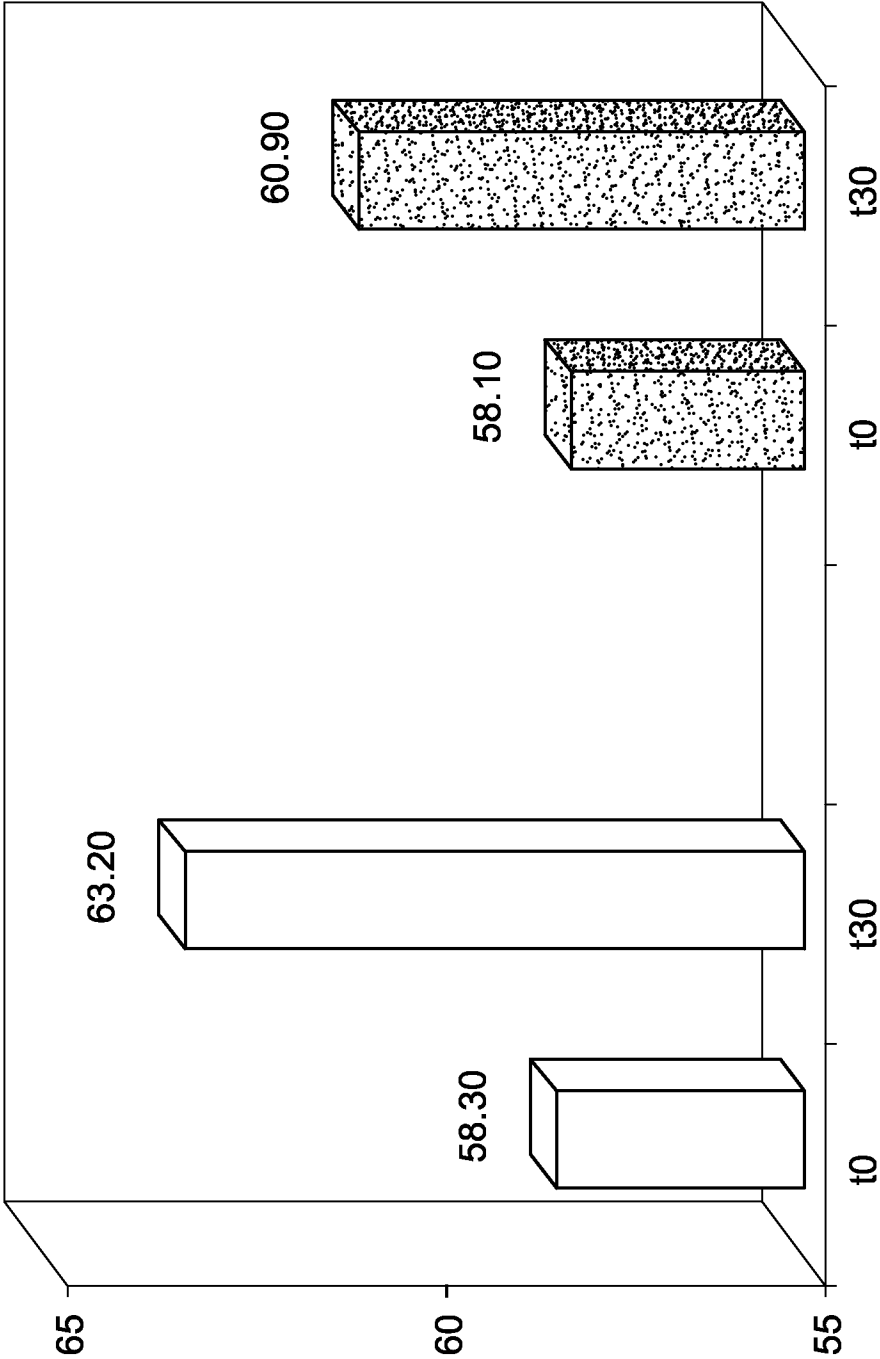


Fig. 4

- COSMETIC PRODUCT CONTAINING PURIFYING THYME ENHANCED BY A BOOSTER AND CELL TURNOVER AND METABOLISM ACCELERATOR
- ▤ COSMETIC PRODUCT CONTAINING PURIFYING THYME WITHOUT BOOSTER AND CELL TURNOVER AND METABOLISM ACCELERATOR

