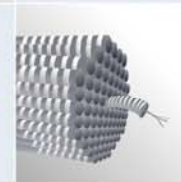


# A TRIFUNCTIONAL INGREDIENT FOR AN INTEGRAL COLLAGEN TREATMENT

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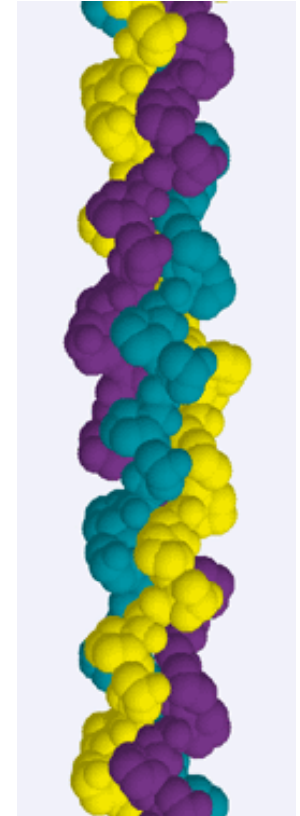


# COLLAGEN FEATURES

Collagen is the most abundant protein in the skin and the major component of the extracellular matrix (ECM)

Gives the dermis its mechanical and structural integrity

A typical collagen molecule has a long, triple-stranded helical structure, in which three collagen polypeptide chains are wound around one another in a rope-like superhelix



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# TYPES OF COLLAGEN

## FIBRILLAR COLLAGENS: I, II, III, V, XI

### Type I:

- ✓the most abundant collagen in the human body
- ✓fibrils have a great tensile strength and elastic resistance

### Type III:

- ✓accumulates around blood vessels and is plentiful in fetal skin.
- ✓youthful skin contains a predominance of Collagen III
- ✓as skin ages type III fibres are replaced by type I fibres

## NON-FIBRILLAR COLLAGENS: IV, VI, VII, VIII, IX, X, XII-XIX

### Type IV:

- ✓the most abundant structural component of basement membranes
- ✓constitutes a stable scaffold for the basal lamina of the DEJ
- ✓serves as anchoring support for cells and other constituents of the basement membrane

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# COLLAGEN - FEATURES OF AGED SKIN

- a) Reduced Collagen synthesis
- b) Increased disorganisation of the fibril network
- c) Increased Collagen degradation: increased MMP (matrix metalloproteinases) expression and collagen glycation

+

UV irradiation accelerates the aging process (photoaging)



Skin loses elasticity



Skin sags and forms furrows



Wrinkle formation

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# TRYLAGEN®

TRYLAGEN® is a combination of active peptides and proteins

- ❑ Provides an efficient treatment to restore the collagen levels of both young and mature skin
- ❑ Maintains an adequate long-lasting collagen function that will ensure a healthy and youthful skin

TRYLAGEN® acts on 3 main functions:

- A) COLLAGEN **BOOSTING**
- B) COLLAGEN **ORGANISATION**
- C) COLLAGEN **PROTECTION**

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# TRYLAGEN®

TRYLAGEN® contains a combination of ingredients that tackle the problems associated with different stages in the collagen cycle and provide an integral treatment to improve overall collagen quality

## BOOSTING

PSEUDOALTEROMONAS  
FERMENT EXTRACT

HYDROLYZED WHEAT  
PROTEIN

HYDROLYZED SOY  
PROTEIN

## ORGANISATION

TRIPLEPTIDE-10 CITRULLINE

## PROTECTION

HYDROLYZED WHEAT  
PROTEIN

HYDROLYZED SOY PROTEIN

TRIPLEPTIDE-1

TRYLAGEN® keeps our skin's collagen in balance

This is macroscopically translated in a significant ANTI-WRINKLE EFFECT

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## A) COLLAGEN BOOSTING

- As skin ages, collagen synthesis is decreased and degradation is increased, resulting in connective tissue damage, and loss of the skin's 3D integrity
- Reduced synthesis of collagen types I and III is characteristic of chronologically aged skin

**TRYLAGEN®** boosts the synthesis of Collagens type I, III and IV, helping to increase the levels of collagen lost due to the aging process

