Skin lightening via natural botanical oily composition

Victoria Donat, Anna Martí - Textron Plimon, Spain

Abstract

We aimed to develop a novel lipid-based skin whitener and personal care product to replace syntethic bioactives. Additional qualities were intended as safety, naturalness, preservative absence and thermal stability. For this purpose, a broad study was performed to identify vegetable oils and botanical extracts, in order to produce a lipid solution showing skin whitening, hydrating and nourishing activities. The product obtained using selected ingredients was subsequently submitted to performance assessment, safety assays and physico-chemical characterisation. Efficacy trials evaluated the whitening activity of the product under *ex vivo* conditions after 4 doses, at 100% concentration, using reconstructed human epidermis and displaying a 47% reduction of melanin quantity versus the negative control. Safety was proved by dermatological assays. Key parameters as density, viscosity, acidity, peroxide and saponification values were determined, fatty composition analysed by gas chromatography, and oxidative stability tested by Rancimat method, revealing full suitability for cosmetic use.

Melanin is the natural pigment of human skin. Tyrosinase is responsible for melanisation in plants and animals, which leads to – sometimes undesirable – browning. Tyrosinase is a melanogenic copper containing enzyme that catalyses the transformation of tyrosine to dopaquinone, eventually leading to melanin secretion and subsequent accumulation in skin cells.[1, 2]

Effects of UV radiation on the skin are both beneficial and harmful. It induces synthesis of vitamin D, killing of pathogens and treating the disorders like psoriasis. On the other hand, they cause photoageing and skin cancer by making alterations at the cellular level.[3] The exposure of UV radiation on the skin generates oxidative stress resulting in structural and functional changes in the epidermis and biomolecules present within the cell.

Various dermatological disorders, such as melasma, age spots, and sites of actinic damage arise from the accumulation of an excessive level of epidermal pigmentation.[1, 4, 5, 6] Although melanin has mainly a photoprotective function in human skin, the accumulation of an abnormal amount of melanin in different specific parts of the skin, resulting in more pigmented patches, might become an aesthetic problem.

In Western culture, it is still considered desirable to obtain a bronze tan. Despite warnings about the consequences of excessive sun or UV exposure, the artificial tanning business has expanded strongly in the last decades. In the Eastern world, however, a centuries long tradition exists whereby a light complexion is regarded as equivalent to youth and beauty. In recent years, the interest in skin whitening has grown tremendously.[4] Traditionally, skin depigmentation has been performed with aggressive chemical agents. Development of preparations for bleaching hyperpigmented lesions or to safely achieve overall whitening is one of the challenges for cosmetic industry.[7, 8, 9]

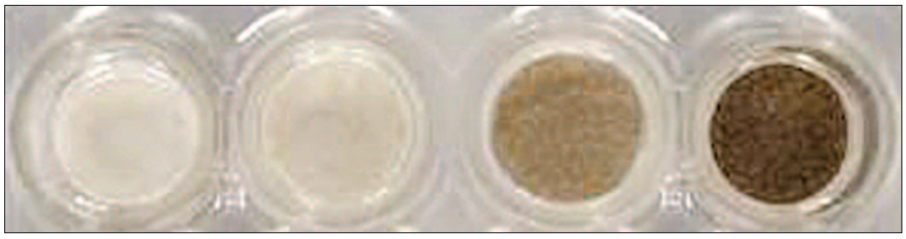

Figure 1: From left to right: tissue without melanocytes; Phototype II tissue; Phototype IV tissue; Phototype VI tissue.

