Capsaicin-free red pepper extract for energized, radiant and non-irritated skin

Audrey Faure-Grousson[1]*, Valérie Cenizo[1], Jinhee Jeong[2], Cédric Khan[1], Hill Kim[3], Iljee Cho[3], Andrea Cavagnino[4] and Pascal Portes[1]

[1] Erborian - L'Occitane Group, Manosque, France; *[2] Erborian - L'Occitane Group, Seongnam, Republic of Korea*; *[3] Radiant INC., Chuncheon, Republic of Korea*; *[4] Oxiproteomics, Paris, France*

*Audrey Faure-Grousson, ZI Saint Maurice – 04100 Manosque – France, +33492706277, audrey.faure-grousson@loccitane.com

Abstract

Background: Pollution generates reactive oxygen species (ROS) which cause cellular dysfunction and premature aging. Indeed, ROS create carbonylation, irreversible changes in proteins, and contribute to dull skin. Red pepper (*Capsicum annuum*) is known in Traditional Korean Medicine for centuries. However, red pepper also has warming properties, supported by capsaicin which can lead to skin irritation and burning sensations. We especially designed a Korean red pepper capsaicin-free extract (RPE).

Methods: A specific gentle RPE was developed by removing irritating capsaicin and tested with a patch test and human repeat insult patch test (HRIPT). We studied RPE antioxidant properties using DPPH, cell ROS and protein carbonylation assays, and its action on cell energy through ATP synthesis in the absence of stress or under pollution. Skin radiance was evaluated in a clinical test with a cream containing RPE.

Results: The developed RPE was non-irritating, antioxidant and also prevented fine dust-induced protein carbonylation. When applied on human dermal fibroblasts (HDF) in absence of any stress, RPE induced more ATP production than Coenzyme Q10. Under fine-dust exposure, RPE also protected ATP synthesis decrease.

Finally, we demonstrated the benefit of RPE into a cream by evaluating skin radiance. After 4 weeks of cream application, complexion brightness and luminosity were increased, then, yellow shade was decreased.

Conclusion: We developed a capsaicin-free Red Pepper extract for better skin tolerance. Red Pepper extract protected proteins and cells from oxidative stress and improved cellular energy suggesting that it could prevent visible signs of pollution and improve dull and tired skin.

Keywords: Red pepper, protein carbonylation, pollution, energy, skin radiance, non-irritant

Introduction

Air pollution is a heterogeneous mixture of chemicals and solid particles, in which chemical composition, size, and sources of origin differ in each microenvironment. Among the wide variety of pollutants, particulate matter (PM) is considered a major concern from a health perspective and has been pointed out as an environmental threat not only to susceptible but also to healthy members of the population [1]. It has been demonstrated that $PM_{2.5}$ induces TNFα expression, which causes filaggrin (FLG) deficiency into the skin and transepidermal water loss (TEWL) increase. FLG is a key protein involved in maintaining normal skin barrier function and hydration. Its deficiency could be responsible of skin barrier disruption and enhancement of skin penetration [2]. A decrease in skin hydration, can result in a rough, dry and dull appearance, with a loss of radiance and suppleness [3].

Particulate matter (PM) is an integral part of air pollution. PM has been shown to penetrate the skin barrier and to induce cytotoxicity, inflammation and reactive oxygen species (ROS) production in human keratinocytes [4]. Protein carbonylation is an oxidative post-translational modification including aldehydes and ketones formed via different mechanisms: (i) direct oxidation of the polypeptide backbone leading to truncated peptides; (ii) side chains oxidation of lysine, arginine, proline, and threonine residues on proteins; (iii) reaction of histidine, cysteine, and lysine amino acid residues with aldehydes, for example, produced by lipid peroxidation; and (iv) glycation (non-enzymatic glycosylation) of lysine residues forming Amadori and Heyns rearrangements products (advanced glycated end products: AGE) [5][6]. Carbonylated proteins become yellowish and contribute to dull skin [7].

In addition, mitochondria, the major source of cellular energy, is a source of cellular ROS generation because of the functioning of the mitochondrial respiratory chain. This is also the first intracellular target damaged by ROS. The $O2^{\bullet-}$ can react with nitrogen oxide (NO), producing peroxynitrite ($ONOO^{\bullet-}$) which can inhibit the respiratory chain and damage various mitochondrial components (respiratory chain complex, membrane, DNA, etc.) [8]. In consequence, ATP production decreases.

ATP, a molecule produced at the end of respiratory chain, is involved in modulating keratinocytes growth and in promoting fibroblasts proliferation and contraction for example [9]. This production decrease could contribute to dysfunctional cells and can alter skin regeneration potential.

Red pepper (*Capsicum annuum*) belongs to *Solanaceae* family. Red Pepper, or *Gochu*, is used internationally as a natural flavoring agent and is renowned as the most important agricultural product in Korea. It is a key ingredient in many kinds of traditional Korean foods such as Kimchi, mainly made with cabbage and red pepper powder [10]. The addition of red pepper suppresses putrefactive bacteria growth and *Lactobacillus* (bacteria useful for fermentation) grows [11]. Red pepper has been used in Traditional Korean Medicine (TKM) for centuries for its antimicrobial and skin regeneration properties. Renowned for its health benefits such as diabetes regulation, protection against cardiovascular diseases, natural antioxidant and energizing, it seems of particular interest for protection and stimulation of tired and stressed skin. However, red pepper also has warming properties, supported by capsaicin that activates the TRPV1 receptor on nerve endings and can lead to skin irritation and burning sensations.

