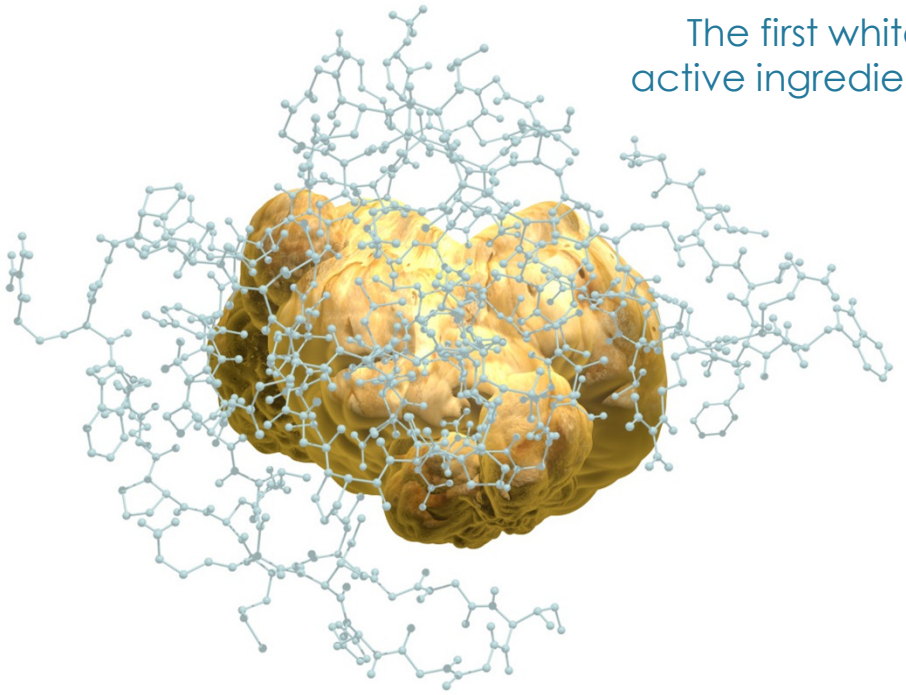
W Tr-Active

The first white truffle derived active ingredient for cosmetics



"Long time ago a thunderbolt hurled by Jupiter created a precious diamond that man has always sought and desired:

the white truffle

so rare, so precious, so rich and powerful, a powerful essence released by the power of enzymes to invigorate the skin and enhance beauty"

gene expression modulation, multiple anti-aging effects

***In vitro*, gene modulation:**

- Extracellular matrix proteins
- Aquaporins
- Matrix Metalloproteinases
- Vitagenes
- Damaged protein degradation process

***In vivo*, anti-aging effects**

- Skin Elasticity
- Filler Effect
- Anti-Wrinkles
- Anti-Eye Bags
- Moisturization

A precious diamond between myth and history

Truffles have been known since the mist of time but the first historical document mentioning it was "*Naturalis Historia*" by Pliny the Elder (79 A.D.) showing that truffles were greatly appreciated by the Romans, who learned about their culinary use from the Etruscans. Juvenal explained the origin of this precious fungus as the result of lightning thrown by Jupiter near an oak, a tree sacred to the Father of all gods. Due to Jupiter's well-known power of seduction, aphrodisiac properties have always been ascribed to truffles.

During the Renaissance truffles were used at the court of the Kings. In 1700 the Piedmont's truffle was considered a delicacy by the European nobility. The composer Gioacchino Rossini was among the admirers of this "fruit of the earth" and referred to it as the "Mozart of all mushrooms". Some scientists of that time described the truffle aroma as a sort of quintessence producing ecstatic effects on human beings: the sublime synthesis of the satisfaction of all senses as the representation of a superior pleasure.

White truffle: the rarest hypogeum fungus

A white truffle is the fruiting body of a subterranean Ascomycete fungus of the genus *Tuber Magnatum*. White truffle is the rarest variety of truffles as it just grows in some extremely limited areas in northern and central Italy, and the area of Alba in Piedmont is worldwide known for the high quality of its white truffle production. White truffle must live in symbiosis with certain trees and shrubs in order to produce the precious fruit. The two organisms, the truffle and the tree, mutually exchange substances through the roots and by means of mycorrhizae. Mycorrhizae are the product of the association between the tubular filaments called hyphae and the root tips of a tree. Thanks to this association, the tree gives nutrients to the fungi, and receives mostly water and minerals in returns.