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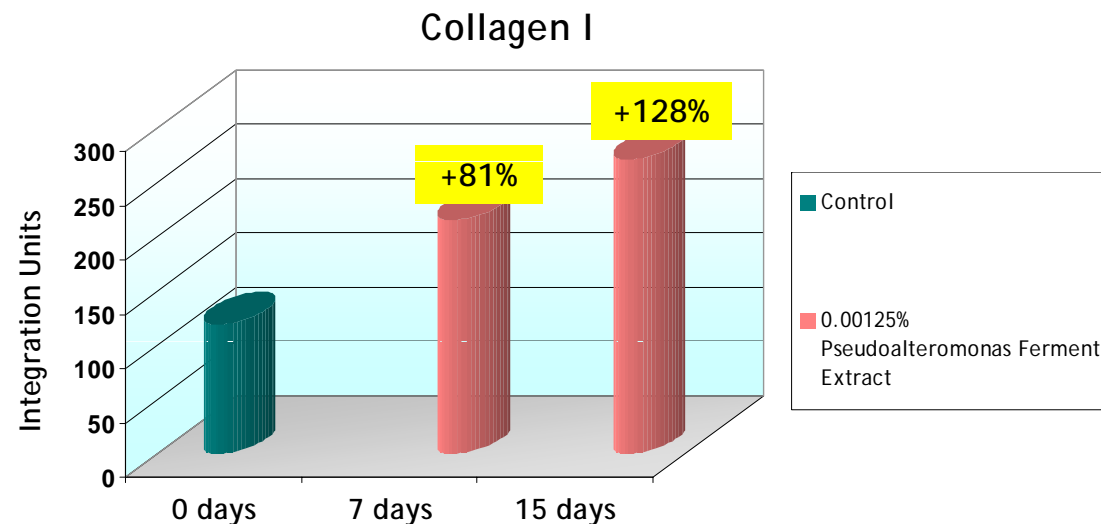
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# *IN VITRO* Increase in Collagen type I synthesis

A cream containing 1.25% PSEUDOALTEROMONAS FERMENT EXTRACT was tested on a reconstituted human skin model (HEKs on a feeder layer of 3T3 fibroblasts)

Collagen levels were assessed using the Dot Blot method on human keratinocytes



At a dose of 0.00125% PSEUDOALTEROMONAS FERMENT EXTRACT, the increase was 81% after 7 days, and 128% after 15 days

5% TRYLAGEN® contains 0.62% PSEUDOALTEROMONAS FERMENT EXTRACT

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Lipotec

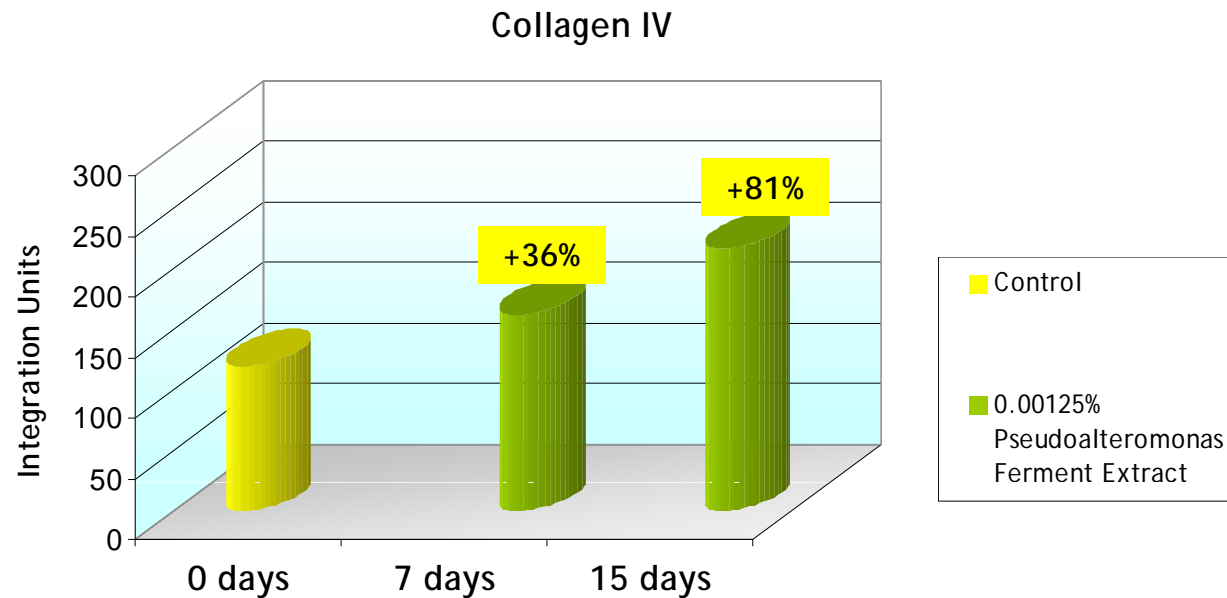
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# *IN VITRO* Increase in Collagen type IV synthesis

The same test was repeated to monitor the synthesis of Collagen type IV



At a dose of 0.00125% Pseudoalteromonas Ferment Extract, the increase was 36% after one week, and 81% after 15 days