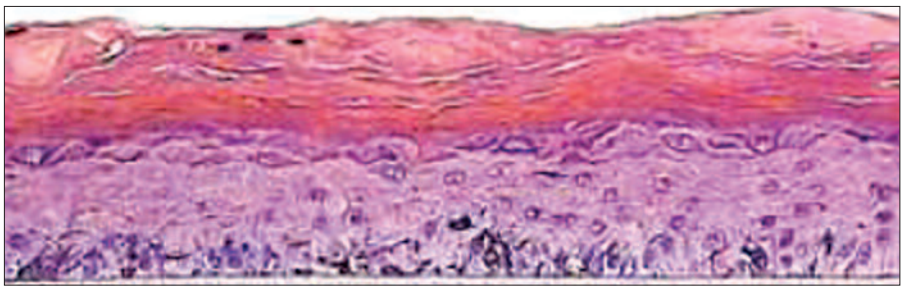

Figure 2: Skin tissue model (RHPE). The test was performed on reconstructed in vitro epithelia, containing fibroblasts and keratinocytes.

One of the most obvious cellular targets for depigmenting agents is the enzyme tyrosinase.[4, 6, 10] The scientific literature on tyrosinase inhibition shows that a large majority of the work has been conducted since 2000 and has mostly been devoted to the search for new depigmenting agents.[11]

Notably, many of these studies deal with tyrosinase inhibitors from natural sources and are mostly of Asian origin. A number of tyrosinase inhibitors from both natural and synthetic sources have been identified.[6, 11]

Botanicals are gaining importance in recent times as active ingredients for cosmetic formulations due to their dermal protective effect against the harmful substances from endogenous and exogenous sources.[1, 3, 5, 8] The use of botanicals as photoprotectives and/or antioxidants has been gaining significant attention of researchers due to multiple biological actions on the skin and positive perception by consumers. The additive

properties exerted by the phytoconstituents of plant actives make them the most suitable ingredient for cosmetic formulations.

Classical skin whiteners, such as hydroquinone, kojic acid or mercury compounds may act as carcinogens or cause other harmful or damaging effects[1, 12-15] and in many cases they are banned in some markets and limited in others.[16, 17]

In this scenario, we pursued to create a new raw material for replacement of synthetic alternatives, aiming also to provide the product with further properties in terms of quality and skin care potential. With this target, an extensive search of plant oils and extracts was carried out, selecting several ingredients eligible to be a part of the formulation, based in their ability to inhibit tyrosinase, according to scientific literature. A lipid composition was prepared using the selected oily extracts and subsequently examined at different levels – functional, dermatological and physico-chemical – to assess skin whitening efficacy, harmlessness and fitness for cosmetic formulation. *Ex vivo* melanin inhibition tests showed remarkable results compared to the negative control; Patch Test revealed the product as non-irritant on human skin, and organoleptic and physico-chemical assays showed an ingredient suitable to be included in a personal care product.

Experimental procedure and results

Formulation

Selected oily extracts of *Aloe barbadensis*, *Rheum rhaponticum*, carotenoids, glabridin, natural tocopherol isolated from *Helianthus annuus*, and up to a 0.1% of dimethylmethoxy chromanyl palmitate were sourced from accredited suppliers and dissolved in fixed vegetable oils, until total homogeneity and clearness of lipid solution.

Efficacy assays

Whitening activity was evaluated on *in vitro* reconstructed tissues of pigmented human skin (phototypes II, IV and VI). Each test substance (test sample, i.e. lipidic solution and positive controls) was topically applied concurrently on five tissue replicates. Ascorbic acid was chosen as a positive control for displaying skin whitening effects previously proven[18] using a concentration higher than that commonly used in marketed skin whitening products to ensure reliable results. After treatment, a visual inspection was performed and one out of five tissues was further evaluated by histology analysis.[19, 20, 21]

The test was performed on reconstructed in vitro epithelia, containing fibroblasts and keratinocytes (model RHPE, Reconstructed Human Pigmented Epidermis) from SkinEthic. When cultivated at the air liquid interface in a chemically defined medium, normal human keratinocytes cultured in the presence of melanocytes of phototypes II, IV and VI from 3D human epidermal tissues. The different tanning degrees of these constructs correspond macroscopically to three different phototypes of human skin.