A mass of branching hyphae is called mycelium. When all the necessary environmental conditions occur, between October and December , a number of hyphae will intertwine and form the fruiting body containing the spores, that's the truffle. Unlike the epigeous fungi which spreads spores form fruiting bodies above ground, the hypogeum fungi cannot disperse the spores via wind. Evolution has therefore equipped them with a strong smell, produced only when they reach maturation. This smell is the secret of truffle reproduction as it is astonishingly able to attracts mammals that feed on truffles and as a result provide for the dispersion of the spores.

That's why truffle hunters work together with their dogs that, after being trained, are able to find truffles by detecting the smell through their keen noses.

MADE IN ITALY

phenbiox

www.phenbiox.it / info@phenbiox.it

W Tr-Active: gene expression modulation, multiple anti-aging effects

The chemistry of white truffle

Truffles strictly depend on other organisms to complete their life cycle as they need to establish symbiotic ectomycorrhizas with plant roots to produce fruiting bodies, and they are unable to spread their spores unless they are found and eaten by mammals. Therefore truffles use volatile signals throughout their life cycle to regulate their interactions with other organisms. Despite this fascinating natural function, the role of truffle volatiles molecules has been mainly investigated just for the commercial value of truffle aroma.

Both kind of interaction, with plant roots and with animals, despite are well substantiated and known, has not been studied in details due to the difficulties in working on these topics with fungi that cannot be easily grown in laboratory conditions. It is known that some of these volatiles affect the root architecture of plants under laboratory conditions, resulting in primary root shortening and root hair elongation. In nature, truffles attract mammals ranging from wild pigs to squirrels, which consume the fruiting bodies and contribute to spore dispersal.

All these interactions are mediated by organic compounds and to date more than 200 different molecules have been identified from truffle species. They are hydrocarbons with a high vapor pressure that might include alcohol, aldehyde, ketone functional groups and often contain sulfur atoms. Some of these molecules are synthetized through the catabolism of the L-methionine, other from the catabolism of the nonsulphur amminoacids, some are derived by fatty acids and finally some are terpenoids belonging to the class of the isoprenoids. Some compounds are common to many species while other are more characteristics of a specific truffle. Bis(methylthio)methane for example is the major contributor to the aroma of the white truffle, and it is not produced in black truffle. Other characteristic molecules of white truffle include 28 sulfur compounds as well as numerous isoprenoids, responsible of the characteristic aroma of this amazing fruit of the earth .

As mentioned some of these compounds are able to attract mammalian due to their similarities to some pheromones. An interesting experiment regarding the ability of the truffle aroma to play a role in man's behavior and preferences was performed through an assessment where pictures of normally clad women were shown to male and female subjects, some of whom were also exposed to a truffle aroma component. The subjects were asked to give a mark to the photographed women for sexual attractiveness. Those who had sniffed the substance gave higher marks than did the others. Such an effect, it is proposed, can explain why the white truffle aroma is so valued.

Gene expression modulation *via* microarrays analysis

The compounds that are constituting the "interactive" phytocomplex of the white truffle are hundreds, difficult to isolate, also because the white truffle cannot be grown in laboratory or artificial conditions, and therefore very difficult to be studied in terms of the interaction that can establish with plants and animals. These practical difficulties in performing laboratory researches explains why the literature regarding the potential biological effects of such an interesting phytocomplex is so poor giving basically no back ground to understand the biochemical bases that must exist due to the indirect evidences related to the fact that animals as well as man are so desperately seeking this truffles.

Using the exclusive bioliquefaction technology we have developed W Tr-Active, a new product that contains the 100 % of the amazing phytocomplex of the Italian white truffle.

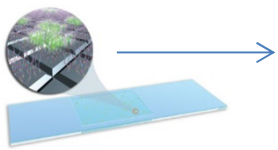
To investigate the effect that the white truffle phytocomplex can deliver to the skin a microarray analysis of treated and untreated cell lines was performed in order to evaluate the gene expression modulation induced by W Tr-Active. Microarray analysis techniques are used in interpreting the data generated from experiments on DNA, RNA, and protein microarrays, which allow researchers to investigate the expression state of a large number of genes, in many cases an organism's entire genome.

