# CHAPTER 6
# THE HISTORY OF EXTRACTION, CHARACTERISTICS AND EFFECTS OF THE APPLICATION OF FISH TROPOCOLLAGEN HYDRATE

Due to unfavourable environmental factors, poorly balanced nutrition and stress and diseases, modern man often suffers from premature protein dissimilation and especially premature collagen biosynthesis inhibition.

Regular intake of protein in the diet is not sufficient for internal cell-construction processes, due to the fact that only up to 30% of these proteins break down into free amino acids to be used in protein creation during anabolic processes. Un-cleaved proteins decay in the large intestine, which intoxicates the body.

Free amino acid complexes are successfully applied in dietetics, supplementation and treatment. The most popular are complex formulations, composed of synergistic ingredients – free amino acids in the form closest to their natural conformation – and substances that support anabolism and the synthesis of the aforementioned amino acids. Complexes containing amino acids, vitamins and minerals are considered to be most effective in supporting therapy, prevention and recovery, since they supply the building material for all proteins, peptides, enzymes, neurotransmitters, RNA, DNA and many other vital substances.

The COLVITA – amino acid-plant-vitamin complex, containing tertiary, biologically active collagen (extracted from fish skin and converted into a freeze-dried form with appendices) is so unique that it merits close examination in this study.

## 6.1. THE HISTORY OF FISH TROPOCOLLAGEN

For many years now, collagen has been known and used in medicine and cosmetology. However, it was generally used in its macromolecular form: fibrous and less often fibril.

Medicine and cosmetology have applied, almost exclusively, quaternary and quinary collagen. Lower order forms (molecular forms) such as tertiary collagen, in the form of a triple helix, have not yet been used. This is primarily due to the fact

that the helical conformation is a very fragile structure, easily breaking down into individual peptides outside the body. As a result, attempts to maintain this structure outside the donor's body, in amounts sufficient for application, have resulted in failure.

After dissimilation, super helix decomposition products do not remain biologically active for a particularly long period: after a certain time, not unlike hydrolysed protein, they become a kind of "peptide carrion" – an unattractive quality for bioengineering drugs or cosmetics.

The breakdown of 3-helix collagen in vertebrates is always completely unpredictable and virtually unique, which has discouraged pharmaceutical and cosmetic companies from experimenting with lower order collagen. Many have adopted the view that the triple helix is unfathomable and impossible to maintain after isolation, and that the individual peptides derived from the disintegration of "living" proteins are no more useful than hydrolysed fibre forms or synthetic forms.

Since the mid-twentieth century, macromolecular collagen in its quaternary – fibrilar and quinary – fibrous form has been used somewhat extensively in implant surgery: suture-dissolving fluids, vascular prostheses, as a component of medicinal ointments for skin burns, to diminish scars and other marks on the skin and to soften the edges of tissues, etc.

It has also been applied in anti-wrinkle creams, after-sun creams, moisturizing lotions, cosmetic masks and many other substances that were expected to help in rebuilding collagen or to increase the growth of systemic collagen, especially skin collagen, which is responsible for the condition and appearance of the skin and the degree of wrinkling.

Collagen fibres have been extracted experimentally from cattle, pigs and other animals, including great apes – which have the most human-like DNA – and even from women's placentae after birth. There have been gruesome reports of collagen fibre extraction from the skin of convicts in China.

It should be known that collagen fibres are "massive". They have a thickness of about 10 microns. Thus, they cannot penetrate the epidermis barrier, the greatest "gaps" in which are measured in nanometres. Therefore, even when finely triturated, unground collagen fibres do not have this transepidermal ability for anatomical reasons. "Collagen" advertised as a cosmetic base is, therefore, merely a marketing technique.

For the above reasons, collagen fragments derived from animal skins proved to be somewhat of a disappointment to cosmetology engineers and billions of their clients. Nevertheless, for marketing reasons, due to the fact that collagen film retains a significant amount of water in the skin, by the end of the '80s, any "high-end" anti-wrinkle cream had to contain INCI – collagen, usually extracted from calves' necks and subjected to hydrolysis.

The outbreak of BSE psychosis supplanted bovine collagen from cosmetics for a certain period: there was a fear of the transfer of BSE prions causing "mad cow" disease. Insurance companies refused to provide cosmetic corporations with cover against this risk, or they demanded unreasonable rates. For many years, this ended the application of collagen in the field of substantive cosmetology. It is only recently that we have begun to see a gradual, albeit timid, comeback of INCI collagen which is still predominantly derived from bovine collagen fibres and used in its hydrolysed form. It is fair to say that persistent attempts to reach new goals with old methods are ongoing...