The aim of the study was to develop a Korean red pepper capsaicin-free extract (RPE), for a safe use in cosmetic products for dull and tired skin.

Materials and Methods

Extraction without capsaicin

The Red pepper powder was purchased from Dae Han Nong San (Department of Korea-farm, Jeongeup, Korea) and extracted with 98% ethanol for 30 minutes at room temperature. 10 g of dried red pepper powder were needed to make 1 kg of RPE. Solvent was eliminated by filtration using filter paper (110 mm; Advantec Toyo Kaisha, Japan) and evaporated using a vacuum rotary evaporator (CVC3000; VACUUBRAND, Germany). Remaining powder was incubated at 70°C for 30 minutes and extracted with Water, Butylene glycol, 1,2-hexandiol and Ethylhexyglycerin for 3 hours at room temperature. The extract was centrifugated and the precipitate was removed. Final extract was sterilized 15 minutes at 121°C.

Analysis of Capsaicin content with HPLC

Capsaicin standard solutions were dissolved with acetonitrile at different concentrations and filtered using 0.22 µm membrane. Red pepper powder was ultrasonically extracted with acetonitrile for 30

minutes. The extraction solutions were filtered the same way. The HPLC system was an Agilent Technologies 1200 series. The column was a C18 (150 mm × 4.6 mm) with pore size of 5 µm. The mobile phase was water/acetonitrile (40/60 isocratic) with a flow rate of 0.8 ml/min. The column was maintained at 40°C. The injection volume was 20 µl and the wavelength was 280 nm.

Patch test and HRIPT

Patch test: 5% of RPE sample was diluted into water and 40 µl were applied to a filter paper disc. Paper disc was attached in a 8 mm chamber to the inner arms of subjects for 24 hours then removed. Skin irritation wass checked after 0, 1, and 24 hours.

Human repeat insult patch test (HRIPT): Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the induction period. The subjects removed the patches 24 hours after each application. 24 hours of rest periods followed each removal. Prior to each reapplication, sites were graded for dermal irritation and sensitization. 14 days after application of the final induction patch, challenge patches were applied to previously unpatched sites, adjacent to the original induction patch sites.

Dermal scores to record irritation test results were distributed from 0 (no visible skin reaction) to 4+ (erythema and oedema with vesiculation).

Evaluation of antioxidant activity

DPPH assay

500 µl of water (control), sample, or ascorbic acid (positive control) were added to 500 µl of 0.2 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) methanol solution per well. After incubation for 30 minutes at room temperature (protected from light), optical density (OD 570 nm) was measured using a microplate reader (Wallac Victor3 1420 Multilabel counter, Perkin Elmer, Boston, USA).

Cellular ROS assay

HaCaT cells were seeded at a density of 1×10^4 cells/well in 96 well-plates in Dulbecco's Modified Eagle Medium (DMEM), high glucose (4.5 g/L), glutaMAX (Gibco, Thermo Fisher Scientific, Inc, UK) supplemented with 2% Fetal Bovine Serum (Gibco, Thermo Fisher Scientific, Inc, UK) and 1% Penicillin-Streptomycin (Thermo Fisher Scientific, Inc, UK). After 24 hours at 5% CO_2, 37°C, the culture medium was replaced by 100 µl of FBS-free medium containing RPE or quercetin (10 µM) and incubated for an additional 24 hours. Then, cells were washed twice with 200 µl PBS and 100 µl

of 20 µM DCFH-DA solution was added. After 20 minutes at 37°C, protected from light, cells were washed twice with 200µl of PBS and 100 µl of 100 µM H_2O_2 solution was added. After 10 minutes at 37°C, protected from light, the fluorescence was measured (λ_{ex} 485 nm/λ_{em} 535 nm) with a plate reader (Wallac Victor3 1420 Multilabel counter, Perkin Elmer, Boston, USA).

Protein carbonylation assay

Cytotoxicity assay

HaCaT cells were seeded at 30.000 cells/well in a 96-well plate and incubated 16 hours with the compounds diluted in Dulbecco's Modified Eagle Medium (DMEM), high glucose (4.5 g/L), glutaMAX (Gibco, Thermo Fisher Scientific, Inc, UK) supplemented with 2% Fetal Bovine Serum (Gibco, Thermo Fisher Scientific, Inc, UK) and 1% Penicillin-Streptomycin (Thermo Fisher Scientific, Inc, UK). Then, fine dust (PM_{10}-like, Ref. ERM-CZ-100) were added and incubated for 24 hours. No treatment was used for the negative control and a toxic concentration of H_2O_2 (1 mM) was used as positive control. Cell viability was assessed using the CellTilter 96® Aqueous One Solution Cell Proliferation Assay kit (Promega Corporation, Madison, WI), according to the manufacturer's instructions. Absorbance was measured (OD 490 nm) on a spectrofluorometer. The viability was calculated as a percentage of the absorbance value of the control. The toxicity threshold was defined beyond 70% of viability.