SEBOMETRY

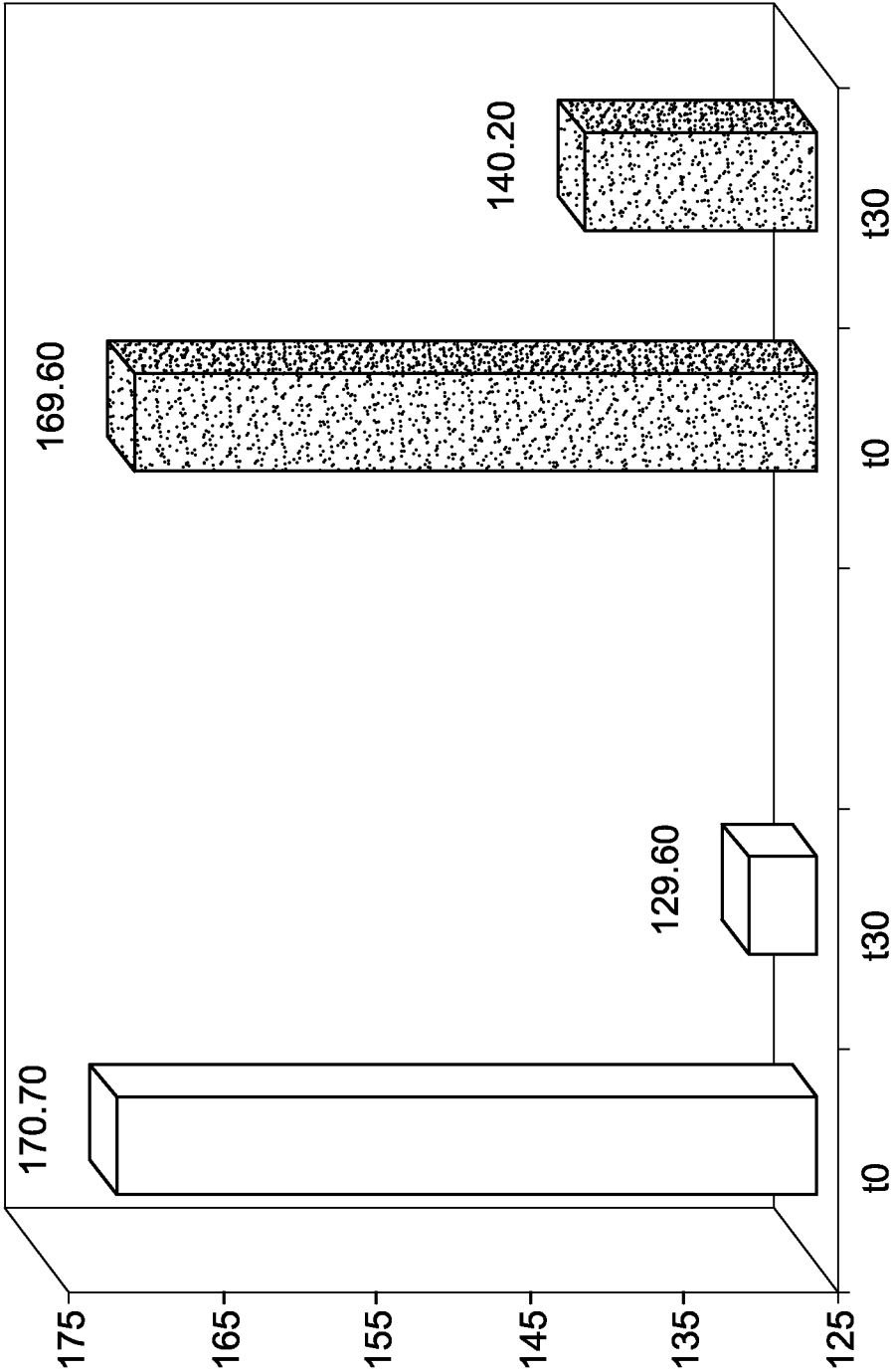


Fig. 5

- COSMETIC PRODUCT CONTAINING PURIFYING THYME ENHANCED BY A BOOSTER AND CELL TURNOVER AND METABOLISM ACCELERATOR
- ▤ COSMETIC PRODUCT CONTAINING PURIFYING THYME WITHOUT BOOSTER AND CELL TURNOVER AND METABOLISM ACCELERATOR