Laboratories of large corporations, in particular cosmetic corporations, have an almost unlimited research budget when it comes to the search for an "elixir of youth" that would effectively stimulate collagen production cell "shipyards" (fibroblasts) to overproduce the protein to such an extent that it would stop the aging process in our skin and other tissues, depending on the collagen exchange. Research has been ongoing for many years for the purpose of finding a non-invasive way to "smuggle" collagen supplies to the fibroblasts. These attempts have so far been unsuccessful.

Another issue, though not covered in great detail in this work, is corrective (plastic) implantology with the use of collagen. Although this method bears a high recoil risk, and it requires repetitive treatments approximately every six months, as well as the fact that it is unavailable to the majority of the population due to high treatment costs, it effectively carries out its function – collagen injections are still the only way to remove existing wrinkles (caused by a loss of dermis) almost immediately.

During the '70s and '80s, The People's Republic of Poland was home to an internationally recognised school of protein biochemistry, especially marine organism proteins. During those years, scientists had access to modern facilities and even the specialised research ship – the "Professor Siedlecki".

For many years, Poland led a very ambitious research program based on the acquisition of food proteins from an inexhaustible reservoir – the sea. As an example, krill plankton protein, which is highly absorbable for the body, was extracted.

This program was interrupted by the economic crisis and social upheaval in the country.

However, Polish biochemists managed to gather valuable experience, which was spread particularly amongst Pomeranian researchers from Gdańsk and Gdynia.

In 1985 and 1986, M. Skrodzki, A. Michniewicz and H. Kujawa, chemists from the Fishing Co-operatives Research Laboratory in Gdynia, came up with a remarkable invention and a pioneering achievement. They isolated tertiary collagen molecules directly from fish skin, which bonded with water molecules (became hydrated) during a process carried out in an organic acid medium, allowing the low order collagen to maintain a stable triple helix conformation outside a living organism in virtually any protein mass.

The outcome of the experiment showed that, when properly filtered, the resulting hydrate is a natural cosmetic with unique moisturizing and anti-wrinkle properties. It serves as an excellent ointment for burns, sores, bed sores, minor wounds and abrasions, dermatitis, atopic and allergic skin reactions and many other diseases.

Sometime later, it was also discovered that, being the richest source of free amino acids, collagen hydrate could be lyophilised or even eaten "raw".

**It turned out that tropocollagen hydrate is capable of transepidermal penetration!** The fish collagen triple helix, together with a small amount of protein residues, formed a natural gel and stabilised with the interspiral and covalent bonds. The maximum temperature at which the gel could maintain stability (so that the collagen bonds are not broken) was dependent on the temperature of the donor fish feeding in the wild and the care with which the manufacturing process was conducted. Initially, this limit did not exceed a few degrees Celsius.

After coming into contact with human skin, body temperature (about 37°C) caused immediate despiralization of the molecules; with the bonds breaking down, tropocollagen dissimilated into peptides and free amino acids, which easily (and rapidly) penetrated the epidermis. These peptides and amino acids would penetrate (extracellular penetration) the keratin deposition, reaching the

source of cytokine formation and some of them would reach even further to fibroblast areas of the intracellular matrix. This stimulated the fibroblasts to overproduce collagen. Provided the described mechanism is sufficiently effective, this is a true breakthrough in cosmetology!

Despite the patenting of this important invention in 1989 (patent no. 144584) M. Skrodzki and his colleagues gained neither fame, nor fortune. During the ongoing turbulent period of political and economic transformation, Poland was not the best place for the market launch of such an invention. No serious investors have shown interest in native fish collagen (until today!). Collagen hydrate, even with very low thermal resistance to denaturation, was demonstrated to foreign companies, but they were all discouraged because the substance's instability posed many logistical challenges for the product's distribution.

Also, the serious misunderstanding that accompanied Polish fish collagen from its inception did not help the lack of interest from investors. The long-term inability to present the product by manufacturers and PR whizzes apparently contributed to reluctance, scepticism and even suspicion of charlatanism whenever collagen was described as "living and biologically active and capable of transepidermal penetration" and a "true elixir of youth".