Our experiments were performed on microarrays containing probes for the complete human genome and were therefore able to assess the modulation of over 42.500 RNA targets. Cell line were treated according to the selected protocol before extracting the RNA, in order to collect through the microarray analysis a complete picture of the modification induced in the gene transcription by our product at the selected time.

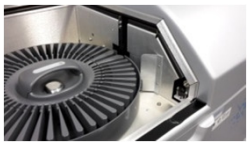
To have a more complete picture of the potential gene modulation that W Tr-Active is able to induce on the skin we have performed microarray analysis on two different cell lines: a keratinocytes model cell line (HaCaT) and a primary human dermal fibroblasts isolated from adult skin. The two cells lines were cultured according to the standard protocols than the samples were treated with W Tr-Active at 1% v/v and incubated for 6 hours before applying the RNA extraction protocol to treated and control cell samples.

The RNA samples is pre-screened to verify the quality of the samples than undergoes to microarray hybridizations on a chip containing the probes for the entire human genome. The chip is than scanned with a microarray scanner (Agilent Sure Scan High Resolution) and the acquired data were analyzed by specific software to evaluate the gene expression modulation.

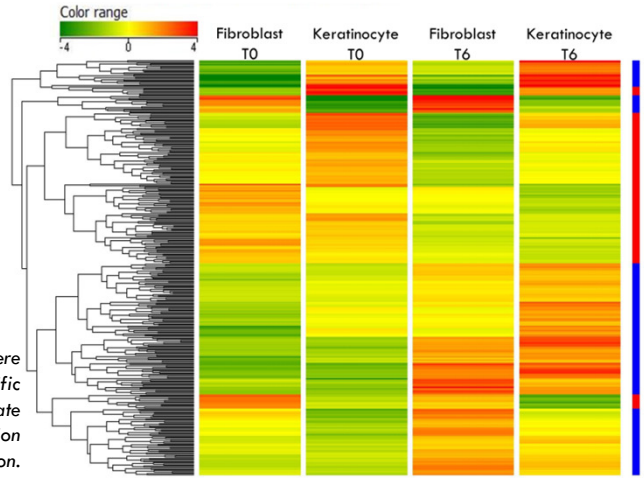
To evaluate the cosmetic potential applications of W Tr-Active we have selected some gene clusters of specific relevance that have been studied in details evaluating their under or over expressions.



Samples of cells models treated with W Tr-Actice undergoes to microarray hybridizations on a chip containing the probes for the entire human genome.



The chip is than scanned with a microarray scanner.



All samples scan were analyzed by specific software to evaluate the gene expression modulation.

W Tr-Active: gene expression modulation, multiple anti-aging effects

gene expression modulation

Up-regulated genes | Down-regulated genes

Vitagenes
Keratinocytes Up-regulated genes
Sirtuins:
SIRT5 sirtuin 5

Heat Shock Proteins:
HSPA4L heat shock 70kDa protein

Telomerases:
TERF2 telomeric repeat binding factor 2
TERT telomerase reverse transcriptase

Fibroblasts Up-regulated genes

Heat Shock Proteins:
HSPA13 heat shock protein 70kDa family, member 13
HSPA6 heat shock 70kDa protein 6
HSPB11 heat shock protein family B (small), member 11
HSPD1 heat shock 60kDa protein 1 (chaperonin)

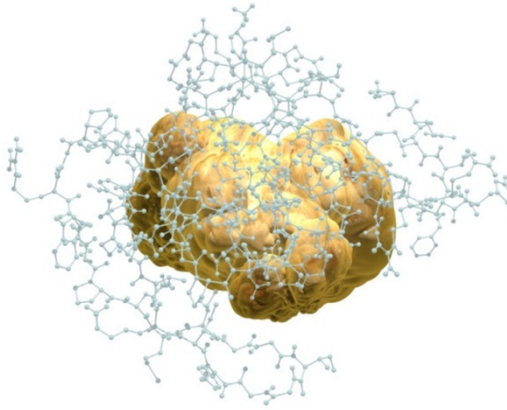
Telomerases:
TEP1 telomerase-associated protein 1

Vitagenes
Modulation of endogenous cellular defense mechanisms *via* the stress response signaling a new approach in preventing aging. Maintenance of optimal long-term health conditions is accomplished by a complex network of longevity assurance processes that are controlled by vitagenes, a group of genes involved in preserving cellular homeostasis during stressful conditions.