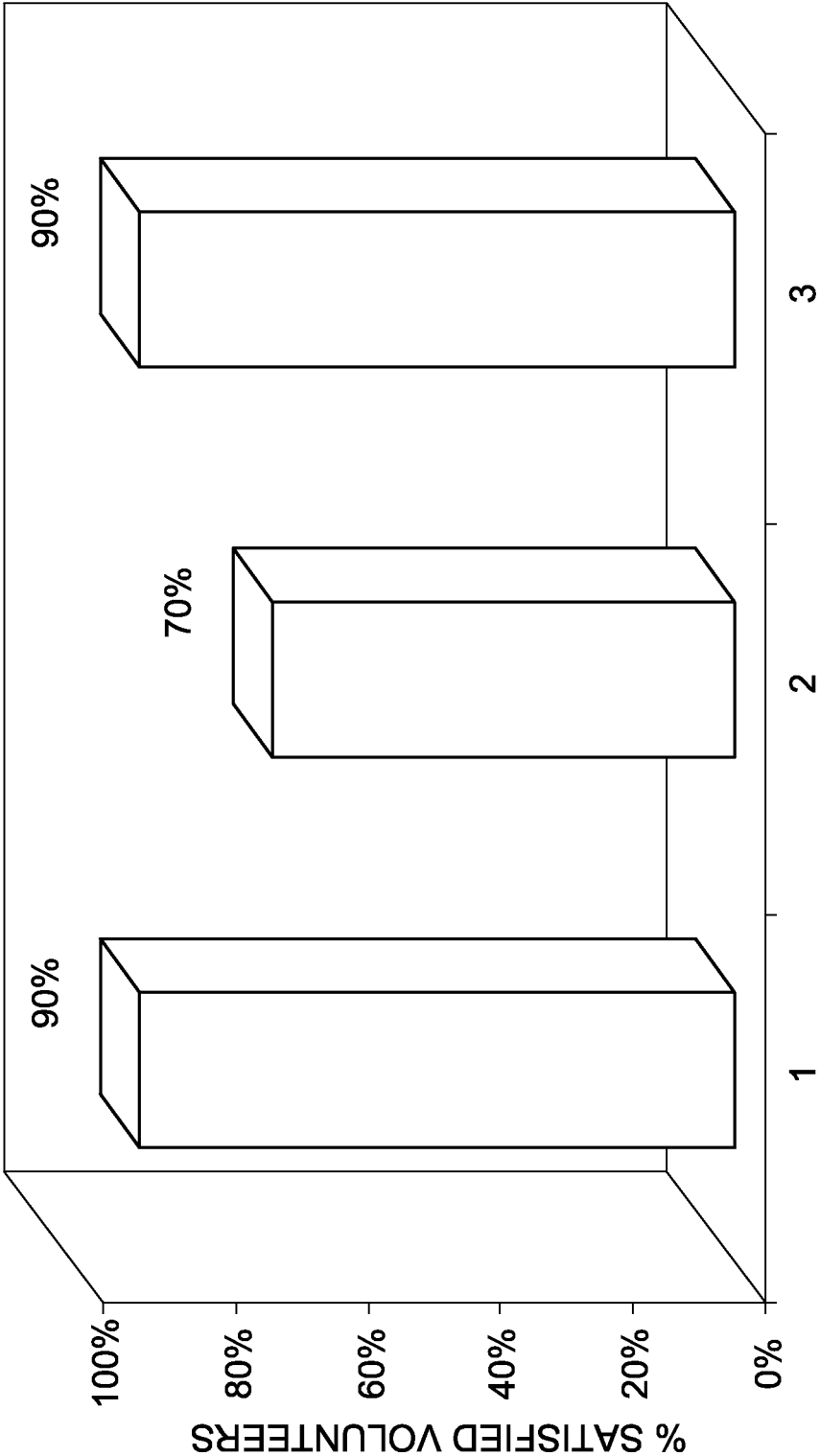