5% TRYLAGEN® contains 0.62%  
PSEUDOALTEROMONAS FERMENT EXTRACT

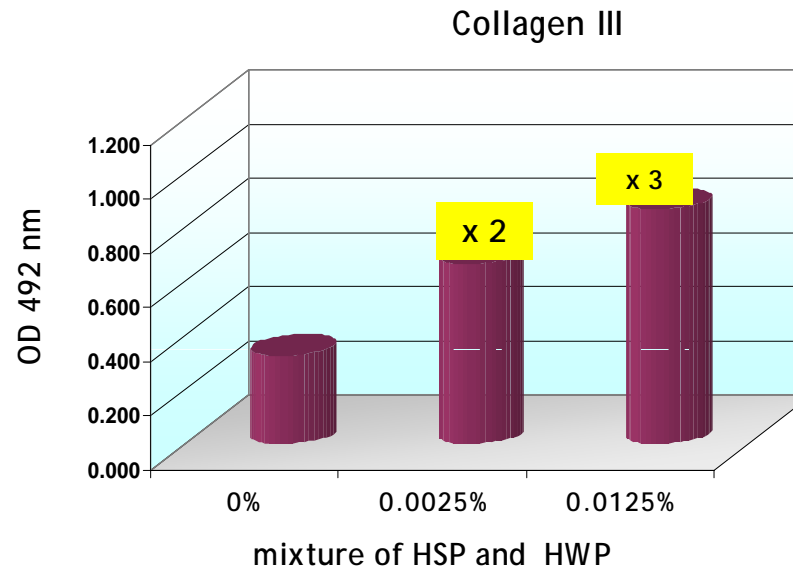
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# *IN VITRO* Increase in Collagen type III synthesis

Human dermal fibroblasts were treated with a mixture of HYDROLYZED SOY PROTEIN and HYDROLYZED WHEAT PROTEIN at two concentrations for 7 days  
Collagen III was detected using an ELISA test with monoclonal antibodies



After 7 days, Collagen III production almost tripled at the dose of 0.0125%

5% TRYLAGEN® contains 0.236% of the mixture  
HYDROLYZED SOY PROTEIN + HYDROLYZED WHEAT  
PROTEIN

trylagen® 

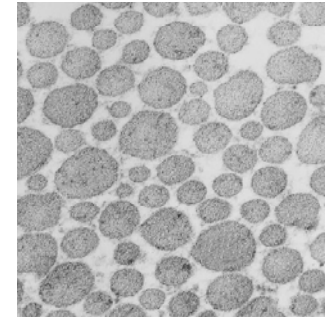
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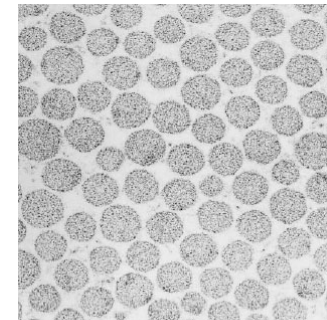
## B) COLLAGEN ORGANISATION

- The process of fibrillogenesis results in the formation of collagen bundles that are responsible for the strength and resiliency of the skin
- Fibrils age-related changes: diameter becomes irregular and distribution is not uniform
- The disturbance of the aging collagen network has a significant effect on skin suppleness and elasticity

**TRYLAGEN<sup>®</sup>** controls collagen fibril dimensions: it uniformises their diameter and spaces them regularly, providing a better cohesion and stabilisation of fibres



Irregular fibrils (old skin)



Regular fibrils (young skin)

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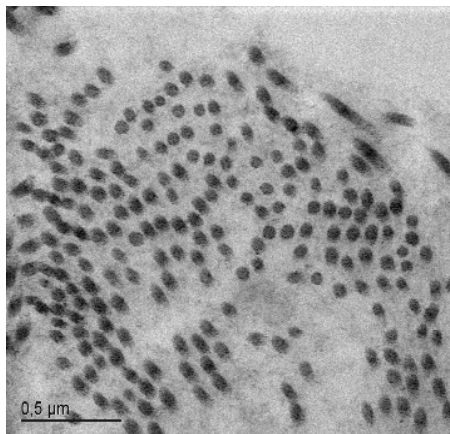


# *IN VITRO*

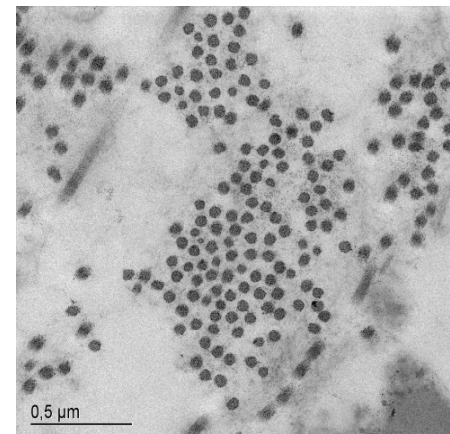
## Collagen fibril study in a human skin model