For more than half a century it has been known that fibrous macromolecular collagen is "too big" to penetrate the dermis barrier. However, this is the only form of collagen known as "collagen" to the average doctor, cosmetologist, or biochemist, capable of providing an expert opinion on this "sensational invention". For a long time, Polish marketers were not able to provide adequate characteristics of the product: a veritable biochemical miracle. This collection of pure collagen molecules (not fibres!) maintains its "living" triple helical structure despite being transported from the bodies of fish into a jar. Once in contact with the skin, the molecules dissimilate into subunits, penetrating the skin like a colander.

There was much confusion surrounding Polish fish collagen. When the experts found out the true value of the invention, though there was still no market demand, more and more "fathers" of the invention began to appear. There were many candidates to share in the spotlight, including the self-proclaimed "professor" as well as various candidates for the production of collagen hydrate as a finished product – each time under a new name.

An unusual situation followed: after the death of two of the three inventors of the original methods and after the expiry of their discovery's legal protection,

the Polish Patent Office allowed the "reinvention" of the hydration method, even though this new method did not differ significantly from the first patented method. All of this gave fish collagen a rather poor reputation.

As usual, market mechanisms decided who would profit from the invention. This ended up being the company that managed to produce the best product and implement the most effective distribution.

Since 2003, the Polish market for fish skin tropocollagen has been dominated by the COLWAY Company, which was established solely for that purpose. In a short time it managed to refine **the fish collagen hydrate formula, making it stable in temperatures of up to 27°C without the need for any preservation!**

The product was called "Natural Collagen" and it soon dominated the market, mainly due to its high-quality, while other competing companies were busy arguing about the worth of the invention. A consumer network was soon created.

Often regarded as controversial, the concept of direct distribution within a multi-level marketing network, in the case of Natural Collagen, has functioned perfectly. Left on the shelves of retail outlets, the product would undergo denaturation during summer periods due to insufficient thermal protection. Moreover, without a very high-budget advertising campaign, it would suffer the same defeat as COLWAY competitors due to higher sales in pharmacies, drugstores or beautician clinics.

Direct sales involves consumer advocacy: one user shares their experience with another potential user, causing an avalanche of demand. If this mechanism usually works in terms of average quality products, for a clearly unique and effective product the effect must have been sensational, which is exactly what happened.

In light of this, COLWAY managed to distribute the first million (!) items without any advertising campaigns or trade credits, relying on the best advertising measure of all: enthusiasm stoked by the tens of thousands of consumer-distributors.

Because they "ran" and supported the network, giving real opportunity to earn a considerable amount of money to hundreds of other people, the Polish owners of COLWAY quickly spread throughout the international market, bringing the "fashion" for Natural Collagen to other countries including the Czech Republic, the Ukraine, Belarus, Russia, Bulgaria, Italy, the UK and beyond...

This is how we learned about Polish fish tropocollagen in 2004/2005 in the Ukraine.

Distributed via direct sales (always looked at with caution), it became famously associated with Panacea and known as a remedy for a thousand diseases. It was registered as a cosmetic and described as transdermal (!), "living" and supposedly biologically active, and it certainly did not come cheap. It was not a good start for COLWAY collagen...

## 6.2. FISH COLLAGEN HYDRATE CHARACTERISTICS

The very first analyses of the tropocollagen hydrate derived directly from the fish skin are surprising. It has to be emphasised, that it is not a hydrolysate, but a hydrate.

Let us explain the difference. Hydrolysis is a chemical reaction, consisting of the breakdown of the molecules of a chemical substance into two or more subunits, occurring in the presence of water. Hydration consists of the formation of water-containing complexes via hydrogen bonds and other intermolecular interactions.

Hydrolysed collagen is simply a form of gelatin. Hydrolysis is always followed by the destruction of crosslinks and peptide bonds of collagen proteins. Thus a hydrolysate is a chaotic mixture of peptide fragments of different masses. Due to the large supply of raw material (waste products of many kinds of skin and other tissues), and a simple extraction technique, collagen hydrolysates are cheap and widely available. They are used as components in foods, feeds and in cosmetics. In the INCI composition, they are to be found under the name "collagen"; however, in this case, the term "collagen" is used only for the sake of marketing.