Fig. 6

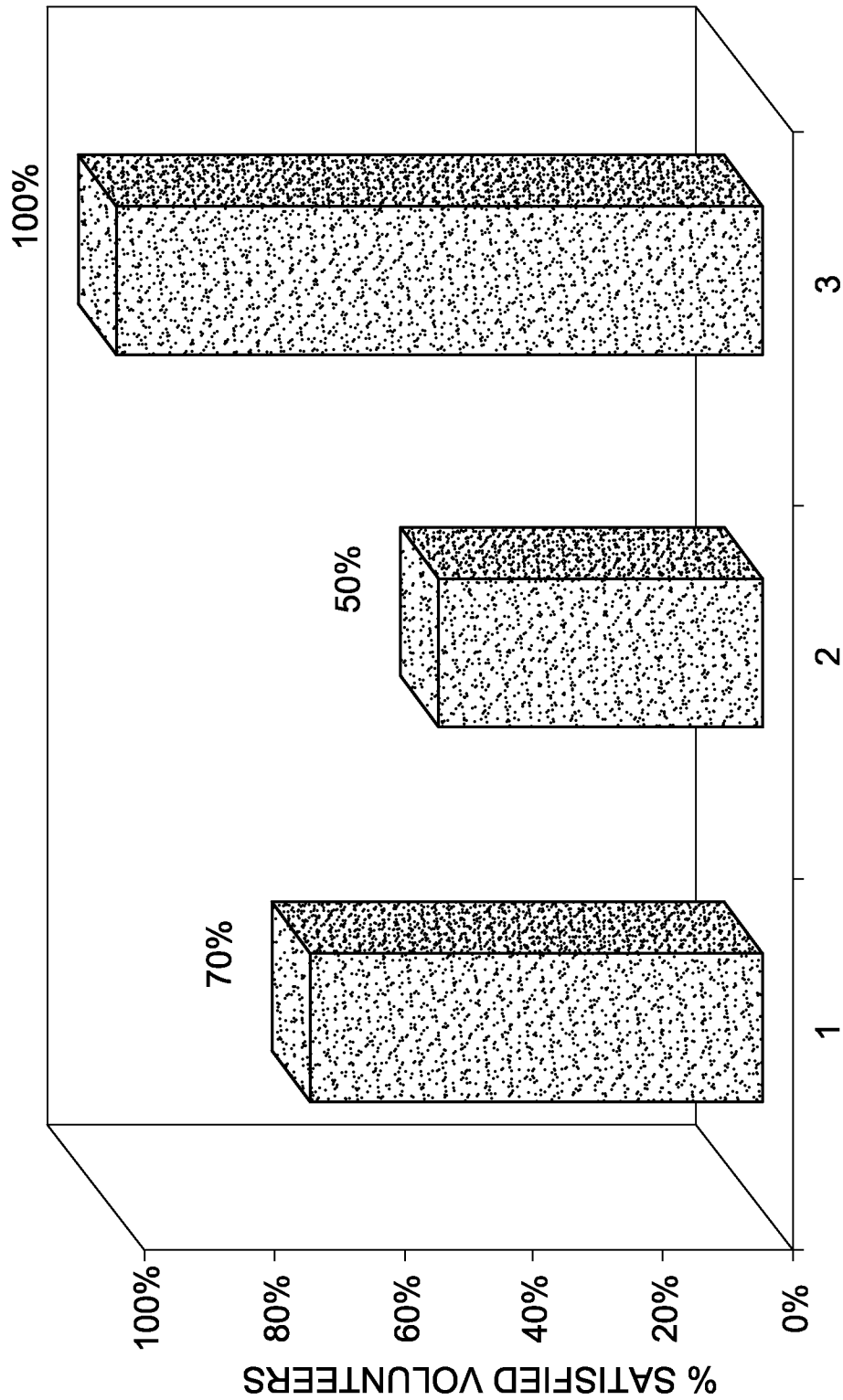
