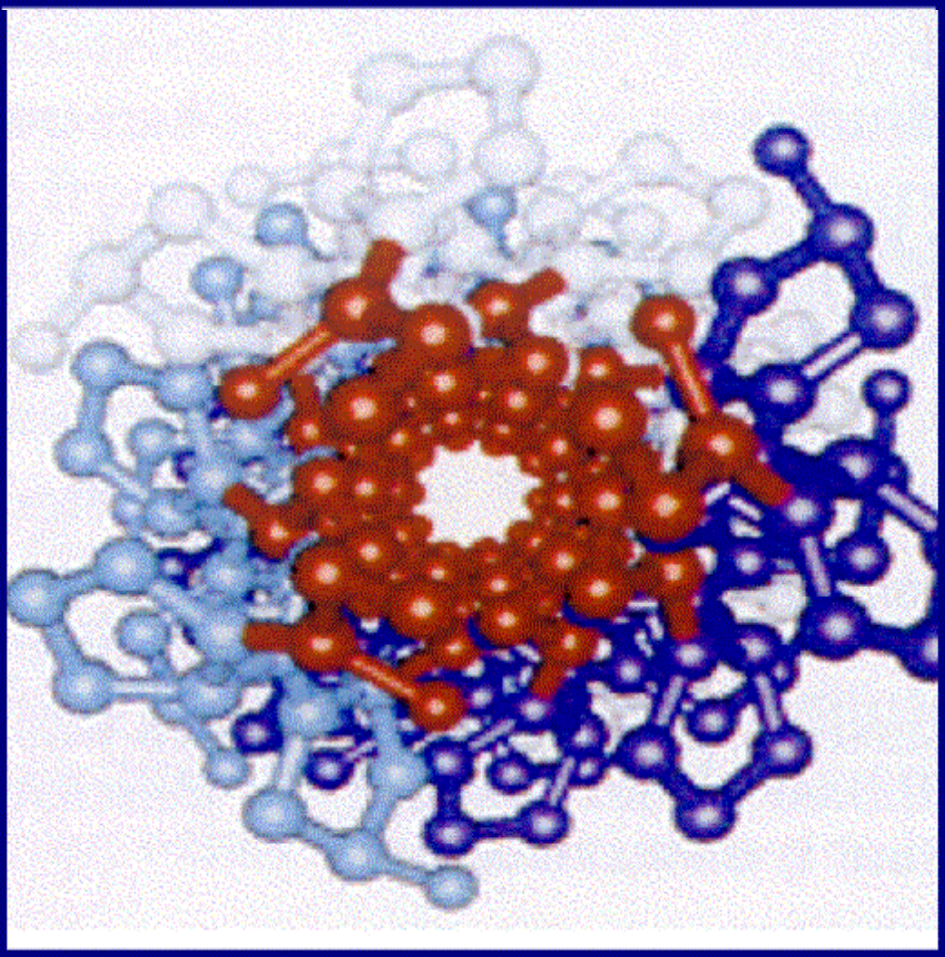
True Human Skin Identical Plant Collagen



novel functional ingredients for multi-purpose formulations

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CAMPO® Multi-Purpose Cosmetic Base Chemicals & Active Ingredients

CAMPO® Novel Functional Active Cosmetic Ingredient & Raw Materials

CAMPO RESEARCH
PRODUCT LITERATURE

What is soluble collagen?

Collagen is the principal protein constituent of the connective tissues, skin, tendon and bone. The corium layer of skin (i.e. the dermis), for example consists of 90 to 95% collagen, on dry basis.

Mature native (undernatured) collagen is largely insoluble but certain solutions such as alkaline buffers, acid buffers and neutral salt solutions are each able to extract a small proportion of collagen into solution. Yields of soluble collagen from skin collagen can vary from less than 1% to about 10% for alkali and neutral salt extractable material and up to 20% for acid extraction. Studies have shown neutral salt soluble and/or alkaline soluble collagen fibres and acid soluble collagen may be an intermediate phase. All these solutions contain collagen in its monomeric form (tropocollagen) which structurally consists of three helical polypeptide chains wound round each other to form a coil. In solution tropocollagen behaves as a rigid rod with molecular weight of approximately 350,000 a diameter of 14°A and a length of 3,000°A irrespective of its source.

Optical rotation, viscosity and ultracentrifugation studies have demonstrated that **Campo Biotechnologic Plant Collagen Extracts** such as **Soluble THSI Plant Collagen I** and Plant **Collagen Freezed-Dried Powdered Extract** possess the characteristics of tropocollagen.

• **Anti-aging / Lifting / Wrinkle filling** -For Improvement of wrinkle appearance and as an aid for more facial collagen density. Visible remodeling of facial contours

• **Reduction of stretch marks** – assist in reduction of stretch marks volume as an aid and with visible improvement of stretch marks disappearance

• **Nail care** - Visible repair of cracked cuticles.