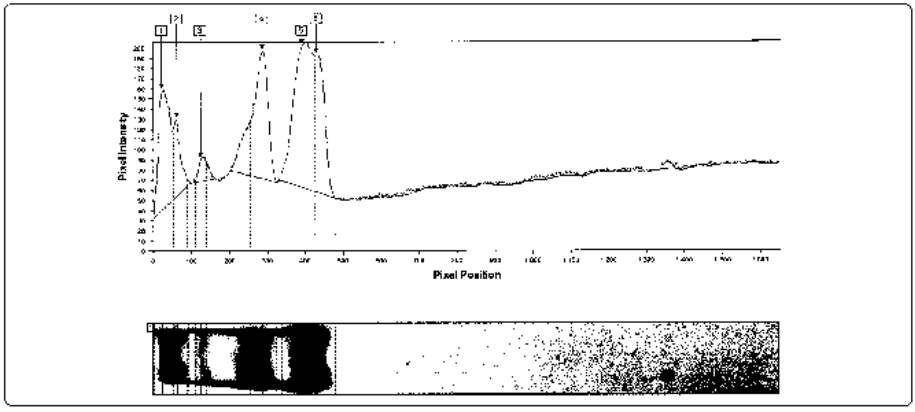
In our opinion this is simply a semantic misuse of the word. (For example, in Spanish, "collagen" refers to some types of sausage casing.)

Hydrate native fish collagen is formed when the triple helical collagen molecules bond with water molecules. It is only through "hydration" that super helices can leave the parent tissue and continue to "bio-exist", maintaining their structure for months or even years outside a living organism while sealed in sterile jars.

Initially this seemed unbelievable.

However, using the photographic laboratory method, it is fairly easily to determine whether the gel in glass jars, mass distributed by the COLWAY Company, is starch or gelatin, and whether it is actually a collection of isolated triple helix

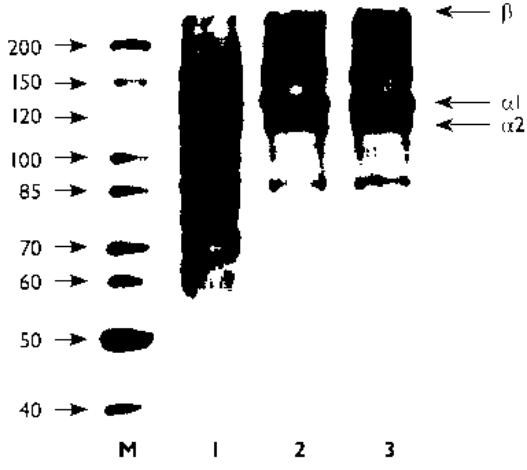
collagen molecules. Using electropherograms or densitometric analysis, we are able to immediately answer any doubt with a considerable degree of precision.




| No | Collagen subunit | V [px] | Distribution in % |
|---|---|---|---|
| 1 | | 783922 | 17.43 |
| 2 | γ | 305390 | 6.79 |
| 3 | | 83663 | 1.86 |
| 4 | β | 951501 | 21.15 |

Picture 6. Densitometric analysis of tertiary collagen hydrate isolated directly from the skin of freshwater fish (study PG-KTCB 01/C1/2007)

Electropherogram of Polish fish collagen, immediately after hydration, produced in a Canadian institution:



The photograph above shows the clearly visible molecular structure maintaining its triple helix conformation. The test material is derived from a typical cosmetic packaging jar, i.e. it is located outside the donor body and outside of laboratory conditions. Surprisingly, the same studies, when repeated after 2 weeks, 3 months and 12 months, showed that the material still retained its triple helical structure.

The form of Collagen invented by Polish chemists is homogeneous and transparent. It is a natural gel with a dynamic viscosity: 2010–2311 [mPa/s] without any mechanical impurities. Depending on the batch of fish skin, there are traces of pigment that give it a certain colour, ranging from graphite to pearl. Research material in individual samples was characterised by the divergence of results typical of the natural products and proteins from different organisms of the same species, all derived using the same method.

The statistical mean of 14 samples gave the following results:

| | |
|---|---|
| Water | 96.2% |
| Dry mass | 3.74% w s.m. |
| Proteins | 3.28% w s.m. |
| Collagen proteins | 2.91% w s.m. |

Besides proteins, there were certain quantities of free amino acids, including peptides, lipids, carbohydrates and amines (mostly prevalent in the bonds stabilizing collagen spirals). Non-collagenous protein components were always elastin and protein residues.

Main amino acids:

| | |
|---|---|
| Glycine | 30.31% |
| Proline | 11.29% |
| Alanine | 11.01% |
| Hydroxyproline | 10.70% |

Moreover: glutamine, arginine, lysine, hydroxylysine.

The dominant fish collagen subunits:

| | |
|---|---|
| Alpha 1 | 37.66% |
| Beta | 21.19% |
| Alpha 2 | 19.89% |
| Gamma 1 | 14.06% |
| Gamma 2 | 6.01% |
| Gamma 3 | 1.19% |

The molecular weight of fish tropocollagen in the test material was usually 357–362 [kDa]; however, for alpha 2 chains the molecular weight was often below 130 [kDa], and for alpha 1 chains it was often below 105 [kDa].