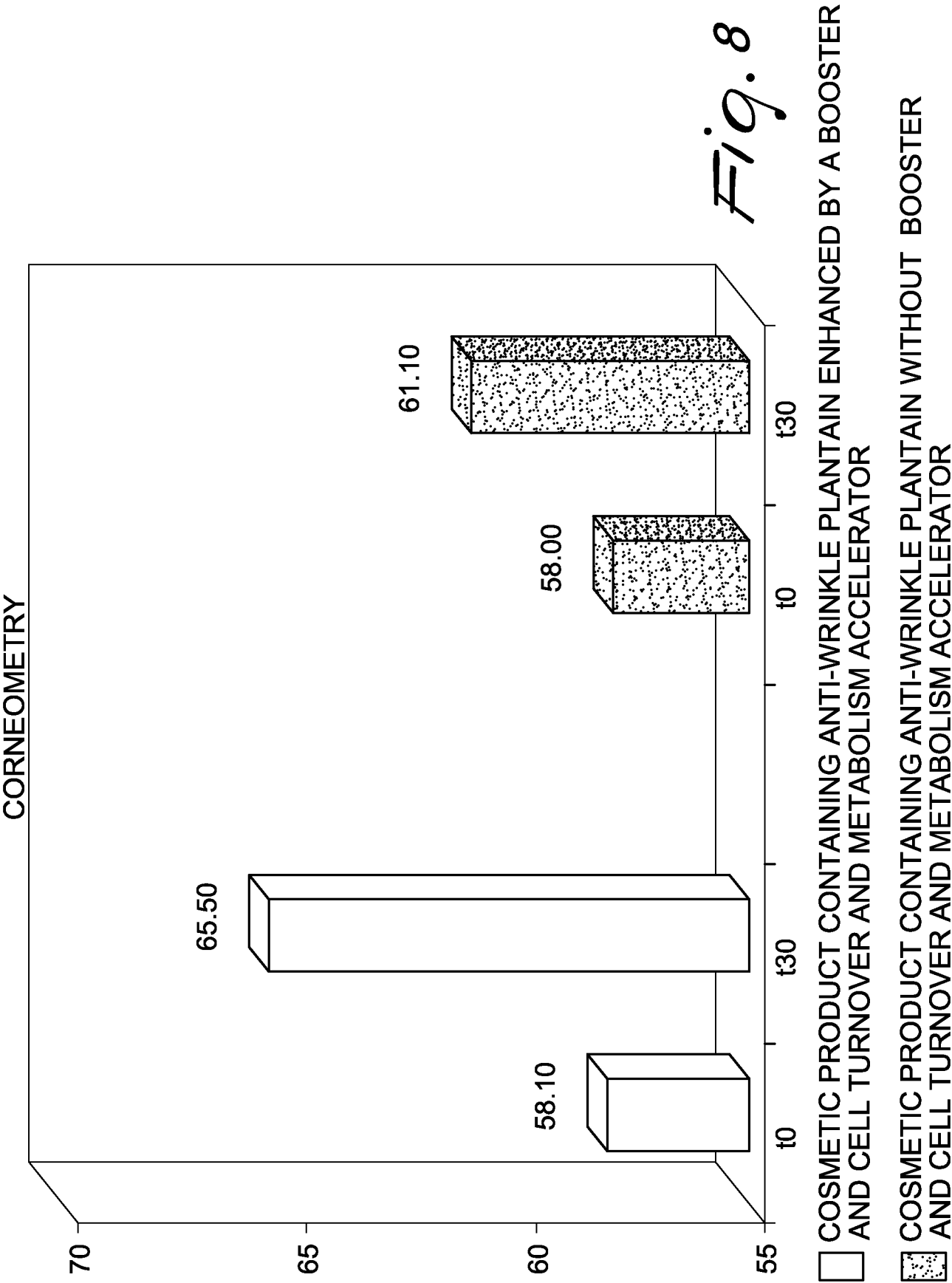