Tissues from a 3D human skin model EFT-200 (MatTek Corporation) were treated with TRIPEPTIDE-10 CITRULLINE 0.01% or non treated (controls)

Tissues were sectioned and observed by Transmission Electron Microscopy (TEM)



Control (untreated)



Treated with TRIPEPTIDE-10 CITRULLINE

Control tissues have irregular fibrils, while tissues treated with TRIPEPTIDE-10 CITRULLINE present more uniform collagen fibrils

5% TRYLAGEN® contains 0.002% TRIPEPTIDE-10 CITRULLINE

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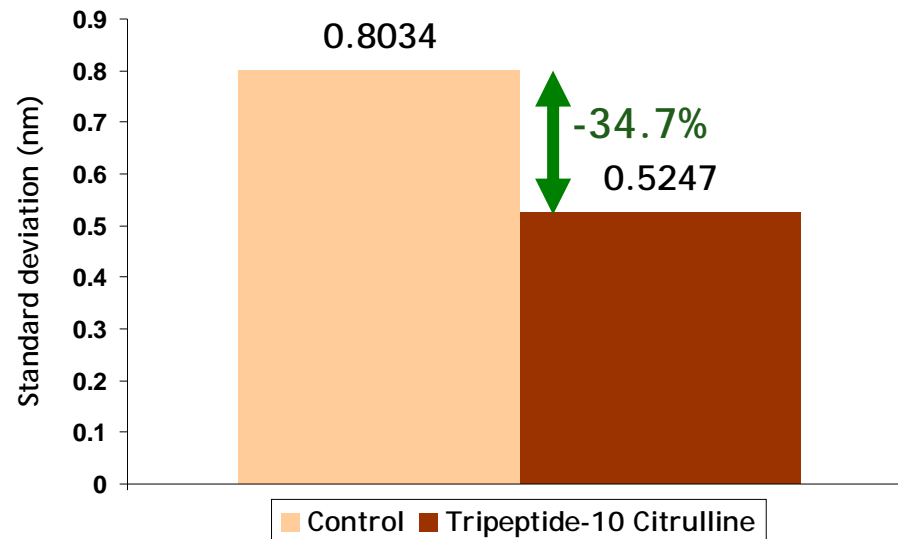
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# IN VITRO

## Collagen fibril study in a human skin model

The diameters of collagen fibrils were measured in each sample and the data obtained was statistically analysed using the *One Way ANOVA* analysis



A 34.7% decrease of the standard deviation was observed for the fibrils treated with TRIPEPTIDE-10 CITRULLINE

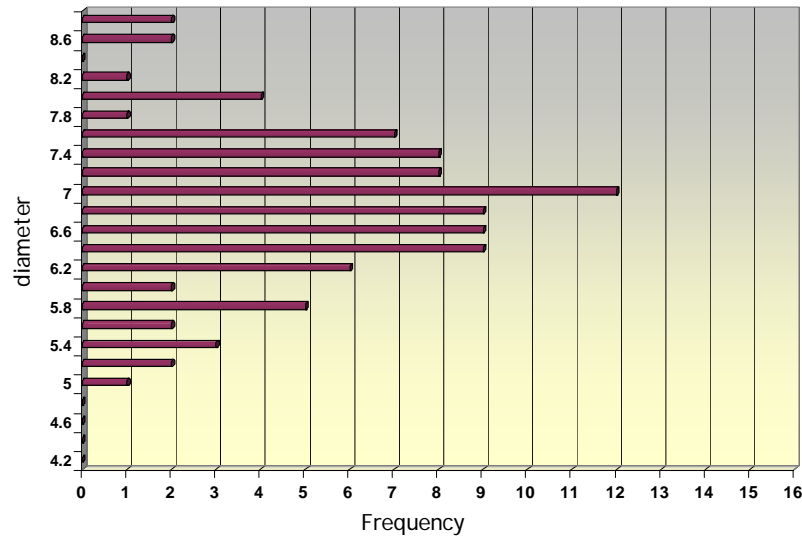
Treated fibrils show a lower variability, they are more uniform