The hydroxyproline content measured to determine the quantity of pure collagen in solution is on average 0.301, which with a ratio of 10.7 (% of all amino acids) allows us to determine that there is about 3.22% of collagen in solution.

| | |
|---|---|
| pH | 3.54 |
| temperature stability | 27.1°C |
| total nitrogen | 14.29% |
| maximum length of the tropocollagen molecule | 300 nm |

At temperatures above the hydrate stability point, the collagen hydrate loses its gel form, turning into a watery liquid, and the electropherogram no longer shows ordered helical structures. The collagen hydrate coagulates and becomes a gelatin. It can be restored to its gel form by cooling, but the breakdown of helical structures is irreversible.

Collagen helical structure degradation mechanisms are totally unique and unpredictable. However, if the temperature is raised 10°C above the stability point, the most abundant helices dissimilation products are amino acids chains and micropeptides <1 nm.

Natural Fish Collagen hydrate, as a finished product, is manufactured in Poland, exclusively for the COLWAY Company, in highly specialised laboratories by Polish INVENTIA Technologies and according to ISO, HACCP, GMP, GSP standards.

The transfer of the collagen triple helix into an aqueous solution requires perfect process sterility. The water used for hydration must be naturally low in minerals

or demineralised via reverse osmosis, and it must be cleansed of any organism using UV rays. All Tools and instruments are sterilised in an autoclave.

The glass jars in which Natural Collagen is produced come with dispensers in order to prevent the "contamination" of the hydrate with bacterial flora from workers' fingertips. Hydrate suction tubes are made of materials that are totally resistant to reactions with acidic substances. Before being packed into cardboard boxes, the bottled product is placed in a styrofoam termobox to provide thermal protection. The same procedure applies to shipping, as there are termoboxes suitable for any quantity of products.

**Note!** All of the aforementioned results and comments are based on the repeated testing of Natural Collagen supplied by COLWAY from the series produced in 2004–2005. These results and comments cannot be compared to the results of standard laboratory samples, nor can they be compared to other collagen hydrates produced in Poland or even to previously manufactured batches of Natural Collagen, due to the fact that the product is subject to continuous improvement.

## 6.3. THE DERMOCOSMETIC EFFECTIVNESS OF FISH COLLAGEN HYDRATE

Natural Collagen is probably the first protein preparation in history to be suitable for dermatological cosmetic use almost immediately after leaving the living organism and without any engineering. It is a pure collagen hydrate; without any preservatives, colours or flavours. It is protected from decay by the total sterility of the manufacturing process and the prevention of bacteria membranes from appearing in the isolated protein with short-chain fatty acids bonded with simple alcohols. Besides protein and water, it contains only lactic acid (an organic acid that occurs naturally in muscles).

Lactic acid occurs naturally in the hydrolipidic layer of the skin. Together with other substances, this acid forms an endogenous protective layer. It moisturises and manages epidermal acanthosis processes. It is involved in the initial process of collagen extraction from fish, and it is also present in the final substance. It enables the collagen hydrate to penetrate the skin by opening the corneocytes, creating additional channels for epidermal penetratation.

Collagen is not capable of transepidermal penetration in the literal sense, and its external application is limited. Even molecular tertiary collagen would have

very limited penetration capabilities in its triple-helical form. Firstly, it is "too big" to pass through the epidermal barrier. Secondly, it is not possible for the triple helical structure, which is extremely fragile outside of the living organism, to "survive the journey through the jungle that is the stratum corneum", even if its channels are opened, for example, by iontophoresis.

The fact that a triplet helical conformation of the protein can be maintained during many years, outside the body of the donor fish, in a plain, disinfected jar is phenomenal. From a biochemical point of view, this is what makes this Polish invention so important.

The way fish collagen hydrate works, when topically applied, is much more complex. Alpha 1, alpha 2 and beta chain dissimilation products – peptides and amino acids – are subunits capable of penetrating the skin, due to their low molecular weight. They access the intercellular matrix area around fibroblasts primarily by extracellular means: "travelling" along gland channels, hair follicles and keratin deposits. Whilst "on the way", they stimulate corneocytes to increase production of cytokines such as FGF and TGF. Few collagen peptides, known in biochemistry as "signal" peptides, are able to reach the very core of fibroblasts. Finally, we see the continuous "support" of fibroblasts in increasing bodily collagen production activity.