Sirtuins: regulate important biological pathways and have been implicated in influencing a wide range of cellular processes like aging, transcription, apoptosis, inflammation and stress resistance, as well as energy efficiency and alertness during low-calorie situations.

Heat Shock Proteins: proteins that are produced by cells in response to exposure to stressful conditions. Many members of this group perform chaperone function by stabilizing new proteins to ensure correct folding or by helping to refold proteins that were damaged by the cell stress.

Telomerases: maintain telomere ends a region at each end of a chromatid, which protects the chromosome from deterioration. Due to each cell division the telomere ends become shorter, cells become senescent and cell division stops. The enzyme adds telomere units to the chromosome to prolong cell life.



Damaged protein degradation process
Keratinocytes Up-regulated genes:

Ubiquitins:
UBA6 ubiquitin-like modifier activating enzyme 6
UBD ubiquitin D
UBE2D3 ubiquitin-conjugating enzyme E2D 3
UBE2E2 ubiquitin-conjugating enzyme E2E 2
UBE2N ubiquitin-conjugating enzyme E2N
UBE2Q2L ubiquitin-conjugating enzyme E2Q family member 2-like
UBE2R2 ubiquitin-conjugating enzyme E2R 2
UBE3A ubiquitin protein ligase E3A
UBIAD1 UbiA prenyltransferase
UBL4A ubiquitin-like 4A
UBQLN1 ubiquilin 1
UBR3 ubiquitin protein ligase E3 component n-recognin 3
UBR4 ubiquitin protein ligase E3 component n-recognin 4

Proteasome
PSMC3IP PSMC3 interacting protein (PSMC3IP)
PSMD5 proteasome (prosome, macropain) 26S subunit, non-ATPase, 5
PSME4 proteasome (prosome, macropain) activator subunit 4

Fibroblast Up-regulated genes

Ubiquitins:
UBA7 ubiquitin-like modifier activating enzyme 7]
UBD ubiquitin D
UBE2DNL ubiquitin-conjugating enzyme E2D N-terminal like
UBE2L6 ubiquitin-conjugating enzyme E2L 6
UBE2Q2L ubiquitin-conjugating enzyme E2Q family member 2-like
UBR4 ubiquitin protein ligase E3 component n-recognin 4

Proteasome:
PSMB10 proteasome (prosome, macropain) subunit, beta type, 10
PSMB2 mRNA for proteasome beta 2 subunit variant protein
PSMB8 proteasome (prosome, macropain) subunit, beta type, 8
PSMB9 proteasome (prosome, macropain) subunit, beta type, 9
PSMD5 proteasome (prosome, macropain) 26S subunit, non-ATPase, 5

Damaged protein degradation process
If any kind of damage occur to proteins folding or structure making it inactive, in order to avoid an accumulation of useless/potentially dangerous protein matter, specific degradation processes that recognize and hydrolyze these materials are present in cells.

Ubiquitins: can mark proteins in many ways and one of the main tasks is recognizing the damaged proteins addressing them for their degradation *via* the proteasome.

Proteasome: main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds.

Extracellular matrix proteins

Keratinocytes Up-regulated genes

Ceramide synthases :
CERS1 ceramide synthase 1

Fibroblasts Up-regulated genes

Collagens :
COL19A1 collagen, type XIX, alpha 1
COL20A1 collagen, type XX, alpha 1
COL23A1 collagen, type XXIII, alpha 1
COL26A1 collagen, type XXVI, alpha 1

Fibronectins :
FNDC3A fibronectin type III

Laminins :
LAMA3 laminin, alpha 3
LAMB3 laminin, beta 3

Integrins :
ITGB8 integrin, beta 8

Hyaluronan synthase :
HAS1 hyaluronan synthase 1
HAS2 hyaluronan synthase 2
HAS3 hyaluronan synthase 3

Extracellular matrix proteins
A collection of extracellular molecules secreted by cells that provides structural and biochemical support to the surrounding cells.