Cell treatment for carbonylated proteins evaluation

HaCaT cells were plated in 6-wells plates at 150 000 cells/cm^2. After 24 hours, RPE or references (N-acetylcysteine (NAC) (2.5 mM), Co-Enzyme Q10 (10 µM)), were added. Cells were incubated for 16 hours (n=3). Then cells were treated with PM_{10}-Like (250 µg/ml) with or without compounds. The control experimental group did not receive any treatment. After 24 hours of treatment, the cells were collected and immediately analyzed for total protein carbonyl quantification or fixed and analyzed for the *in-situ* carbonyl visualization.

Quantification of carbonylated proteins

Proteins were extracted and quantified by the Bradford method and split into equal amounts for analyses. Carbonylated proteins were labeled with a resolvable fluorescent aminooxy probe [12] and samples were resolved by high-resolution electrophoresis onto 4-20% gradient SDS-PAGE. Proteins were fixed to the gel and carbonylated proteins were evidenced by fluorescence scanning. Total

proteins were post-stained with SyproRuby™ protein gel stain. Gel images acquisition for carbonylated and total proteins by differential fluorescence was performed using the Ettan® DIGE imager (GE Healthcare). Densitometric analysis of protein bands was performed using Image J analysis software. Carbonylated proteins fluorescence signal was normalized by total protein signal for each sample.

Visualization of carbonylated proteins

Cells were fixed on the plate with a solution containing 95% Ethanol and 5% Acetic Acid. Carbonylated proteins were labeled *in situ* using the above-mentioned fluorescence probe and DAPI for nuclear labeling. Fluorescent images were collected with an epi-fluorescent microscope and treated with ImageJ software. Image comparisons between different conditions were achieved using identical conditions of acquisition (100 ms and 63X).

Evaluation of ATP synthesis

Human dermal fibroblasts (HDF) cells were seeded in 6-well plates and incubated. After 24 hours, 3 ml RPE or Coenzyme Q10 (positive control) diluted with FBS-free medium were added and incubated for 48 hours. For the stress conditions, HDF were seeded the same way. After 24 hours, fine dust (PM_{10}-like, 100 µg/ml) were added together with RPE or Coenzyme Q10. After 48 hours, cell culture supernatants from each well were removed. ATP synthesis in cells was measured following the ATP determination Kit (Molecular Probes™ A22066, Invitrogen) protocol. Luminescence was measured using a luminometer (Synergy™ Mx Monochromator-Based Multi-Mode Microplate Reader, BioTek, Winooski, USA).

Statistical analysis

Data management and statistical analyses were accomplished using GraphPad Software (La Jolla, California, USA). To assess statistical differences between experimental groups, the analysis of variance (ANOVA) with Dunnet's post-hoc test was performed for RPE multiple doses testing. For single-dose treatments (positive controls), unpaired Student t-test was performed.

Clinical evaluation of skin complexion

32 women aged between 18 and 45 years old, of Asian skin type, phototype III or IV according to Fitzpatrick's scale, having uneven skin tone and dull complexion, applied a cream containing 5% RPE daily on the face over a 4-weeks period.

Evaluation of the skin complexion

Skin complexion evaluation is based on a visual sensory analysis of six descriptors by three trained judges, according to a specific scale: Colors (olive/yellow/pink), Luminosity, Brightness and Transparency of the skin on the face (CLBT^{TM} Method [13]). Colors of the skin: the colors "olive", "yellow", and "pink", were fixed to describe the various complexion shades. Physical characteristics (LBT) were Luminosity: intensity of light spots on the salient areas of the face, Brightness: synthesis of the uniformity of the skin color and regularity of the skin texture (= skin homogeneity) and Transparency: characteristic of the skin through which we can see the veins.
In vivo visual evaluation was based on a structured visual scale presenting a range of the 3 main colors of facial skin. For each descriptor the mean of the three judges' evaluation was calculated. For the colors descriptors, the results were given in terms of percentages of color saturation. For the LBT descriptors, the results were given in terms of marks out of 10. Statistical analysis followed Student *t*-test, two-tailed for paired groups at 5%, after checking the normality of the distributions by a Shapiro-Wilk test at 1%.

Results

Development of RPE without capsaicin

HPLC analysis confirmed the retention time of capsaicin at 4.2 min and the content of capsaicin in RPE was of 0.57 ppm, confirming the removal of capsaicin thanks to the extraction process. The detection limit was 0.2 ppm (***Figure 1***).