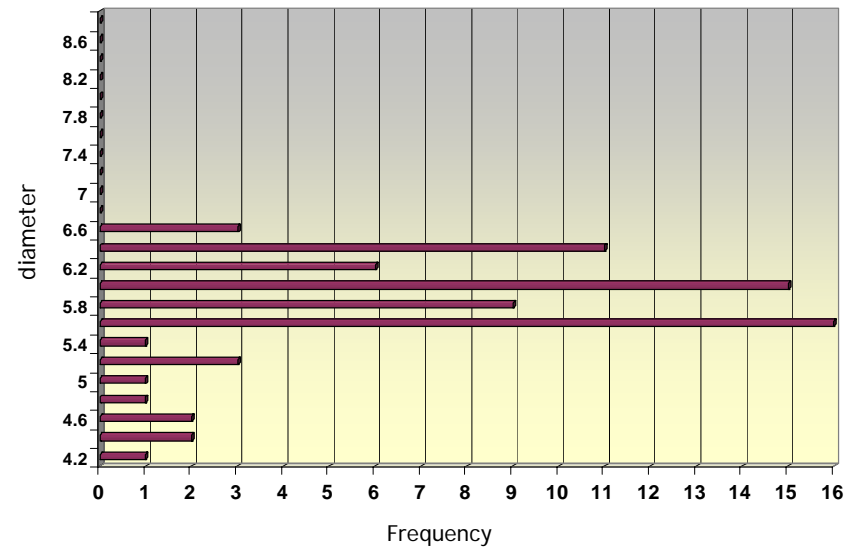
# *IN VITRO*

## Collagen fibril study in a human skin model

Tissues treated with TRIPEPTIDE-10 CITRULLINE show a perfect normal distribution with a narrower range compared to control tissues



Control (untreated)



Treated with TRIPEPTIDE-10 CITRULLINE





## C) COLLAGEN PROTECTION GLYCATION INHIBITION

- Glycation is a damaging reaction to proteins, increased with aging. It is a non-enzymatic reaction between free amino groups in proteins and reducing sugars such as glucose
- In skin, glycation creates new residues or cross-linking of collagen (Advanced Glycation End products, AGEs)
- AGEs increase during intrinsic aging of normal human skin
- Intermolecular cross-linking in aged skin results in the loss of elasticity and the stiffness of tissues observed during aging

**TRYLAGEN®** inhibits glycation, avoiding the formation of AGEs, whose accumulation irreversibly induces the loss of skin elasticity

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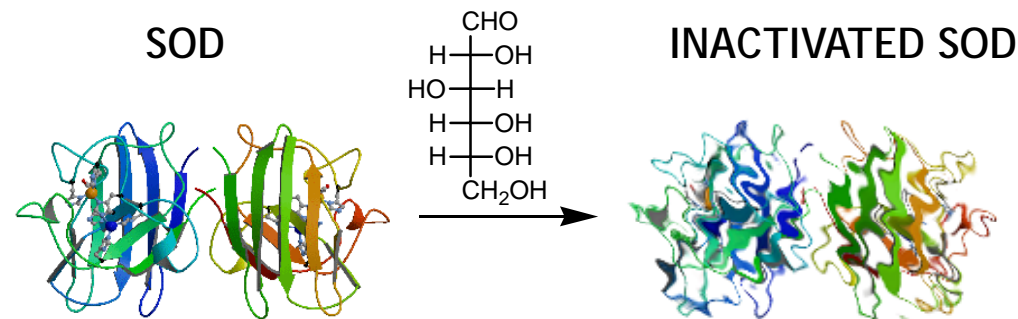


# *IN VITRO* Glycation inhibition

A usual glycation test is performed with the enzyme SOD. This enzyme is naturally deactivated by glycation when incubated with glucose or other monosaccharides

Some compounds can inhibit SOD glycation and, therefore, maintain the enzyme activity

In this study, the effect of TRIPEPTIDE-1 as an inhibitor of glycation is evaluated