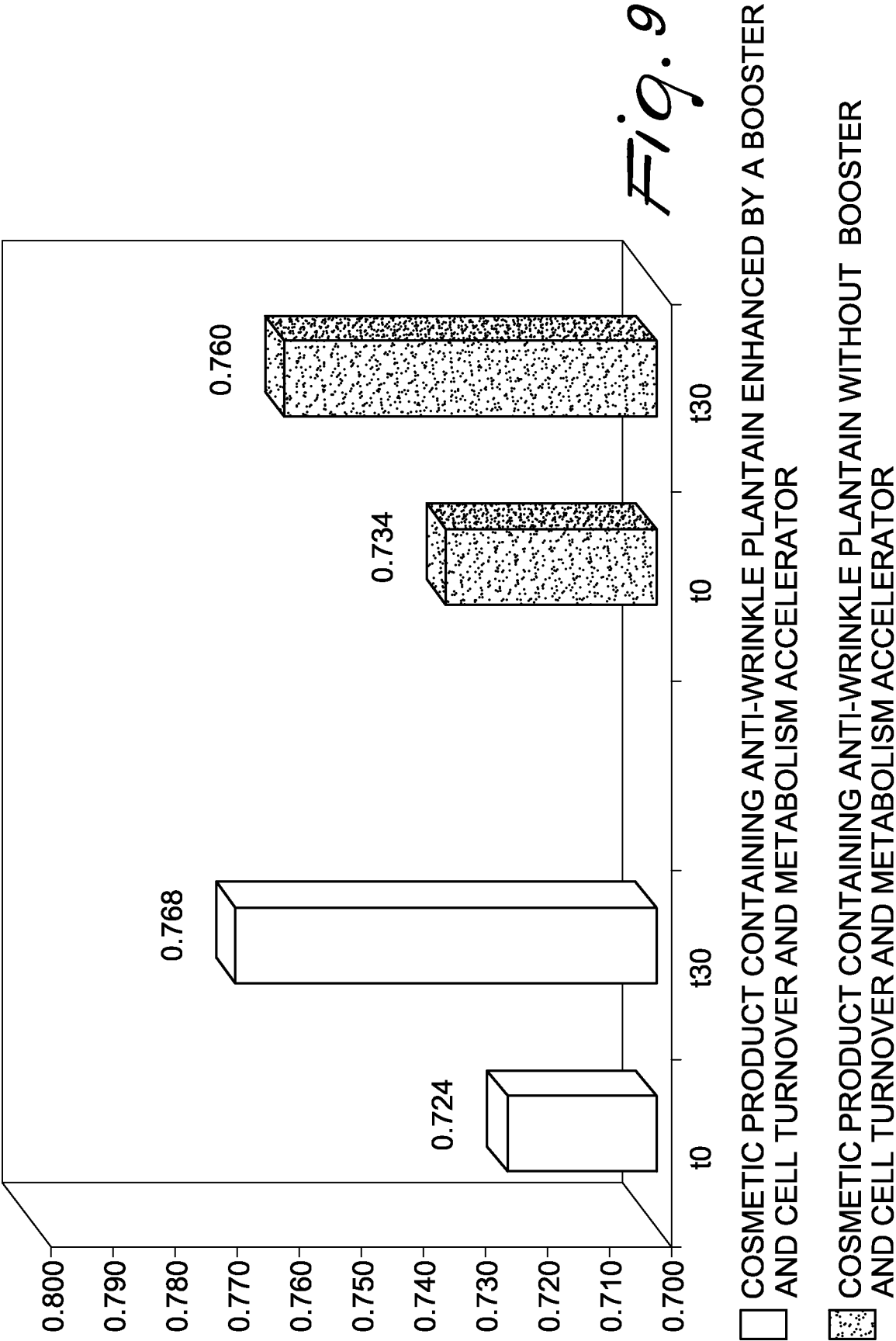