The transdermal penetration mechanism of the degradation products of the triple-helix is no longer a topic of debate. It can be demonstrated by irradiating the hydrate before epicutaneous application and by following its "journey" into the skin. This can also be demonstrated by biopsy, which shows a dramatic increase in the level of hydroxyproline in fibroblast ECM samples several minutes after dermal application.

It has not yet been explained why it is so important that alpha and beta chain degradation products should come from the most recent dissimilation processes. In other words: why amino acids that dissimilate as a result of the denaturation of collagen in contact with the skin penetrate the epidermis incomparably better than "peptide carrion" amino acids, which never form collagen molecules ex vivo.

This is very important in practice, as fish collagen hydrate is an extremely fragile substance which can easily undergo thermal denaturation, resulting in irreversible despiralization due to improper storage. What was once a collection

of molecules, bonded together in a gel form, turns into a random bundle of disordered proteins in a watery liquid – a kind of "peptide soup".

We do not know exactly why amino acids that dissimilated from the triple helix, e.g. three days before, are less biologically active than those dissimilating directly on the surface of the skin, even though they come from the same product batch.

This fact is quite detrimental to the commercial success of Polish fish collagen. Maintaining the hydrate in temperatures below the denaturation point presents some logistical problems and it also significantly increases the costs of conventional distribution (wholesale-store-client). Among other things, this is where direct sales are an advantage. Being responsible for their goods, distributors take good care of the hydrate's storage and transport conditions and give clear instructions to the final consumer.

However, direct sale results in a form of distrust towards the product amongst many potential customers, opinion-forming circles, doctors and cosmetologists.

And so the self-limitations of this sensational product come full circle, which is in fact a major breakthrough in contemporary cosmetology.

The target effects of cosmetic products are also worth mentioning: effective skin hydration, thickening of the skin and increased mucin and mucous in the papillary dermis. Within 110–130 days, the systematic application of collagen hydrate removes virtually 100% of "dry" wrinkles resulting from inadequate water and lipophilic substance retention in the epidermis. It can the formation of new "senile" wrinkles for several years (!). It temporarily tightens skin defects such as mimic wrinkles and eye bags – which are not in fact wrinkles, but rather genetic or acquired weaknesses of facial micromuscles.

Collagen affects all skin types. The risk of reaction to fish protein is, in contrast to animal proteins, measured in per mils.

Based on customer surveys carried out on a huge group of respondents (several thousand), the Polish distributors of Natural Collagen created the following list of the effects of this dermal application:

- It forms a water retaining film on the dermis,
- It sends polypeptides and amino acids to the extracellular space of the dermis,
- It stimulates fibroblast and keratinocyte activity,
- It increases elasticity and softens the epidermis,

- It smoothens minor "senile" wrinkles,
- It fully removes "dry" wrinkles,
- It firms up and moistens the skin,
- It delays aging of the skin,
- It helps in most cases of acne during youth,
- It supports acne treatment,
- It supports cellulitis treatment,
- It fully removes the results of insect stings,
- It fully removes minor chafes, bruises and bedsores at an early stage,
- It fights most nail conditions,
- It regenerates and strengthens hair,
- It delays the appearance of grey hair,
- It supports the reproductive system's immunological protection,
- It removes heel spur,
- It makes furrows less visible,
- It eliminates blackheads,
- It treats scleroderma,
- It brightens nevuses and senile nevuses,
- It soothes neuralgia and chronic osteomyelitis,
- It lowers skin PH,
- It accelerates the burning of lactic acid in the muscles,
- It supports treatment of varicose veins in the legs,
- It softens the edges of old scars and smoothens new scars,
- It supports alergic skin treatments,
- It prevents stretch marks,
- It soothes bone, joint, arthritic and rheumatic pain,
- It delays osteoporosis,
- It eliminates swelling,
- It eliminates skin inflammations,
- It prevents the dilation of capillaries and telangiectasias,
- It removes trophic changes,
- It prevents melanoma,
- It supports rehabilitation after fractures, sprains and childbirth,
- When applied to eyelids, it improves sight,
- It regenerates vaginal mucosa,
- It prevents vaginal mycosis,
- It treats circinate psoriasis,

- It stops alopecia areata,
- It soothes periodontitis,
- It supports the treatment of diseases of the whites of the eyes,
- It improves venous and arterial circulation,
- It symptomatically relieves back pain and nerve root pain,
- It is more effective than nose drops in stopping a runny nose,
- It heals frostbite,
- It regulates excess sweating,
- It is the best balm after shaving and depilation,
- It is very effective after plastic surgery,
- It helps the skin to recover after invasive scrubs.