• **Tattoo aftercare** - Immediate soothing effect on tattooed skin

Biotechnologic Plant Soluble collagen differs from **gelatin of Plant sources (offered as vegetal collagens)** in possessing a helical structure and the difference can be seen in the high viscosity and high optical rotation values of **Soluble THSI Plant Collagen I** compared to gelatin **(gelatin offered as Vegetal Collagen).**

Soluble THSI Plant Collagen I produces a pronounced and immediate effect when included in skin care compositions, leaving the skin feeling smooth and velvety. It is complex triple helix structure gives it high moisture retention properties. It acts as a reservoir, improving elasticity and combating dry flaky skin conditions.

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As standard, Biotechnologic Plant (soluble) collagens are available from Campo as Plant Collagen Freezed Dried Powder Extract , a concentrated 100 % which is totally soluble in aqueous dispersion and **THSI Plant Collagen I**, a clear aqueous solution. Other forms of Biotechnologic Plant soluble collagen is achieved by reprecipitation of the solubilised collagen at pH 4 and this also provides an additional purification stage.

For ease of handling, **THSI Plant Collagen II** is supplied at pH 4, in the form of a paste of highly swollen, precipitated soluble collagen.

Above pH 4.5 or below pH 3.8 **THSI Plant Collagen II** becomes completely soluble, forming very viscous solutions.

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However, due to the presence of fatty acids naturally associated with Biotechnologic plant native collagen tissue extracted with the soluble collagen from the starting material, diluted collagen solutions are translucent.

THSI Plant Collagen I is supplied as a clear aqueous solution of pH 3.5. It is recommended for applications where the clarity of solution is an important attribute and where for technical reasons pH adjustment is not possible. Apart from clarity of aqueous solution, the properties of **THSI Plant Collagen I** and **THSI Plant Collagen II** in solution remain the same.

Amino acid composition

Amino acid analysis on **THSI Plant Collagen I**, **THSI Plant Collagen II** and **Plant Collagen Freeze Dried Powder Extract** showed the following composition.

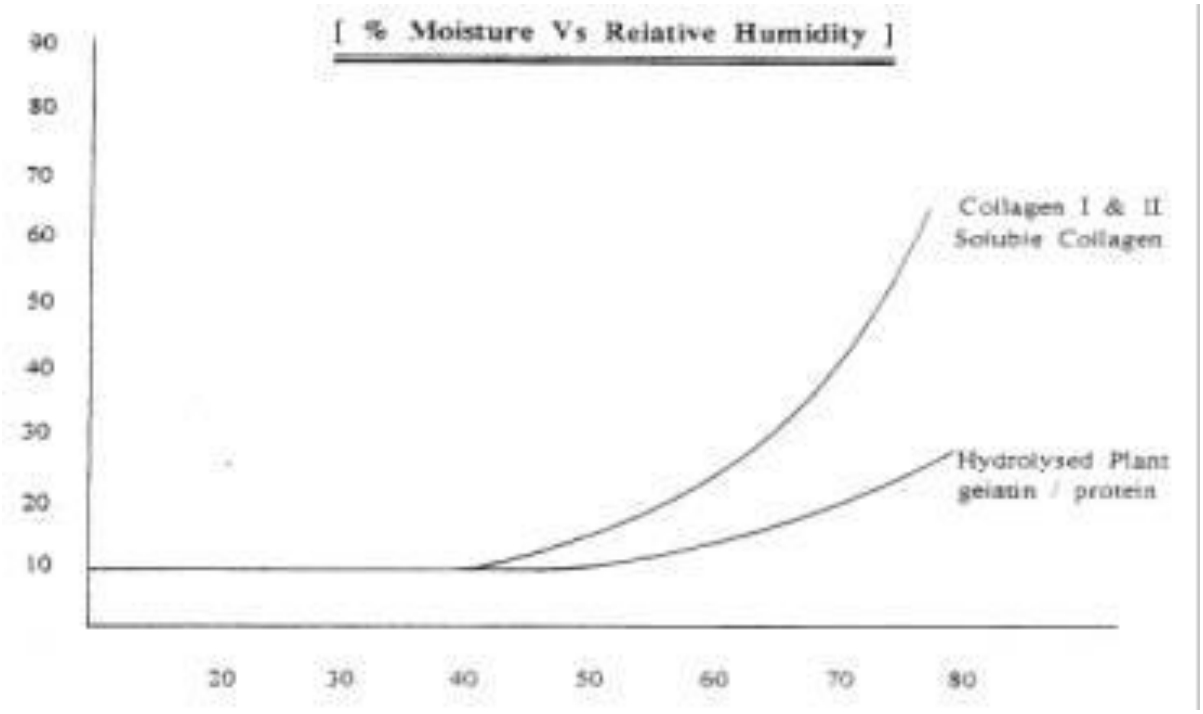
Amino acid	Amino acid residues per 1000 residues
Alanine	107.3
Glycine	310.3
Valine	22.9
Leucine	25.6
Isoleucine	11.9
Proline	144.8
Phenylalanine	10.7
Tyrosine	1.8
Serine	28.3
Threonine	15.2
Methionine	3.9
Arginine	46.5
Histidine	4.8
Lysine	27.7
Aspartic acid	44.1
Glutamic acid	75.4
Hydroxyproline	105.8
Hydroxyiysine	8.3