Fig. 7



ELASTOMETRY



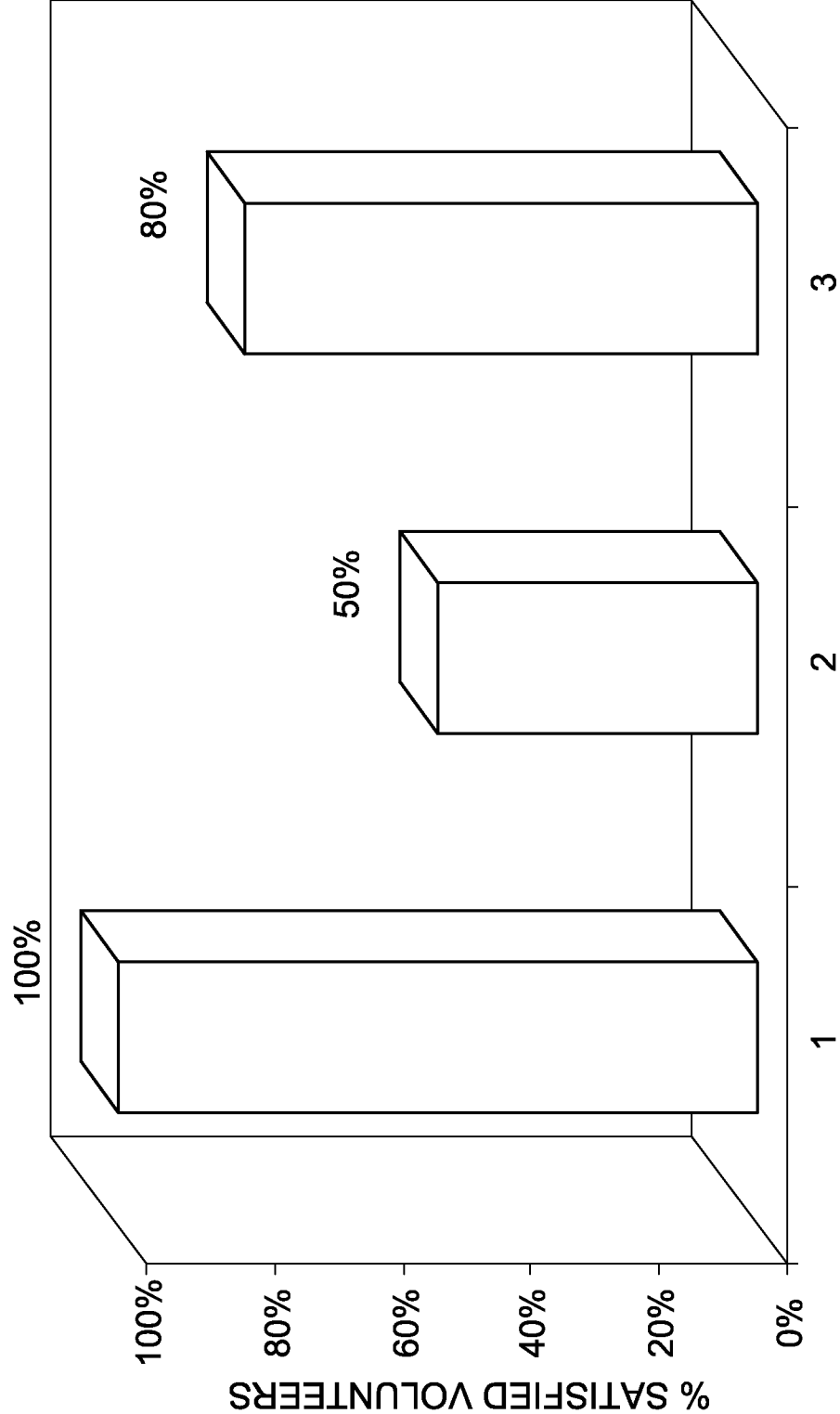