Regardless of the quality and effectiveness of its medical applications alone, fish collagen hydrates – including Natural Collagen – are registered as cosmetics in the country of manufacture, Poland. This is due to the fact that the registration of natural cosmetics is very simple (almost everywhere), in contrast with the registration of medicines in the European Union, which is costly, long and also requires complex clinical trials.

In such circumstances, the product administrators refrain from publicizing its therapeutic effects. EU regulation does not recognise the category of **cosmeceuticals** – cosmetics with uncontested healing properties. This is namely the group that would include fish collagen hydrate.

There are also possible contraindications to the use of Natural Collagen:

- Low protein diet in serious (requiring dialysis) kidney disorders,
- Chemotherapy,
- Radiotherapy,
- Collagenosis-related disorders,
- 4th-8th month of pregnancy (on legal grounds),
- Egzema resulting from protein allergies,
- Alergy to fish protein (around 0.008% of the population).

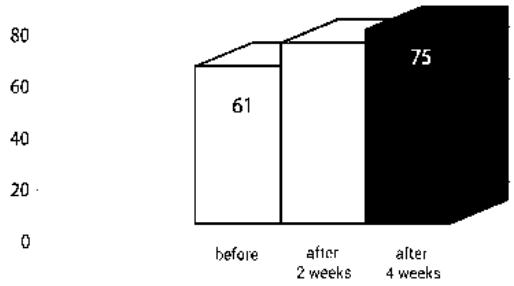
We would like to add at this point that we believe these contraindications are rather far-reaching assurances for the legal concerns of their authors rather than objective contraindications. Perhaps Polish doctors, whose knowledge of fish tropocollagen is, in our opinion, very inadequate, made suggestions to the distributing company of any theoretically possible contraindications. Our research's present state does not allow us to confirm any of them. However, as is always the case in medicine, one can be wrong.

## 6.4. FISH COLLAGEN HYDRATE APPLICATION AND INSTRUMENTAL RESEARCH

Numerous studies have been conducted on the application and effects of collagen hydrate on the skin. Below is a plain outline (without any comments) of the research results during the so-called "short period": 2 weeks and 4 weeks. In terms of Polish law, this is not the Research Report, which can be reproduced only in its full version.

Access to the full report: Colway Sp. J. 84-207 Koleczkowo, Hippiczna Str. 2, telephone: 0048 58 6762262 www.colway.net.pl

Averege results of cheek skin moisturization before and after 2 and 4 weeks of the preparation's application (in units of measurement)




| | SKIN MOISTURIZATION (UNITS OF MEASUREMENT) | | |
|---|---|---|---|
| $X_{śr}$ [j.p.] | 61 | 70 | 75 |
| Average increase of skin moisturaization (units of measurement) | | 9 | 14 |
| average increase of skin moisturaization [%] | 100* | 15 | 23 |

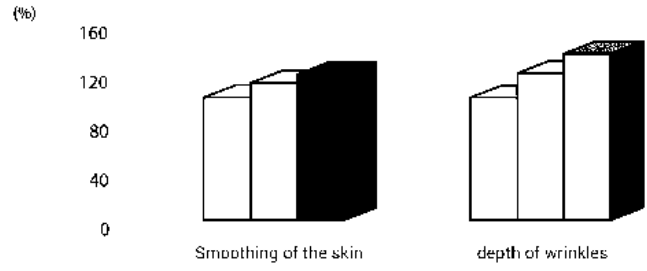
Average results of biomechanical skim parameters of cheek skin before and after 2 and 4 weeks of the preparation's application (in %)


| | SKIN FIRMNESS | | | TOTAL SKIN ELASTICITY | | | SKIN PLASTICITY | | |
|---|---|---|---|---|---|---|---|---|---|
| | $P_0$ | $P_k^2$ | $P_k^4$ | $P_0$ | $P_k^2$ | $P_k^4$ | $P_0$ | $P_k^2$ | $P_k^4$ |
| $X_{av}$ [units of measurment] | 0,1546 | 0,1964 | 0,2191 | 0,6597 | 0,6965 | 0,7322 | 0,3266 | 0,3058 | 0,2590 |
| Average increase [%] | 100* | 26 | 42 | 100* | 6 | 11 | 100* | 6 | 17 |