Treatment of the tissues:

- Five tissues per test substance and for each control were used.
- 1 µl of the test sample (lipidic solution) and positive control (2% ascorbic acid solution) were dosed per 0.5 cm2 tissue.
- The lipidic solution was used directly, without dilution (100% concentration).
- 4 applications were performed for each test, incubating at 37°C, 5% CO_2, < 80% RH during 72 h, followed by visual inspection, and melanin quantification, as follows: day 1) dose 1; day 2) dose 2 and dose 3; day 3) dose 4; day 4) incubation at 37°C, 5% CO_2, < 80% RH; day 5) incubation at 37°C, 5% CO_2, < 80% RH; day 6) visual inspection; cell viability evaluation by MTT assay, histology and melanin quantification.
- Untreated tissues were used as negative control.
- Tissues treated with 1 µl of 2% ascorbic acid solution were used as positive control. The treatment of positive control tissues was performed as described for the test product.

Melanin quantification

Three tissues for each of the test conditions (test sample and controls) were evaluated at the end of the incubation period. Tissues were processed and extracts obtained were analysed in a spectrophotometer at 490 nm for melanin quantification. Synthetic melanin was used as standard. Results were expressed as Optical Density (OD) and as mg/ml melanin.

Under the experimental conditions, the analysed sample of the lipidic solution did not show cytotoxic effects on the in vitro-RHPE model. The positive control did not show cytotoxic effects on the in vitro-RHPE model.

Reduction on melanin quantity was calculated for the positive control and for

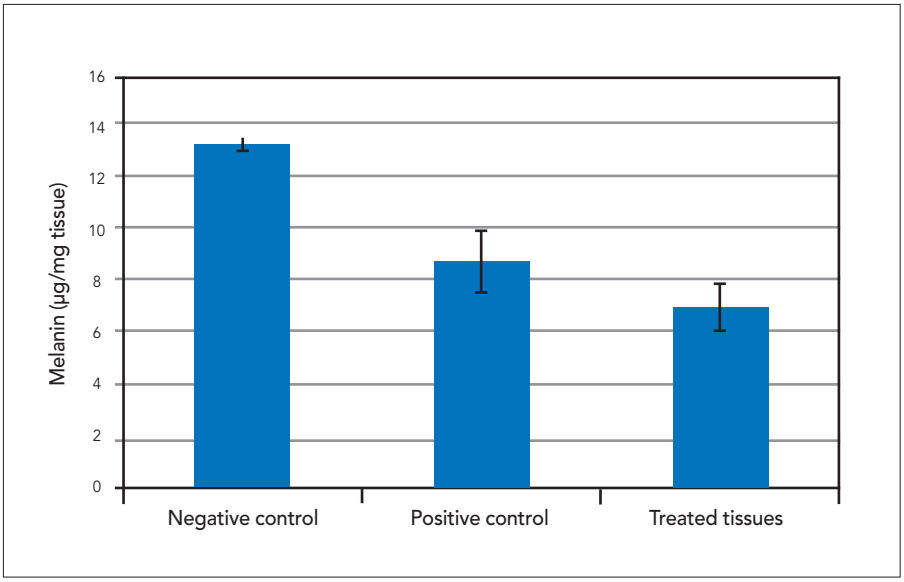

Figure 3: Melanin quantification in RHPE treated tissues. The quantity of melanin in the average value of three replicates (three treated tissues).

Table 1: Raw data – melanin quantification.

Experimental Condition	Optical Density				Melanin quantification (ug/ml)				
	Tissue 1	Tissue 2	Tissue 3	Tissue 4	Tissue 1	Tissue 2	Tissue 3	Average (%)	SD (%)
Negative control	0.184	0.201	0.177	0.188	262.63	294.93	248.69	268.75	23.72
Positive control (2% ascorbic acid)	0.158	0.165	0.151	01.58	213.22	226.52	199.28	213.01	13.62
Sample (lipid solution)	0.150	0.141	0.161	0.150	196.75	179.94	218.29	198.23	19.36

Table 2: Results of semi-occluded patch test with 11 volunteers, mixed, no specificity of the panel.