Fig. 10

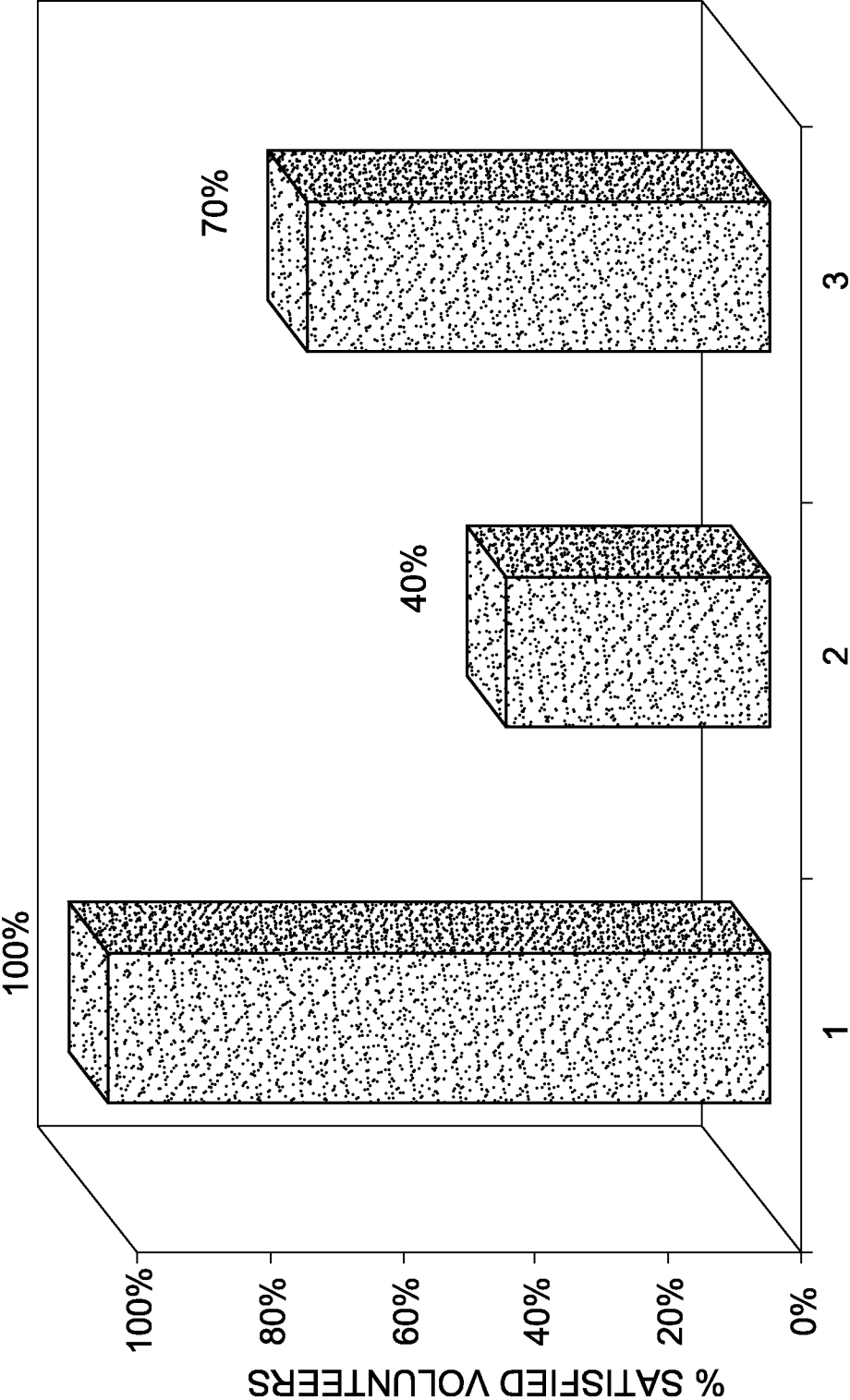


Fig. 11