Info: $P_0$ – results before the application,
$P_k^2$ – results after 2 weeks of the preparation's application,
$P_k^4$ – results after 4 weeks of the preparation's application,
* – $P_0$ taken as 100%

| | SMOOTHING OF THE SKIN | | |
|---|---|---|---|
| | $P_0$ | $P_k^2$ | $P_k^4$ |
| $X_{av}$ [units of measurment] | 15 | 13 | 12 |
| Average increase in smoothing of the skin [%] | 100* | 12 | 19 |

Average results for cheek skin smoothing and decrease in wrinkle depth (around the eyes) before and after 2 and 4 weeks of the preparation's application (in %)




| | DEPTH OF WRINKLES | | |
|---|---|---|---|
| | $P_0$ | $P_k^2$ | $P_k^4$ |
| $X_{AV}$ [units of measurment] | 0,15 | 0,12 | 0,10 |
| Average increase in smoothing of the skin [%] | 100* | 21 | 36 |

Info: $P_0$ – results before the application,
$P_k^2$ – results after 2 weeks of the preparation's application,
$P_k^4$ – results after 4 weeks of the preparation's application,
* – P0 taken as 100%

Average results for cheek skin moisturization (around the eyes) before and after 2 and 4 weeks of the preparation's application.

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| Proband's code [number-initials/age] | Skin moisturization [units of measurement] | | | Increase in skin moisturization [%] | |
|---|---|---|---|---|---|
| | $P_0$ | $P_k^2$ | $P_k^4$ | after 2 weeks | after 4 weeks |
| 1-TM/60 | 56 | 66 | 75 | 18 | 34 |
| 2-MS/39 | 66 | 74 | 75 | 12 | 14 |
| 3-ChM/60 | 58 | 68 | 72 | 17 | 24 |
| 4-MA/39 | 64 | 74 | 77 | 16 | 20 |
| 5-MS/39 | 65 | 72 | 75 | 11 | 15 |
| 6-MM/60 | 58 | 68 | 77 | 17 | 66 |
| $X_{Average}$ | 61 | 70 | 75 | 15 | 23 |

Info: $P_0$ – results before the application,
$P_k^2$ – results after 2 weeks of the preparation's application,
$P_k^4$ – results after 4 weeks of the preparation's application.

Average results for cheek skin firmness (around the eyes) before and after 2 and 4 weeks of the preparation's application.

| Proband's code [number-initials/age] | Skin firmness [units of measurement] | | | increase in skin firmness [%] | |
|---|---|---|---|---|---|
| | $P_0$ | $P_k^2$ | $P_k^4$ | after 2 weeks | after 4 weeks |
| 1-TM/60 | 0,1305 | 0,1600 | 0,1865 | 23 | 43 |
| 2-MS/39 | 0,2550 | 0,3350 | 0,3650 | 31 | 43 |
| 3-ChM/60 | 0,1367 | 0,1550 | 0,1633 | 13 | 20 |
| 4-MA/39 | 0,1067 | 0,1467 | 0,1700 | 37 | 59 |
| 5-MS/39 | 0,1185 | 0,1450 | 0,1667 | 22 | 41 |
| 6-MM/60 | 0,1800 | 0,2366 | 0,2630 | 31 | 46 |
| $X_{Average}$ | 0,1546 | 0,1964 | 0,2191 | 26 | 42 |

The results of the systematic application of fish collagen hydrate on the skin during 110–130 days are far more spectacular. The examination results obtained in the Ukraine, in Poland and in Russia coincide completely. There is, however, still no investor willing to support such a controlled examination, during 110–130 days, of a number of probands, which would enable the study of clinical applications.

Systemic collagen in the skin is completely replaced over the course of a 100–190 day cycle, depending on age, gender, race, skin type and other factors.

In the case of north-central European women, aged 35–40, with a rather bright carnation and skin type, defined by cosmetologists as "normal" or "mixed", who, during the test period:

- do not expose their dermis to sun, wind, frost;
- do not smoke;
- maintain the right nutritional amino acid balance;
- maintain a stable level of ascorbic acid (vitamin C), vitamins E, A, $B_6$ and basic micronutrients;
- do not follow a low calorie diet;
- remain generally healthy;
- remain stress-free,

it is impossible, in our opinion, that a 110–130 day dermal application of fish collagen hydrate would not stop, for a few years, the formation of new wrinkles in the areas which received regular (1–3 times a day) application of the protein preparation on wet skin.

These conclusions are probably quite bold, but they are supported by many observations and interviews with patients during 2005–2008.