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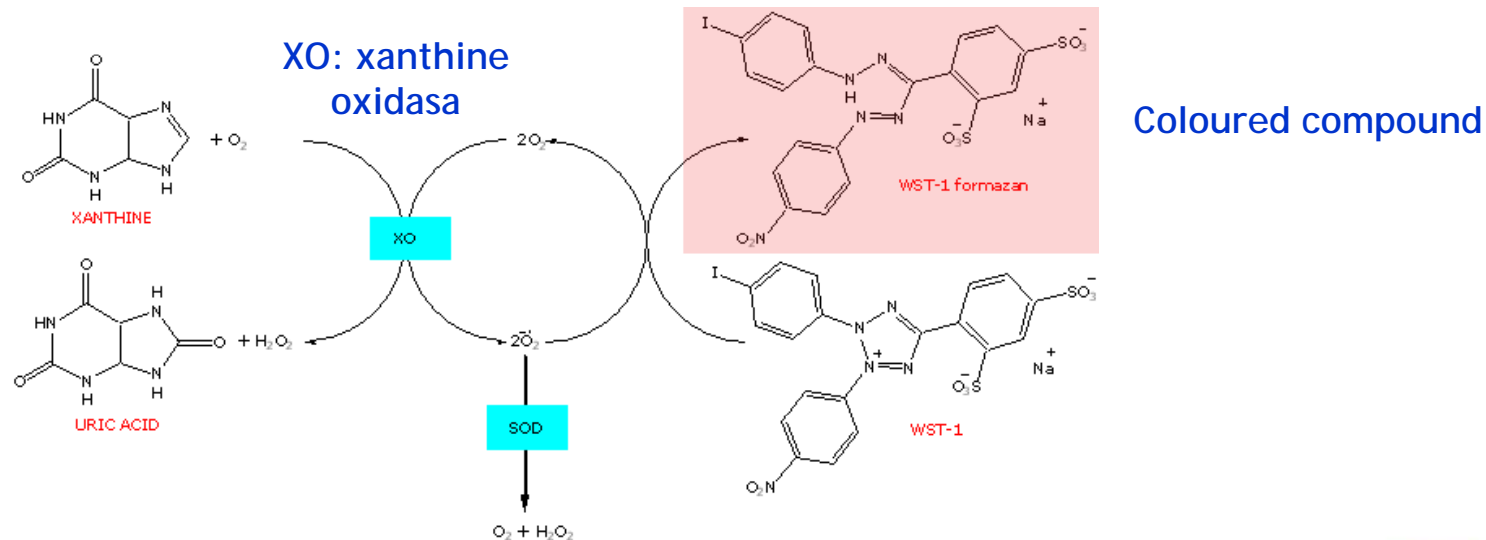
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# IN VITRO Glycation Inhibition

Activity of SOD can be measured with a colorimetric method:

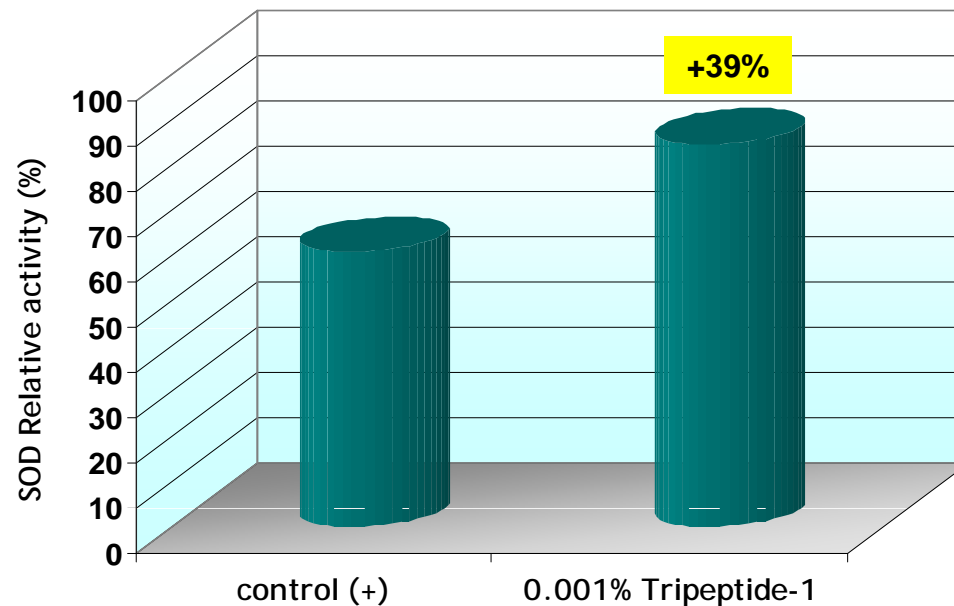
- SOD captures the radicals generated by the oxidation of Xanthine.
- If SOD is deactivated, the radicals generate a coloured formazan salt





# *IN VITRO* Glycation Inhibition

The samples treated with 0.001% TRIPEPTIDE-1 experienced an increase in SOD activity, which means that TRIPEPTIDE-1 inhibited SOD glycation



5% TRYLAGEN® contains 0.0005% TRIPEPTIDE-1

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## C) COLLAGEN PROTECTION

### COLLAGENASE – HUMAN MMPs INHIBITION

- Skin aging is associated with increased MMP (matrix-degrading metalloproteinases) expression and enhanced collagen degradation
- MMPs are a family of proteolytic enzymes that specifically degrade collagen, elastin and other proteins in connective tissue

Human MMPs:

**MMP-2:** cleaves type IV collagen and it is also capable of degrading type I collagen

**MMP-3:** degrades type IV collagen

**TRYLAGEN®** inhibits the activity of metalloproteinases (MMP-2 and MMP-3), protecting collagen from degradation

trylagen®



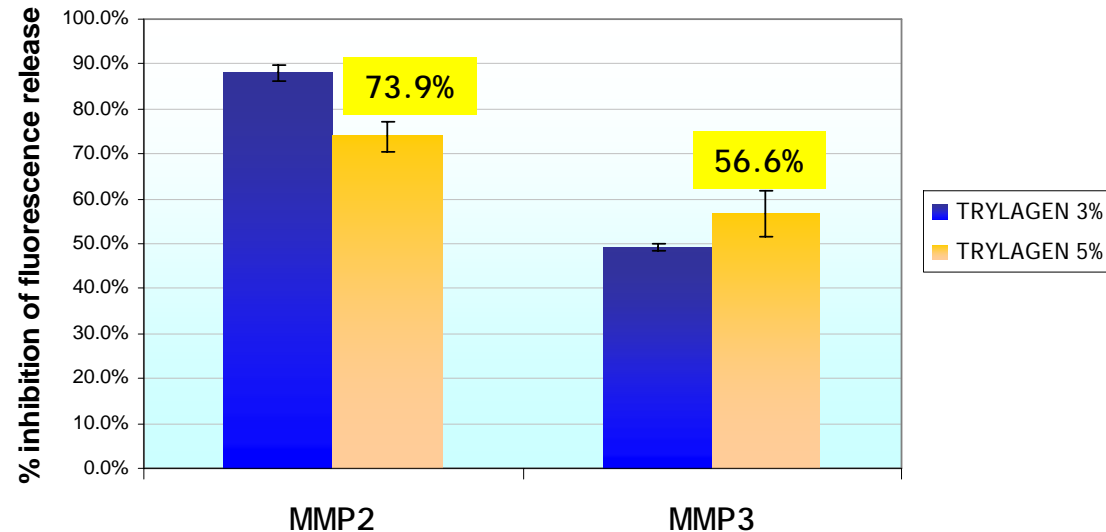
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# *IN VITRO*

## Human MMPs inhibition activity

The fluorescence released by quenched gelatin (denatured collagen) when digested with human MMP-2 and MMP-3 was measured



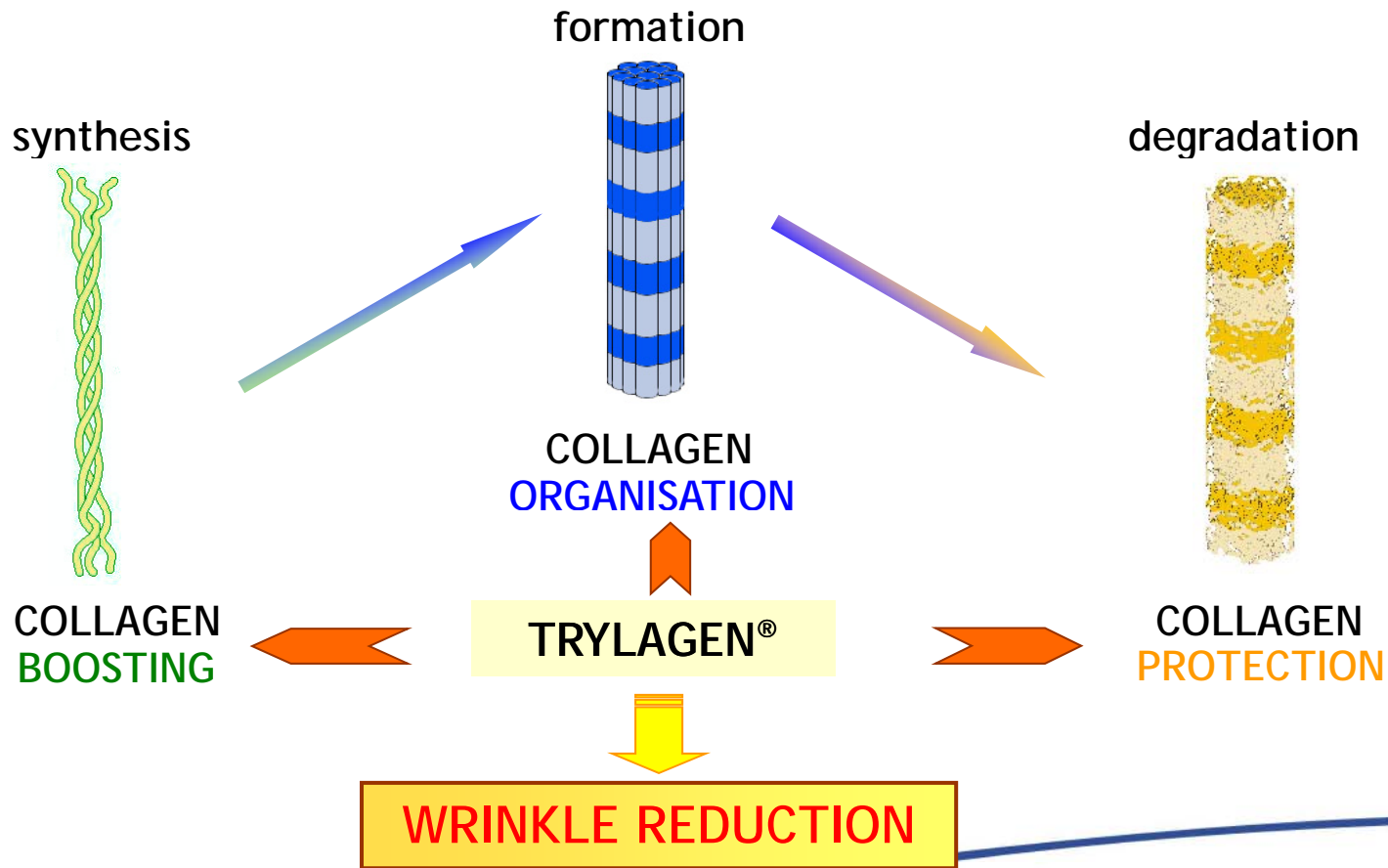
TRYLAGEN<sup>®</sup> showed to inhibit human MMP-2 activity by 73.9%, and human MMP-3 activity by 56.6% at the recommended dose of 5%