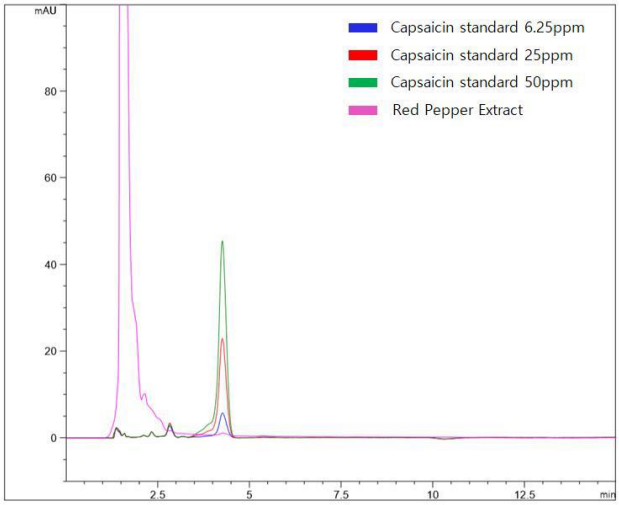

Figure 1: HPLC Chromatogram of capsaicin standard and Red Pepper Extract.

RPE is non-irritating

We first verified the absence of irritation generated by the capsaicin-free RPE. A 5% solution of RPE was tested on human volunteers with an acute skin irritation patch test and a repeat skin irritation patch test (HRIPT). In both tests, RPE did not induce any skin irritation on any of the 10 volunteers, as described into ***Figure 2***.

ACUTE SKIN IRRITATION – PATCH TEST	
Subject number	Scores
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0

HUMAN REPEATED SKIN IRRITATION - HRIPT												
	Induction scores									Challenge scores		
Subject n°	1	2	3	4	5	6	7	8	9	24 H	48 H	72 H
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0

Figure 2: Patch test and HRIPT results with 5% RPE.

As the first extraction step consisting in removing capsaicin could have reduced the known energizing and antioxidant activities of the red pepper, we tested RPE efficacies in several assays.

RPE is antioxidant

RPE significantly increased DPPH radical scavenging activity in a concentration-dependent manner, as shown on ***Figure 3Figure 3***. 1% and 2% of RPE exhibited antioxidant activity at a similar level as the positive control, ascorbic acid.

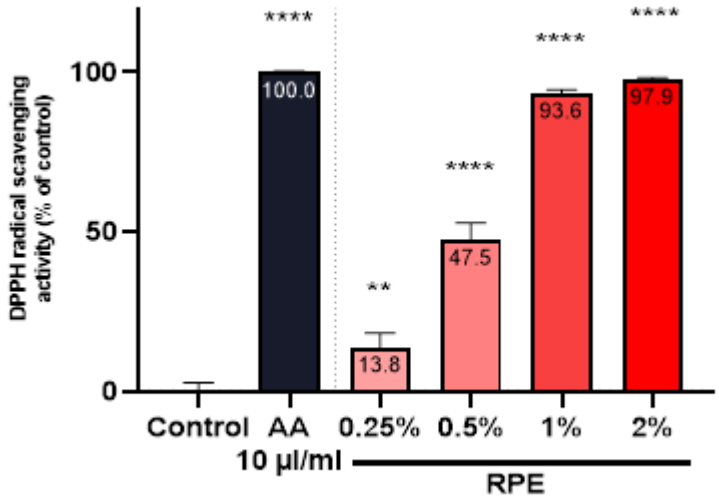

Figure 3: Antioxidant properties of Red Pepper extract. DPPH assay. Plotted values represent the mean of 3 measurements and standard deviation. Values are expressed as a percentage of the control. AA= ascorbic acid, RPE: Red pepper Extract.

We then tested RPE antioxidant properties directly on HaCaT cells. When H_2O_2 was applied onto cells, intracellular ROS were increased by 83%. Positive control Quercetin reduced ROS generation. In the same way, RPE significantly decreased ROS production in a dose-dependent manner and completely prevented ROS generation when used at 0,5% and higher concentrations (***Figure 4***).

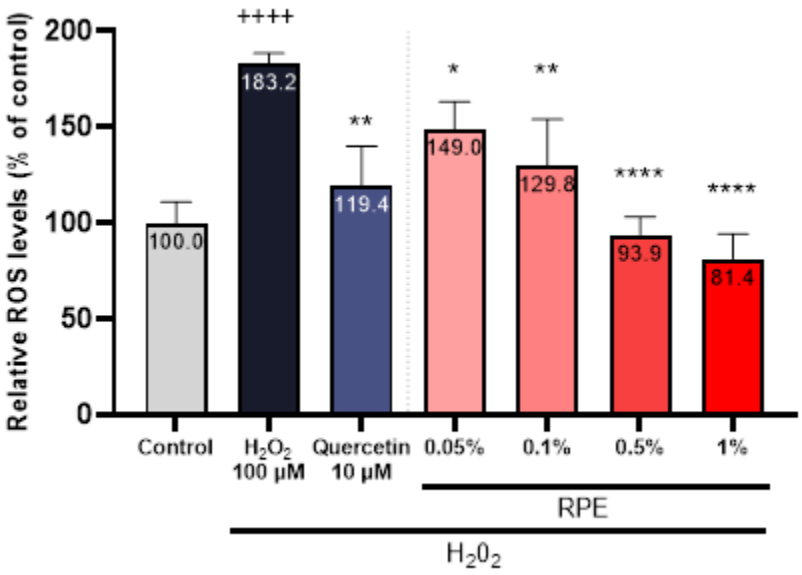

Figure 4: Cellular antioxidant properties. Cellular ROS were measured with the DCFH-DA fluorescent probe assay. Plotted values represent the mean of 4 different culture wells and standard deviation. Values are expressed as a percentage of the control. AA= ascorbic acid, RPE: Red pepper Extract. ++++p<0.0001 unpaired t-test vs control; *p<0.05, **p<0.01, ****p<0.0001 unpaired t-test or one way ANOVA vs H_2O_2 stress.

