RESVERATROL 2010 1st international conference of resveratrol and health

# GRAPE VINE RESVERATROL

## Health and Beauty Benefits

## VINEATROL® the only extract which contains the Red Wine Resveratrol Derivatives

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## Wine and Health

The ***French Paradox*** refers to the statement that people in France suffer at relatively low incidence of coronary heart diseases despite their diet being rich in saturated fats. Several epidemiological studies have shown the correlation between this paradox and a regular red wine consumption.
The phenolic components of red wine, in particular the Resveratrol and the Resveratrol Derivatives, have been identified as the major agents in helping to maintain a good cardiovascular health and to reduce the risk of neuro-degenerative diseases.

**RED WINE RESVERATROL DERIVATIVES**

Resveratrol is present in red wine in different forms: *trans*- and *cis*-forms, glycoside forms, oligomers and glucoside oligomers. The following resveratrol derivatives have been identified in red wine [1,2,3,4 ]: piceatannol, epsilon-viniferin, delta-viniferin, pallidol, vitisin A and hopeaphenol.

**RESVERATROL DERIVATIVES BIOLOGICAL ACTIVITIES**

The biological activities of the resveratrol derivatives are less studied than those of trans-resveratrol because the standards are not available; a few studies prove that these derivatives have biological activities similar to that of trans-resveratrol..
Here is a not complete list of the biological activities of the resveratrol derivatives:

**Epsilon viniferin :** Antioxidant [5], Anti-inflammatory [6], Anti-tumor effects [7] , Neuroprotective [8] , Sirt1 activator, 5-alpha reductase inhibitor [9] ,

**Piceatannol :** Antioxidant [10] , Anticancer agent [11], Anti-inflammatory [12], Vasorelaxant [13] , Neuroprotective [14], Sirt-1 activator [15],

**Hopeaphenol:** Antitumour [16], Anti- inflammatory [17],

**Ampelopsin A:** Antitumour [18], HIV-1 infection inhibitor [19],

**R-Viniferin ( Vitisin B ) & R2-Viniferin ( Vitisin A ) :** Antioxidant and Cardio-Protection [20], Neuroprotective [21].

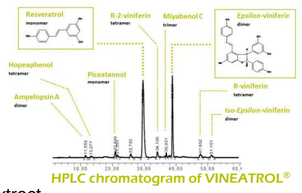
**ORIGIN OF RESVERATROL DERIVATIVES IN RED WINE**

Resveratrol derivatives are constitutive stilbenes of the woody parts of the plant. They are present in the grape skin as induced substances. The resveratrol derivatives concentration in the grape skin is 1000 times less than those in the woody parts.
The origin of resveratrol derivatives in wine is most likely due to the extraction from grape skin occurring during alcoholic fermentation. The grape stems are also a potential source of resveratrol derivatives in red wine [22]. The amount of stems in the fermentation process depends on the way of wine-making practises: for example manual or mechanical harvest, destemming or not.
An old wine-making practise which is still widely used even in stainless steel tanks consists in putting a bundle of vine shoot at the bottom of the tank. It is used as a filter before the bottom valve. The presence of vine shoots which contain a large amount of resveratrol derivatives can also contribute to resveratrol derivatives in red wine [23]

## Grape Vine Resveratrol

Actichem produces Grape Vine Resveratrol since 10 years. The process and the product are protected by a patent [24]. So, Actichem is the only French Grape Vine Resveratrol manufacturer.
Grape Vine Resveratrol is a vine shoot extract, because vine shoot is the richest part of the plant in terms of resveratrol derivatives content. Grape Vine Resveratrol is made up of trans- resveratrol, epsilon-viniferin, and other resveratrol oligomers found in red wine.
The vine shoots are selected in the famous wine region of Bordeaux in the South West of France. The vine shoots are collected from January to March. The bundles are dried before storage. The dried vine shoots are crushed and then extracted and purified using ethanol and water.

Nine resveratrol monomers and oligomers have been identified in the Grape Vine Resveratrol:
- 2 monomers: resveratrol and piceatannol
- 3 dimers: Ampelosin A , Epsilon-Viniferin, Iso Epsilon-Vinifern
- 1 trimer: Myiabenol C which we have isolated for the first time in the Vitis Vinifera Species,
- 3 tetramers: r-Viniferin ( Vitisin B ), r2-Viniferin ( Vitisin A ).



HPLC chromatogram of VINEATROL®

These molecules represent around 40 % of the total weight of the extract.
All these molecules have been isolated, characterised by mass spectrometry and NMR analysis.
They are now currently quantified in the extract by HPLC analysis.
A research program ( ORFFI project ) is being carried out in order to identify other resveratrol oligomers in the extract.