# TRYLAGEN® TRIFUNCTIONALITY

TRYLAGEN® acts at 3 different stages in the life of Collagen:





## *IN VIVO* Anti-wrinkle effect

- ❖ A panel of 20 female volunteers aged 35 to 55 used a cream containing 5% TRYLAGEN<sup>®</sup> twice daily during 30 days
- ❖ The volunteers applied a placebo cream on one side of the face (around the eye) and the cream containing TRYLAGEN<sup>®</sup> on the other side
- ❖ The depth of wrinkles was measured using the optical 3D PRIMOS technique

trylagen<sup>®</sup>



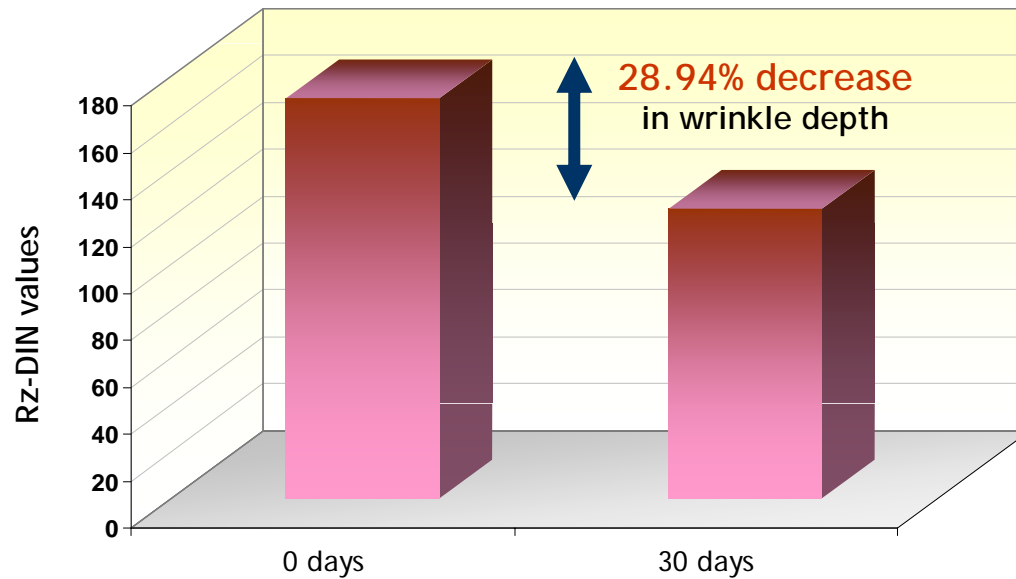
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# IN VIVO Anti-wrinkle effect

After 30 days, a 28.94% decrease in wrinkle depth was accomplished for the cream containing 5% TRYLAGEN<sup>®</sup>, with maximum values up to 35.32% decrease

Improvement in skin roughness



0 days



30 days

Volunteer 12

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