Test	Results
Type of patch	Semi-occluded
Number of volunteers	11
Sex of the panel	Mixed
Specificity	None
Average irritation score	0.00
Classification	Non irritant

the test product. The positive control reduced a 34% of melanin quantity versus the negative control. The lipidic solution reduced a 47% of melanin quantity versus the negative control.

Figure 3 shows the quantification of melanin in treated RHPE tissues; melanin was quantified in the negative control, in the positive control and in the test sample treated tissues. Average and standard deviation from three tissues were calculated. A significant decrease in the quantity of melanin was detected in the positive control and in the treated tissues with the test sample (Student's T test $P<0.05$).

Safety assays

A study report was carried out for assessment of skin tolerance of the cosmetic product developed in the present work, after a single application under semi-occluded patch during 48 hours on 10 volunteers by patch test method. Assessment of the skin local tolerance of the studied product after an epicutaneous test performed in semi-occluded conditions, after a single application on the skin of the back and under semioccluded patch. According to the experimental conditions of the study, the product can be considered as non irritant regarding its primary skin tolerance.

Table 3: Results of physico-chemical characterisation

Parameter	
Appearance	Oily liquid, slightly fruity odour
Acidity (mg KOH/g)	0.25 – 0.30
Peroxide value (meg0_2/Kg)	max. 14
Saponification value	170 – 185
Density (20°C)	0.910 – 0.915
Viscosity	66.6 cps
Oleic acid	35 – 50%
Linoleic acid	20 – 35%

Physico-chemical characterisation

Acid, peroxide and saponification values, as well as density measurement, were assessed by methods of analysis set by European Pharmacopeia (EP). Fatty acids were determined by Gas Chromatography, also according to EP standards. Viscosity was measured in a Selecta ST2001 viscometer by Brookfield rotating method (following 2.2.10. protocol of European Pharmacopoeia), with R1 spindle, at 23.5°C and 100 rpm.

Organoleptic properties were noted by visual inspection.

Oxidative stability

The Oil Stability Index (OSI) was determined using a Rancimat instrument. The rapidity of oxidation of an oil depends on the degree of unsaturation, the presence of antioxidants, and prior storage conditions. In the OSI analysis, the rate of oxidation is slow until resistance to oxidation is overcome. This time is known as the oxidation induction period and it is a tool to determine the useful life of the oil. OSI was determined at 100°C and 110°C, according to ISO 6886 (1996) procedure 'Animal and vegetable fats and oils. Determination of oxidation stability'. Experimental conditions were as follows:
Sample amount 2.5 ± .01 g
Temperature 100°C ± 0.2°C
Gas flow: 20 L/h
Vessel: 50 mL distilled water
Evaluation Conductivity
Induction time (tangent method)

The induction time obtained was 6.87 h at 100°C and 3.39 h at 110°C, value that can be extrapolated by Rancimat software, giving a shelf life of 24 months. This predicts a good stability for a vegetable oil to be marketed as cosmetic raw material, provided proper handling and storage as per manufacturer recommendations when supplied.

Conclusions

The product developed in the present work achieved the intended properties. Composed of 100% natural oils and 99.9% natural actives, ingredients were specially selected for optimum whitening performance, intensive skin care, and self-preservation.

This combination of selected plant oils and bioactive botanical extracts showed whitening activity at 100% concentration, when dosed 4 times and reduced a 47% of melanin quantity versus the negative control under the *ex-vivo* test conditions on live human skin cell cultures. It has been tested on epithelium phototype VI, that is, black or dark brown skin.