RPE protects proteins from fine dust-induced carbonylation

We then evaluated the ability of RPE to prevent the oxidative process leading to protein carbonylation in HaCaT cells. When tested alone, RPE did not present any cytotoxic effect up to 5%. In the presence of fine dust (PM_{10}-Like), the maximum non cytotoxic concentration was 2% (data not shown). This concentration was thus chosen as the maximum concentration for the evaluation of protein carbonylation prevention.

When HaCaT cells were treated with RPE, NAC or Coenzyme Q10 before fine dust exposure, RPE prevented protein carbonylation induced by fine dust by 38%, while NAC and Coenzyme Q10 led to 28% and 57% protection respectively (***Figure 5***). The concentration of 0,5% RPE was thus chosen for the carbonylated proteins visualization assay.

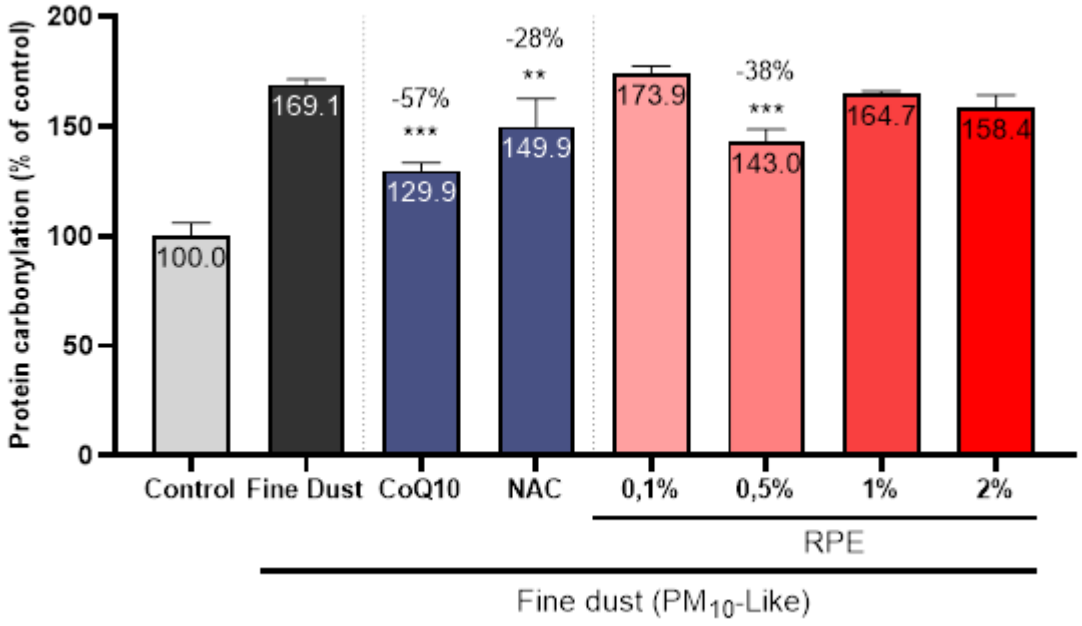

Figure 5: Prevention of protein carbonylation: the levels of carbonylated proteins for each experimental condition are plotted with standard error from the average values (n=3). *p<0.05, **p<0.01, ***p<0.001 ANOVA Statistical analysis with Dunnett's post-hoc multi-comparison analysis versus fine dust condition.

In situ fluorescent labeling of carbonylated proteins on cells confirmed the presence of protein damages under fine dust exposure, as shown by the increase of the oxidized proteins staining (red) (

Figure 6). In these conditions, 0,5% RPE prevented the proteins oxidative damage with a similar effect than those observed with the positive controls NAC and Coenzyme Q10.

