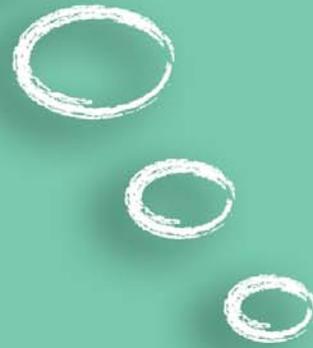


ARTHRED



Cargill Texturizing Solutions.
NUTRITION FOR HEALTHY JOINTS.

Cargill[™]

Table of Contents

Healthy Thinking	3
Introduction	3
Joint problems, one of the leading risks of disability	3
What is Arthred™ hydrolyzed collagen?	3
Functionality of Cartilage	3
Bioavailability of Arthred™ hydrolyzed collagen	4
▪ Preclinical Studies	4
▪ Clinical Studies	5
Hydrolyzed Collagen and Joint Health	6
▪ Clinical Studies	6
Mode of Action of hydrolyzed collagen	7
Suggested Intake	8
Technical Data	8
Safety and Regulatory Aspects	9
Summary	9
References	10

ARTHRED



Healthy Thinking

Thinking about health and nutrition? Keen to use nature-based food ingredients to produce new products targeted for health and wellness? Looking for innovative and scientifically-based approaches to developing dietary supplements, functional foods, or sports nutrition? Let's work together to develop products that contribute to a healthy and comfortable life style for your customers.

Educated consumers expect nutritionally valuable foods - foods that are vital to a well-balanced diet while providing science-backed health benefits. Moreover, the market for nature-based supplements and functional food ingredients is growing rapidly.

Cargill has responded to these needs with a range of innovative solutions based on plant or animal derived ingredients that can help promote health and wellness. Moreover, with our global networking, focus on quality, and strong research and development capabilities, we are well prepared to help you meet future challenges.

We provide all major segments of the food, beverage, personal care, and pharmaceutical industries with specialized individual ingredients. And we offer custom-developed solutions for producing fresh, tasty, and health-promoting food. In addition, our worldwide network of application service centers, technical service laboratories, and production facilities enables Cargill to maintain an active presence both internationally and locally.

Introduction

This brochure is designed to provide you, a valued Cargill business customer, with an overview of the current state of scientific data on hydrolyzed collagen and the direction in which it is headed. Results from *in vitro* studies, preclinical research, and clinical studies in generally healthy populations are discussed. The results should be regarded as preliminary in many of the considered areas. As with all emerging science, the information and interpretation of the data may change with further research. Thus, this science review is offered for your information and consideration. It is not intended to serve as the sole basis for any food labeling claims.

Joint Problems - One of the Leading Risks of Disability

Early protection of cartilage is very important to ensure its health over time. Everyone is at risk of decreasing joint health as a result of the natural ageing process, environmental influence, and congenital or inherited factors. Individual lifestyles that may influence joint health include obesity and strenuous physical activities, e.g. professions involving activities, exercises, or sports that continually stress joints or cause injury. Proper nutrition, lifestyle choices to maintain or achieve a healthy body weight, and protection from abrasion with exercise, rest, and relief from stress are important factors in maintaining joint health [1, 2]. In addition, cartilage should be stimulated to regenerate itself as much as possible [3].

What Is Arthred™ hydrolyzed collagen?

Arthred™ hydrolyzed collagen is produced by enzymatic hydrolysis of collagen from pigs and cattle under controlled conditions [4]. Hydrolyzed collagen or hydrolyzed gelatin is a special type of gelatin with a much lower molecular weight (MW). This enzymatic hydrolysis process yields short-chained peptides having a low mean MW of approximately 3 kilodaltons (kDa) (mean chain length of 25 - 30 amino acids). Upon further processing, the product is spray-dried into an agglomerated form which offers excellent solubility. Because it is sourced from animal collagen, the amino-acid composition of the peptides resembles that of collagen in human articular (joint) cartilage. The product has a high content of the amino acids hydroxyproline and hydroxylysine as is characteristic of collagen. The predominant amino acids in Arthred™ hydrolyzed collagen – glycine, proline, hydroxyproline, lysine, and hydroxylysine – comprise approximately 50% of the substance, by weight.