This whitening product is safe and can be applied directly to skin, since efficacy tests

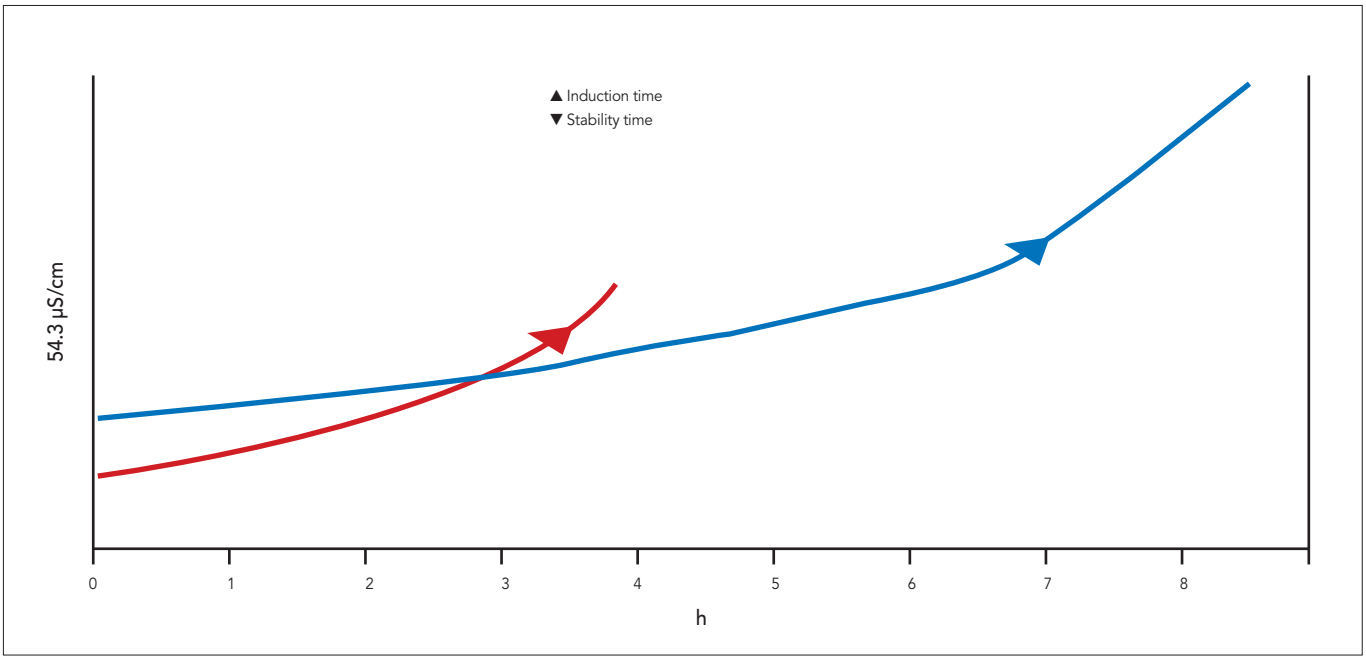

Figure 4. Oil Stability Index (OSI) determined by Rancimat method at 100°C and 110°C; blue line: determination at 100°C, induction time 6.87 h; red line: determination at 110°C, induction time 3.39 h.

have been performed using the original product and Patch Test result is non-irritant, performed with 11 human volunteers.

Formulated on an oil basis, unlike most whitening products in the market – water based – the developed product incorporates lipidic phase and bioactive principles simultaneously, thus warranting their stability and leading to a highly-stable, preservative-free product.

Oil-based, water-free formulation warrants microbiological preservation by avoiding aqueous media causing microbial degradation, even being preservative-free. Avoidance of aggressive chemical agents present in classical skin whiteners that usually cause undesirable side-effects leads to a more advantageous safety profile.

The present product incorporates lipidic phase and bioactive principles at a time, allowing cosmetic manufacturers to replace chemical raw materials and synthetic bioactives by a vegetable-based raw material and bioactive principles in one step. Manufacturing process would then become easier, avoiding the steps of solubilisation/stabilisation of whitening active principles.

Addition of chemical lightening agents is not necessary and might be detrimental to naturalness and harmless of the product, without improving significantly its efficacy or even causing a potential competitive inhibition that could lead to a reduction of effectiveness.

In conclusion, a novel plant-based, oil-based skin whitener has been developed, with a promising potential in cosmetic applications to achieve simultaneously skin depigmentation and intensive skin care. PC

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