Collagens: main structural proteins of the various connective tissues in animals.

Fibronectins: glycoproteins of the extracellular matrix that bind to integrins and to other extracellular matrix components such as collagen, fibrin etc.

Laminins: high-molecular weight proteins, a major component of the basal lamina (one of the layers of the basement membrane), a protein network foundation for most cells and organs.

Integrins: transmembrane receptors that are the bridges for cell-cell and cell-extracellular matrix (ECM) interactions.

Hyaluronan synthase: are membrane-bound enzymes which produce the glycosaminoglycan hyaluronan at the cell surface and extrude it through the membrane into the extracellular space.

Ceramide synthases: are integral membrane proteins of the endoplasmic reticulum that catalyzes the synthesis of ceramide.

Matrix Metalloproteinases
Keratinocytes Down-regulated genes:
MMP12 matrix metallopeptidase 12
MMP13 matrix metallopeptidase 13
MMP24 matrix metallopeptidase 24 (activates MMP2 by cleavage)

Fibroblasts Down-regulated genes:
MMP11 matrix metallopeptidase 11 (stromelysin 3)
MMP16 matrix metallopeptidase 16 (activates MMP2 by cleavage)
MMP24 matrix metallopeptidase 24 (activates MMP2 by cleavage)

Matrix Metalloproteinases
Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases.
Collectively, these enzymes are capable of degrading all kinds of extracellular matrix proteins, but also can process a number of bioactive molecules.

Aquaporins
Keratinocytes Up-regulated genes:
AQP3 aquaporin 3
AQP6 aquaporin 6
AQP7 aquaporin 7

Fibroblasts Up-regulated genes:
AQP9 aquaporin 9

Aquaporins
Aquaporins are integral membrane proteins from a larger family of major intrinsic proteins (MIP) that form pores in the membrane of biological cells. These proteins are membrane water channels that play critical roles in controlling the water contents of cells.

W Tr-Active: gene expression modulation, multiple anti-aging effects

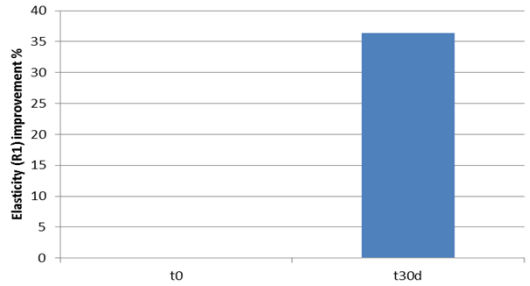
MULTIPLE ANTI-AGING EVALUATION *IN VIVO* ASSESSMENT

To evaluate the performances of W Tr-Active as multi target anti-aging active ingredient for cosmetic applications some instrumental and clinical trials has been performed. 20 female patients, aged between 25 and 59 years old (45 ± 11,2), were selected and told to apply a simple gel formulation containing 1% w/w W Tr-Active twice per day for 30 days.

Skin Elasticity

The effect of W Tr-Active to increase the skin elasticity was assessed performing some specific measure with a cutometer.

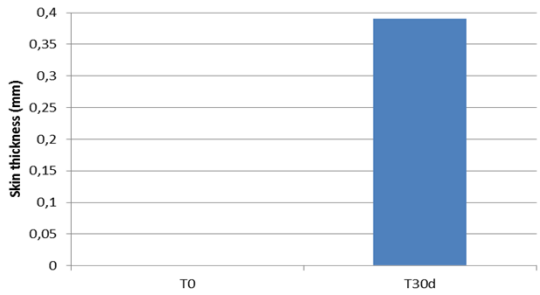
After 30 dys of treatment the product was able to act the R1 parameter (i.e. the ability of the skin to return to its original state) measured by the cutometer. The product is therefore able to increase the elastic properties of the skin (+35 % vs.T0) improving its ability to promptly go back to the original state after a deformation.



Skin thickness (filler effect)

W Tr-Active was tested through plicometric measurements in order to assess its ability to increase the skin thickness (filler effect).