Collagen is the only mammalian protein containing large amounts of hydroxyproline and therefore this amino acid can be considered specific for collagen.

Characteristics

Moisture retention

The following graph illustrates the water retentive properties of **THSI Plant Collagen I**, **THSI Plant Collagen II** and **Plant Collagen Freezed Dried Powder Extract** compared with a typical hydrolysed collagen derived from plant gelatin/protein of molecular weight 4000.



Stability

Soluble **THSI Plant Collagen I** is stable at room temperature and below, and the high viscosity is thereby maintained indefinitely. At somewhat higher temperatures soluble **THSI Plant Collagen I** denatures (that is, it loses its characteristic triple helix structure) and converts to gelatin. The stabilising bonds responsible for maintaining the triple helix structure are largely secondary forces - hydrogen bonds - and consequently denaturation occurs at comparatively low temperatures. As a consequence of the change in behaviour in solution - that is, from a rigid rod to a random chain - denaturation is accompanied by a marked decrease in viscosity and optical rotation.

The rate at which denaturation occurs is dependent upon the temperature (Tp) of soluble **THSI Plant Collagen I** is defined, somewhat arbitrarily, as the temperature at which its intrinsic viscosity falls to half its original value after 30 minutes. (Appendix 2 explains the derivation of intrinsic viscosity and shows a typical viscosity/temperature curve for **THSI Plant Collagen II**).

Unlike gelatin, which is readily hydrolysed by proteolytic enzymes such as pepsin and trypsin, **THSI Plant Collagen II** is largely unaffected by these enzymes.

Applications

THSI Plant Collagen II and **THSI Plant Collagen I** are ideal for skin care preparations including, conditioners, cleansers, moisturisers, sun care products, eye wrinkle and facial creams, night creams, anti-ageing and nutrient creams.

• **Anti-aging / Lifting / Wrinkle filling** -For Improvement of wrinkle appearance and as an aid for more facial collagen density. Visible remodeling of facial contours

• **Reduction of stretch marks** – assist in reduction of stretch marks volume as an aid and with visible improvement of stretch marks disappearance

• **Nail care** - Visible repair of cracked cuticles.

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Suggested formulations can be provided upon request.

Solubility

THSI Plant Collagen II at pH 4 is in the form of a dispersion of precipitated soluble collagen. If the pH is adjusted below 3.8 or above 4.5, the collagen becomes completely soluble. The change in viscosity with pH is illustrated in the table below. The results refer to a 0.1% solution of **THSI Plant Collagen II** at 25°C.

pH	Viscosity (mps)
3.35	32.5
3.70	26.7
4.15	*9.7
5.00	35.0
6.10	34.8
7.50	35.5

*Soluble collagen precipitated.

Since the solubility of **THSI Plant Collagen II** is minimal at pH 4, then for maximum moisturizing properties, formulations containing **THSI Plant Collagen II** should be above pH 5.0.

It is not normally essential to use to buffer to incorporate **THSI Plant Collagen II** into a cosmetic formulation, but buffers can be used to advantage. Examples are

1. 0.15M citric acid plus sodium citrate to pH 6.5
2. 0.15M sodium citrate plus citric acid to pH 3.7

The above does not apply to **THSI Plant Collagen I** which is offered in a soluble, rather than precipitated form.

Compatibility with other materials

Apart from denaturation of soluble collagen by heat, the stabilising bonds responsible for the collagen structure can also be destroyed by chemicals possessing hydrogen bond breaking properties. For this reason **THSI Plant Collagen II** and **THSI Plant Collagen I** should not be processed with urea, guanidine hydrochloride or other Hydrogen bond breakers.

THSI Plant Collagen II and **THSI Plant Collagen I** should not be formulated with materials capable of either precipitating protein (e.g. ethanol) or cross-linking it - to give insoluble collagen (e.g. formaldehyde or those preservatives capable of releasing it, and tannins).