The characteristics of the commercial product for nutraceuticals VINEATROL® are:
**t-resveratrol** > 5% (more on request)
**epsilon-viniferin** > 5%
**total resveratrol momomers and oligomers** > 20% or 30 %

VINEATROL® WD is a water dispersible form of VINEATROL® for beverages.

### Some wrong statements about Resveratrol from grape vine

**It is possible to produce resveratrol from red wine:** WRONG
To produce 1 kg of an extract containing 5% resveratrol, you need 10 000 litre of red wine.
**It is possible to produce resveratrol from grape**: WRONG
The resveratrol content of grape extracts is between 100 to 2500 ppm, 20 times less than VINEATROL®
**It is possible to produce a grape extract containing only trans-resveratrol:** WRONG
Epsilon-viniferin is the main metabolite of resveratrol produced by *Vitis Vinifera*. All the extracts of the plant *Vitis Vinifera* which contain resveratrol, also contain epsilon-viniferin: for example red wine, grape extracts, Grape Vine Resveratrol. The separation of Resveratrol and epsilon-viniferin is very hard and is only possible by preparative HPLC. In a *Vitis Vinifera* extract, the epsilon-viniferin content must be minimum higher than 0.2 time the resveratrol content. ***If not, the extract obviously contains additional t-resveratrol from another source than Vitis Vinifera***

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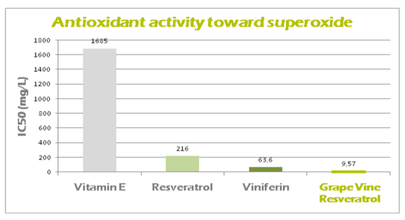
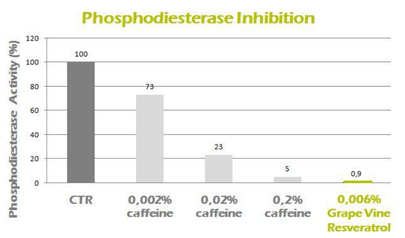
## Biological Activities of Grape Vine Resveratrol




### *Antioxidant Activity*

The Antioxidant Activity is quantified by Electron Paramagnetic Resonance.
**In this test, VINEATROL® is more efficient than trans-resveratrol and epsilon-viniferin and 170 times more active than Vitamin E.**

The Antioxidant activity of VINEATROL® has also been quantified by ORAC Europe BV. The Total ORAC Value of VINEATROL® is 15000 µmol TE/g ( Data from Breko GmbH).

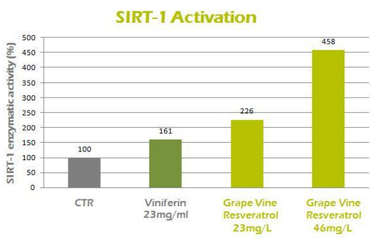



### *Anti-inflammatory activity*

Epsilon-viniferin possesses anti-inflammatory properties by inhibiting PDE subtypes: 1 to 6, with a significant selectivity for the PDE4 subtype [6].
In this study we compare the inhibitory effect on the cyclic 3':5' AMP phosphodiesterase activity of the Grape Vine Resveratrol to that of Caffeine

**In this test, VINEATROL® proved to be 30 times more active than Caffeine in inhibiting PDE activity.**

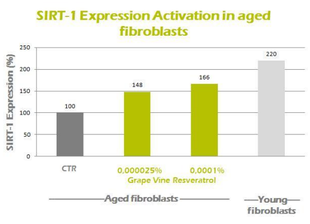
### *SIRT-1 Activation*




### SIRT-1 Activation

SIRT-1 activity in the presence of Grape Vine Resveratrol is measured with a Biochemical model composed of the SIRT-1 enzyme and a fluorescent substrate.

**The SIRT-1 enzymatic activity is increased by 358 % using 46 mg/l VINEATROL® solution**

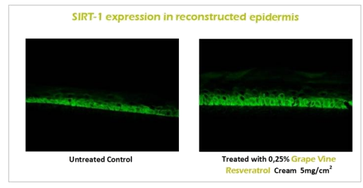



**Effect of Grape Vine Resveratrol on the expression of SIRT-1 in aged Fibroblasts**

The SIRT-1 expression in fibroblasts is measured by quantitative real-time polymerase chain reactions

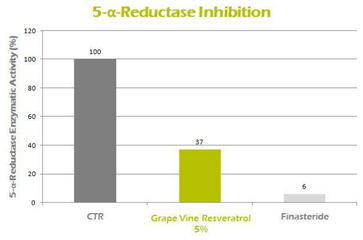
The SIRT-1 expression in senescent fibroblasts decreases by 45 % compared with the expression in normal fibroblasts ( CTR ).