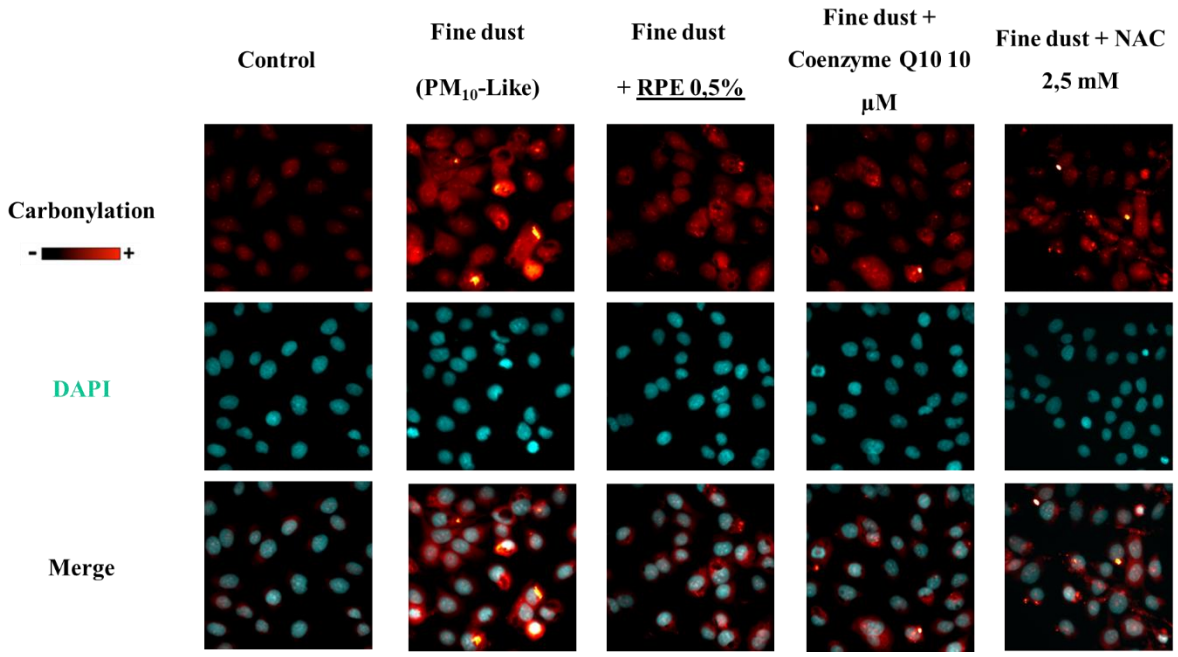

Figure 6: In situ visualization of carbonylated proteins. Images show oxidized proteins (red), nuclei (DAPI signal, cyan) and composite images of independent fluorescent signals (merge). Magnification x63.

RPE increases ATP synthesis in the absence of stress

Red pepper energizing properties is usually attributed to capsaicin. We evaluated the capacity of the capsaicin-free RPE to increase energy production on HDF. ATP production was induced in a dose-dependent manner upon RPE treatment, with a maximum a 5-fold increase with 1% RPE compared to the control, while Coenzyme Q10 induced a two-fold increase at the tested concentration (***Figure 7)***.

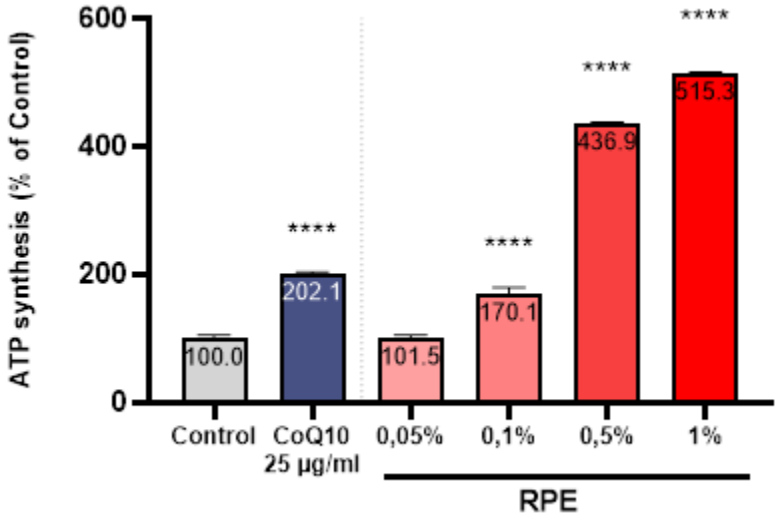

Figure 7: ATP synthesis production. Plotted values represent the mean of 3 different culture wells and standard deviation. Values are expressed as a percentage of the control. ****p<0.0001 unpaired t-test or one way ANOVA vs control.

RPE preserved ATP synthesis under fine dust stress

The mitochondria is the main producer of ATP in cells, and is also the main target of ROS damages which lead to a decrease in ATP synthesis. Given the antioxidant and energy inducing properties of the RPE, we evaluated its ability to prevent ATP decrease under oxidative stress. When fine dust were applied on HDF, ATP production was decreased by 64% compared to the control. In the presence of 1% RPE, ATP production was less decreased by fine dust (-30,2%), resulting in a 53% protection and Coenzyme Q10 by 23% (***Figure 8)***.