After 30 days of treatment the product was able to increase thickness of the skin of 0.39 mm. This result confirms that W Tr-Active is able to deliver on skin an interesting filler effect.



Anti-wrinkles (Visiometer measurement)

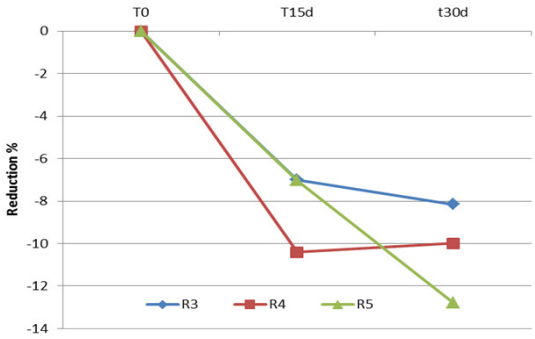
An instrumental assessment on the ability of W Tr-Active in reducing wrinkles was performed using a skin visiometer. The parameters assessed during the instrumental evaluation were:

R3: mean depth of roughness

R4: smoothness depth

R5: arithmetic average roughness

The product W Tr-Active was able to perform an anti-winkle effect that was already measurable after 2 weeks of treatment with a reduction of the assessed parameters of – 7 / 7 / 10,4 % respectively. In 30 days of treatment the reduction of wrinkles was further improved to a – 8,2/ 10 /12,8% respectively.

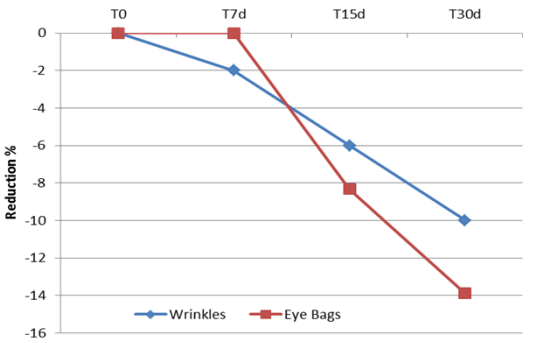


Anti-wrinkles anti-eye bags (Clinical score)

The wrinkle severity and the degree of the eye bags were evaluated with a clinical examination performed by skilled dermatologists on a standard visual scale.

The wrinkle reduction assessed during the clinical examination was confirming the results obtained with the instrumental measurements performed with the skin visiometer. A slight anti-wrinkle effect was already noted after a single week of application of W Tr-Active and after two weeks the reduction was -6%. At the end of the treatment at 30 days the overall wrinkle reduction was - 10 %, confirming the results obtained during the instrumental assessment.

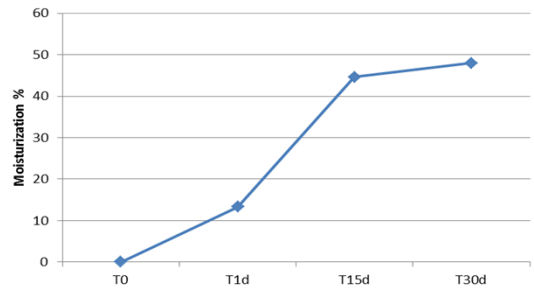
W Tr-Active was starting acting against eye bags after 2 weeks of treatment with an interesting reduction of eye bags degree of - 8 %. In four weeks of treatment the product was able to reduce the eye bags of - 14%.



Moisturization

The effect on skin moisturization that can be delivered by the daily application of W Tr-Active was instrumentally assessed using a corneometer.

W Tr-Active was able to increase the skin moisturization of over 13 % in just 24 hours. The strong moisturizing effect delivered by the active was confirmed by the measurements performed after 2 and 4 weeks of treatment where the moisturization was increased of 45 % and 48% respectively.



<u>The results obtained with the instrumental and clinical dermatology examination assessemnts were confirming the ability of W Tr-Active to act as a multi-functional anti-aging active ingredient.</u>

W Tr-Active technical specifications:

INCI name: TUBER MAGNATUM EXTRACT

Composition: Glycerin, Tuber Magnatum Extract, sodium benzoate, potassium sorbate.

Suggested concentration of use: 1% w/w