**VINEATROL® increases SIRT-1 expression in senescent fibroblasts with a dose dependent effect.**




**Effect of a cream containing grape vine resveratrol on SIRT-1 expression in reconstructed human epidermis**
A cream containing Grape Vine Resveratrol was tested on a human reconstructed epidermis model. The expression and the localisation of the SIRT-1 in the epidermis is done with immune-labelling *in situ* .
**The quantification of the fluorescence intensity showed an increase of 55 % of the SIRT-1 expression by the cream.**

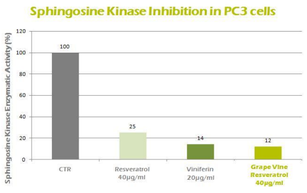
### *Prostate Health*




### 5-alpha-reductase inhibition

The enzyme 5-alpha-reductase transforms testosterone into di-hydro-testosterone, the active form of testosterone.
5-alpha-reductase activity in the presence of Grape Vine Resveratrol is measured on a reconstructed skin model which over-expresses a radioactive isotope.

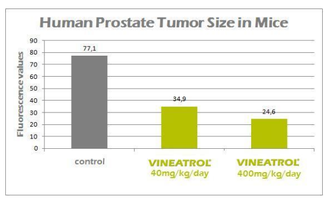
**In this test, VINEATROL® inhibits 5-alpha-reductase by 63%.**




**Sphingosine Kinase inhibition [25]**

Sphingosine kinase activity in the presence of Grape Vine Resveratrol is measured on PC3 prostatic cancer cells, by radioactive dosage.

**VINEATROL® is more efficient than resveratrol and epsilon-viniferin. It inhibits sphingosine kinase by 88% after 72 hours of treatment.**




**In Vivo Study [25]**

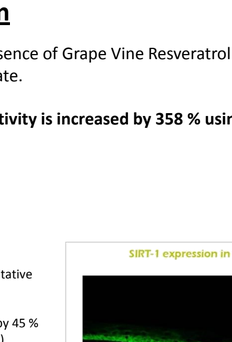
VINEATROL® reduces the size of human prostate tumours implanted in mice with a dose dependant effect.

### *Anti-Ageing / Anti Wrinkle activity*

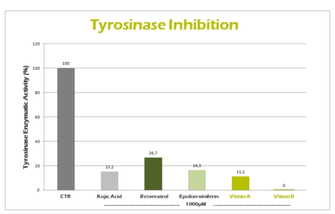
**Clinical Test**
The deeply wrinkled skin of a panel of 20 women aged from 49 to 69, were treated with a cream containing 3 % Grape Vine Resveratrol for 56 days.

Crows-foot measurements demonstrate

**a decrease of 22,9% on the wrinkled surface area**
➔ **80 % of the women** showed an improvement
**a decrease in the number and the surface area of the wrinkles**
➔ up to **71 %** fewer wrinkles
➔ up to **77 %** less wrinkled surface area



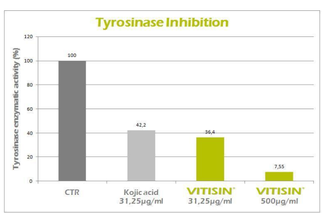

### *Skin lightening Activity*




**Inhibition of mushroom tyrosinase activity *in vitro***

Vitisin A inhibits tyrosinase by 89 % and Vitisin B by 100%.

**Vitisin A and Vitisin B are more potent tyrosinase inhibitors than kojic acid, epsilon-viniferin and resveratrol.**




VITISIN® is a solution of grape root extract containing 1% Vitisin A+ Vitisin B.

**At low concentrations, VITISIN® is more potent than Kojic acid.**

*Expressed in dry matter

## Research Partnerships

In partnership with BREKO GmbH, ACTICHEM participates at the research program: "The Usage of resveratrol oligomers in functional food and nutraceuticals with a focus on cancer prevention." funded by the German Federal Ministry of Education and Research ( ORFFI Project ), in collaboration with three scientific partners:
Prof. Dr. Prof.Dr.Sabine Kulling, MRI, Federal Research Institute of Nutrition and Food, Karlsruhe
Prof. Dr. Peter Winterhalter, Institute of Food Chemistry, University of Braunschweig
Prof. Pablo SteinbergUniversity of Veterinary Medicine Hannover.

**ACTICHEM is looking for new partnerships to test the biological activities of Grape Vine Resveratrol Derivatives**