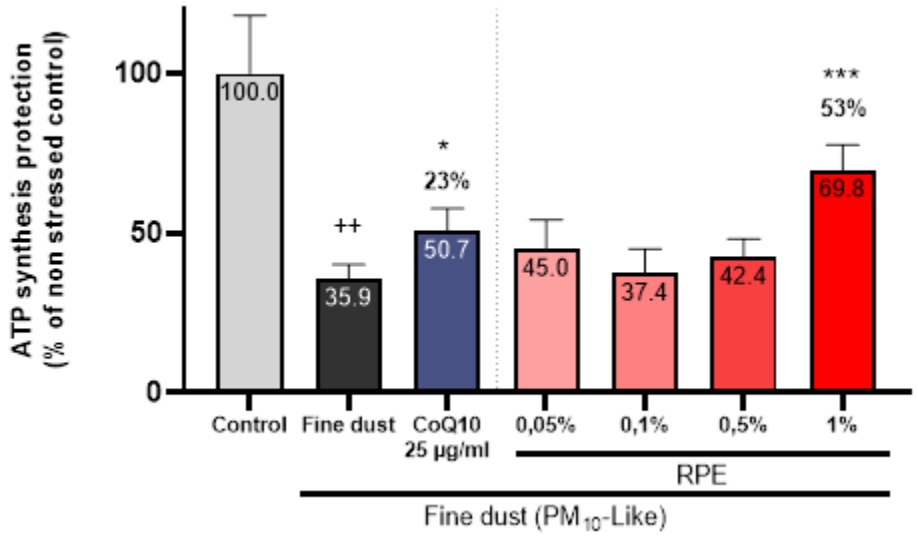

Figure 8: RPE maintains ATP production under pollution. Plotted values represent the mean of 3 different culture wells and standard deviation. Values are expressed as a percentage of the control. ++p<0.01 t-test vs control; *p<0,05 t-test vs PM_{10}; ****p<0.0001 One way ANOVA vs fine dust.

<u>Clinical evaluation of a cream containing Red Pepper Extract</u>

Taken together, antioxidant and energizing properties of RPE, without irritant effect, suggested that RPE could improve skin radiance. In order to demonstrate this hypothesis, we evaluated a cream specifically designed for skin radiance improvement containing RPE at 5%. Results are described into ***Figure 9***.

	Evolution of the studied parameters (in %)					
	LBT (mean %)			Colors (mean % of saturation)		
	Luminosity	Brightness	Transparency	Olive	Yellow	Pink
Between T0 and T+1 week	**+7%** *p<0,001*	**+7%** *p<0,001*	**+7%** *p<0,001*	-2% *(ns)*	+2% *(ns)*	-1% *(ns)*
Proportion of subjects with improvement	77%	81%	77%	42%	35%	32%
Between T0 and T+4 weeks	**+10%** *p<0,001*	**+13%** *p<0,001*	**+13%** *p<0,001*	**-9%** *p<0,001*	**-7%** *p<0,001*	-4% *(ns)*
Proportion of subjects with improvement	81%	94%	90%	71%	65%	35%

Figure 9: Facial complexion radiance evaluation using CLBT method. Percentages of variation of the descriptors of the skin complexion 1 week and 4 weeks after application of the cream containing 5% RPE as well as percentages of subjects having shown an improvement in each graded descriptor. (ns: the difference is not significant).

After 4 weeks of cream application, on one hand, complexion brightness was increased by 13%, luminosity by 10%, and transparency by 13%. On the other hand, yellow shade was decreased by 7% and olive shade by 9%. Interestingly, effect on luminosity, brightness and transparency occurred from 1 week of use, and was visible by more than 80% of subjects after 4 weeks of cream application.

Based on above data, it was possible to conclude that the Red Pepper Cream was able to significantly improve the radiance of the skin complexion after 4 weeks of application.

Discussion

The newly developed RPE demonstrated antioxidant properties and decreased ROS production into cells under pollution stress. In addition, RPE protected proteins from carbonylation induced by pollution, represented here by fine dust (PM_{10}-like). Pollution can induce ROS generation which create oxidative stress and increase pro-inflammatory cytokines and nitric oxide (NO) release [14]. When exposed to oxidative stress, proteins can be altered by an irreversible carbonylation mechanism. In the *stratum corneum*, carbonylation leads to the alteration of its physicochemical properties, such as optical transparency or extensibility of the skin. Moreover, carbonylated proteins accumulate into photoaged skin. Yuki and al. demonstrated that carbonylated proteins accumulation is involved in the yellowish color change in photoaged skin [7]. It was already admitted that dull skin appearance is often linked to the yellow components of skin tone [15]. RPE has antioxidant properties and could

prevent protein carbonylation. Therefore, this could limit yellowish color change of the skin induced by ROS, which are produced under pollution for example.

Skin radiance is a complex parameter which includes the quantity of light reflected by the skin, but not only. It seems to be a balance between color, influenced by microcirculation and inner skin structures, light reflection and skin texture [16]. Baret and al. organized a brainstorming with volunteers to select some items which could be used to assess clinically skin radiance. It seems that for younger people, skin radiance is more dependent on skin luminosity and color, and for older one, it was characterized by irregular surface skin aspects and color variations. These observations confirmed that skin radiance is multifactorial and can be influenced by many parameters [17].