Detection of active collagen in cosmetic formulations

The determination of hydroxyproline content gives a measure of the amount of collagen used in a cosmetic formulation. However, the **hydroxyproline content alone cannot be taken as a direct measure of active soluble collagen content** since **denatured soluble collagen** (**plant gelatin**) will also possess **the same hydroxyproline content** but will lack the characteristic triple helix collagen structure. It is therefore desirable to check that the characteristics of soluble collagen are still detectable, and the simplest indication of this is the viscosity/temperature curve (see Appendix 2).

It is not possible to suggest a universally applicable method for extracting soluble collagen from cosmetic formulations, but for creams containing 0.1% or more active soluble collagen the following extraction procedure has been successfully used.

30g cream is shaken with 200ml petroleum spirit in a separating funnel, whilst maintaining the temperature at 25°C or below. After allowing to settle (10 minutes), the upper ethereal layer is decanted and the lower aqueous layer transferred to approximately 20mls 0.2M citrate buffer (pH 6). Any undissolved material is filtered through muslin and the filtrate tested for hydroxyproline content and for viscosity/temperature relationship (see Appendix 2). A fresh solution should be taken for viscosity measurement at each temperature, but it is still possible to progressively higher temperature for viscosity measurements.

Physiological data

THSI Plant Collagen I, **II** are well established cosmetic raw material and presents no special hazards. **THSI Plant Collagen I**, **II** did not produce any irritation to the skin or eyes (rabbit) when tested as a 10% dispersion. **THSI Plant Collagen I**, **II** was also found to be a non-primary irritant, non-sensitizer when applied to human skin (Repeated insult Patch Test).

The LD50 for **THSI Plant Collagen I** & **II** is greater than 40g/kg bw (rats).

Plant Collagen FD Powder Extract (powder collagen) can be expected to possess similar properties to **THSI Plant Collagen I** (standard collagen dispersion).

Stability

One of the characteristics of soluble collagen is that it will denature at modest temperatures. This is in contrast to both insoluble collagen, in which the helix structure is 'locked-in' by cross links formed during ageing, and gelatin in which the helix structure has already been destroyed. Care must be taken to avoid temperatures above ambient.

For example it is possible to heat **THSI Plant Collagen II** and **THSI Plant Collagen I** to denaturation temperature whilst still retaining the major proportion of the collagen in the undenatured form provided the time involved is not more than 5 minutes.
Particular care should be taken with formulations with pH's below 4.0 where the Tp is slightly lower. In such cases, the premixed emulsion should be cooled to below 28°C before adding the **THSI Plant Collagen II** or **THSI Plant Collagen I**.

Maximum heat stability of **THSI Plant Collagen II** and **THSI Plant Collagen I** is obtained for formulations with pH between 5.0 and 8.5.

Preservation

THSI Collagen I & **II** are supplied suitably preserved. The standard preservative systems are as follows:

THSI Plant Collagen 1	None	magnetized vacuum-preserved
THSI Plant Collagen II	0.3% 0.1% 0.1%	Lonicera jap.Extr. Sorbic acid Neem seed Extr.

Storage **THSI Plant Collagen I** & **II** should be stored between 5°C and 25°C and be protected from direct sunlight. Under these conditions they have a shelf life of approximately 24 months.

Specifications

	THSI Plant Collagen II
Appearance	White paste
% anhydro residues	0.37 min
% ash	1.0 max
PH	3.7 - 4.5
% soluble collagen	95% min
Microbiological Total Count	100 opg max.
Yeast and moulds	100 opg max.
	THSI Plant Collagen I
Appearance	Clear, slightly opalescent liquid (white to white buff)
%hydroxyproline	0.12 - 0.15
% ash	1.0 max.
pH	3.2 - 3.8
% total solids	1.0 - 3.0
% soluble collagen*	0.86 - 1.07
% nitrogen	0.16 - 0.20
Microbiological Total Count	500 opg max.
Yeasts and moulds	100 opg max.

***soluble collagen content is calculated from the relationship**
% soluble collagen = % hydroxyproline x 7.14
(If the hydroxyproline content is expressed in terms of an hydro residue instead of the free amino acid then the conversion factor is 8.15).

COLLAGEN BIOSYNTHESIS

The synthesis of the different polypeptide strands that are combined to make different types of collagen is genetically regulated by the production of messenger RNA. The synthesis of polypeptide strands occurs on membrane-bounded polysomes, but the the hydroxylation of lysine and proline occurs after the strands are assembled. Ascorbic acid is required for the hydroxylation of lysine and proline. Polypeptide strands enter the cisternae of the endoplasmic reticulum (amembraneous assembly labyrinth within the cell), the terminal extensions of the strands are aligned, and then the strands spiral around each other. Procollagen or immature collagen has long terminal extensions protruding from each end of the newly formed triple helix. Procollagen moves to the golgi apparatus and is packaged into vesicles that are moved to the cell surface, probably by microtubules.