In addition, it was demonstrated that when the skin surface is irregular, light is not well absorbed and reflects in many directions. Radiant skin is usually considered as smooth and homogeneous [7]. The skin is an organ with a continuous regenerating epidermis. The epidermal progenitors are highly proliferative and active and need ATP for their energy requirements. Oxidative stress can also modify mitochondrial function, resulting in ATP depletion and modification of the respiratory chain. Therapeutic targeting of mitochondria in the skin involves either boosting ATP production or scavenging the excess of free radicals [14][15]. We proved that RPE can increase ATP production in HDF in absence of any stress. Moreover, RPE protected ATP synthesis when cells were exposed to fine dust, inducing an ATP production decrease. Interestingly, RPE showed better results that CoQ10 both in the absence or in the presence of pollution stress. It was already demonstrated that CoQ10 could restore ATP production and alleviate oxidative stress in aged skin cells [16]. Thanks to its capacity to increase and protect ATP synthesis, it is conceivable that RPE could act on cell renewal and so improve skin surface, for a smoother skin. This parameter is the second major one involved into skin radiance definition, with skin color. Smooth skin can better reflect the light, this leads to brighter and more transparent skin, as looked for a radiant skin.

We hypothesized that these two components of skin radiance: color and skin surface, could be improved by RPE. We wanted to confirm visible results by testing 5% of RPE into a finish product. Tested cream was a mix of several active ingredients chosen for their action of skin radiance and dull skin correction, including RPE, as a major of them.

According to CLBT evaluation, the cream increased brightness and transparency, while olive and yellow shades decrease. This cream improves skin radiance and color complexion, and RPE could be an important player of these results.

RPE is the first cosmetic active ingredient from red pepper on the market that was especially developed to avoid skin irritation thanks to capsaicin removal. Indeed, capsaicin is known to induce ATP production by TRPV1 pathway [18]. Many properties are generally attributed to capsaicin, but we demonstrated here that the efficacy of RPE could come from a *totum* of actives molecules contained into the plant.

Conclusion

We developed a non-irritating Korean red pepper extract showing antioxidant and energizing properties through ATP synthesis. These properties confer to RPE specific efficacy on dull skin, to enhance skin radiance and preserve it even under pollution stress.

Acknowledgments. The authors warmly thank Hill Kim (Radiant) and Iljee Cho (Radiant) for their major contribution into RPE development.

Conflict of Interest Statement. NONE.

References

1. World Health Organization (2014) Burden of disease from the joint effects of household and ambient air pollution for 2012. Public Health, Social and Environmental Determinants of Health Department, WHO.
2. Kim BE, Kim J, Goleva E, et al (2021) Particulate matter causes skin barrier dysfunction. JCI Insight 6(5).
3. Bianchini JM, Zhang Q, Hanna G, et al (2019) A unique gel matrix moisturizer delivers deep hydration resulting in significant clinical improvement in radiance and texture. Clin Cosmet Investig Dermatol. 12:229-239.
4. Jin SP, Li Z, Choi EK, et al. (2018) Urban particulate matter in air pollution penetrates into the barrier-disrupted skin and produces ROS-dependent cutaneous inflammatory response in vivo. 91: 175-183.
5. Thornalley, P.J. (2008) Dicarbonyls linked to damage in the powerhouse: glycation of mitochondrial proteins and oxidative stress. Biochemical Society Transactions. 5(36):1045-1050.
6. Uchida K (2003) 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Progress in Lipid Research. 42(4): 318-343.

7. Ogura Y, Kuwahara T, Akiyama M, et al. (2011) Dermal carbonyl modification is related to the yellowish color change of photo-aged Japanese facial skin. J Dermatol Sci. 64: 45-52.
8. Shiva, S., et al. (2004) Mechanisms of the interaction of nitroxyl with mitochondria. Biochem J. 379(2): 359-366.
9. Holzer, Aton M. and Granstein, Richard D (2004) Role of Extracellular Adenosine Triphosphate in Human Skin. J Cutan Med Surg. 8(2): 90–96.
10. Kim S, Park J, and Hwang IK (2004) Composition of Main Carotenoids in Korean Red Pepper (*Capsicum annuum, L.*) and Changes of Pigment Stability During the Drying and Storage Process. J of Food Sci. 69(1).
11. Kwon, DY, et al. (2014) History of Korean gochu, gochujang, and kimchi. Journal of Ethnic Foods. 1(1): 3-7.
12. Baraibar MA. (2013) Proteomic quantification and identification of carbonylated proteins upon oxidative stress and during cellular aging. J Proteomics. 63-70.
13. Musnier C. and al. (2004) Visual evaluation in vivo of complexion radiance using the CLBT™ sensory methodology. Skin Res Technol. 10:50-56.
14. Tiwari BS, Belenghi B, Levine A. (2002) Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. Plant Physiol. 128(4):1271.
15. Sreedhar A, Aguilera-Aguirre L, Singh KK. (2020) Mitochondria in skin health, aging, and disease. Cell Death Dis. 11(6):444.
16. Jeudy A., Ecarnot V., Humbert P. (2015) Measurement of Skin Radiance. Agache's Measuring the Skin. Springer, Cham.
17. Baret M, Bensimon N, Coronel S, et al. (2006) Characterization and quantification of the skin radiance through new digital image analysis. Skin Res Technol. 12(4):254-260.
18. Vahidi Ferdowsi P, Ahuja KDK, Beckett JM, Myers S. (2021) TRPV1 Activation by Capsaicin Mediates Glucose Oxidation and ATP Production Independent of Insulin Signalling in Mouse Skeletal Muscle Cells. Cells. 10(6):1560.