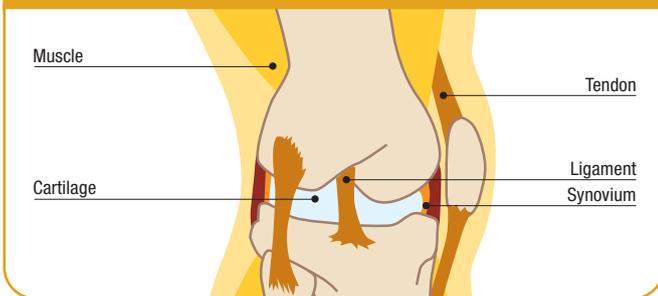
Functionality of Cartilage

Joints are movable interfaces between bones. They are intended to enable a high degree of mobility but, at the same time, must ensure adequate stability. There are different types of joints for different types of movement. Ball-and-socket joints, such as the hip and shoulder, allow full rotation of the joint; hinged joints, such as the knee, allow movement in back and forth directions (See Figure 1).

Muscles and ligaments hold the bones in place and allow joints to bend and move as nature intended. Tendons are tough, fibrous cords



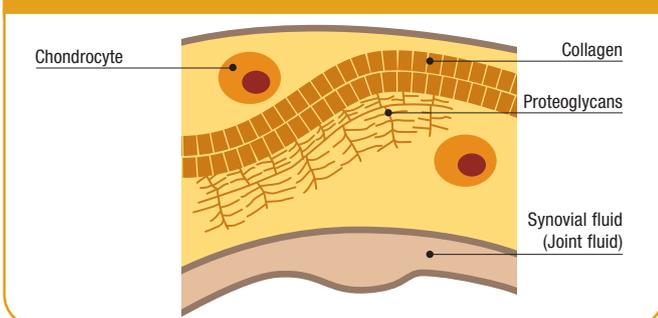
Fig. 1 - Anatomy of a knee joint (side view)



that connect muscles to bones. Together, tendons, ligaments, and muscles help to control and support the joints while allowing movement and range of motion.

In general, joints consist of two or more bone ends, a rounded ball and a hollowed counterpart that fit together [1]. Both bones are coated with a smooth, elastic layer of cartilage that protects the joint cavity. Since cartilage contains no blood vessels, the synovial fluid, which bathes the cartilage, supplies it with nutrients and removes waste. The synovial membrane (synovium) produces the synovial fluid which is continually renewed so it can provide continuous lubrication between the opposing layers of cartilage. Cartilage is composed mostly of water, as well as other components such as collagen, proteoglycans, and chondrocytes described below (See Figure 2).

Fig. 2 - Components of cartilage



Collagen:

This fibrous extracellular protein is the building block of skin, tendons, bones, and other connective tissues. In cartilage, the predominant collagen is collagen type II which has a triple-helical conformation and forms a meshwork that offers support and flexibility to joints.

Proteoglycans:

The basic structure of these macromolecules includes a core protein with at least one (but frequently up to tens or hundreds) carbohydrate

chain, the glycosaminoglycans. In cartilage, strands of proteoglycans and collagen are interwoven to form a meshwork that allows cartilage to flex and absorb physical shock.

Chondrocytes:

These cells are embedded in cartilage and help cartilage to grow and remain healthy. Chondrocytes synthesize collagen and proteoglycans that form the extracellular matrix – the basis of the cartilage tissue.

Cartilage remodeling with continuous turnover of collagen and proteoglycan components may be more dynamic than traditionally thought. Anabolic (regenerative) and catabolic (degenerative) processes normally balance to ensure homeostasis in response to conditions such as altered gait, weight distribution, traumatic injury, and others [5]. However, many lifestyle choices lead to scenarios where joint disuse, overuse, and/or stress can contribute to increased catabolic processes that are not fully ameliorated by the homeostatic anabolic processes [6]. As a result, a continuum of joint health is possible among the generally healthy human population. Preliminary evidence suggests Arthred™ hydrolyzed collagen may help to support the resynthesis and preservation of cartilage and, thus, maintenance of joint health in light of some of the effects associated with normal abrasion of the joints [7].

Bioavailability of Arthred™ hydrolyzed collagen

The term bioavailability describes the degree to which a substance can be absorbed and is available to interact in metabolism. The bioavailability of protein from protein fragments is influenced mainly by size and amino-acid composition. Absorption and distribution of Arthred™ hydrolyzed collagen clearly differs from that of free amino acids and, due to its low mean MW (2-3 kDa), from that of conventional collagen (30-90 kDa). As a concentrated source of typical collagen amino acids (glycine, proline, hydroxyproline, lysine, and hydroxylysine) and peptides, Arthred™ hydrolyzed collagen helps to supply them to collagen-containing tissues, such as cartilage, throughout the body [3].

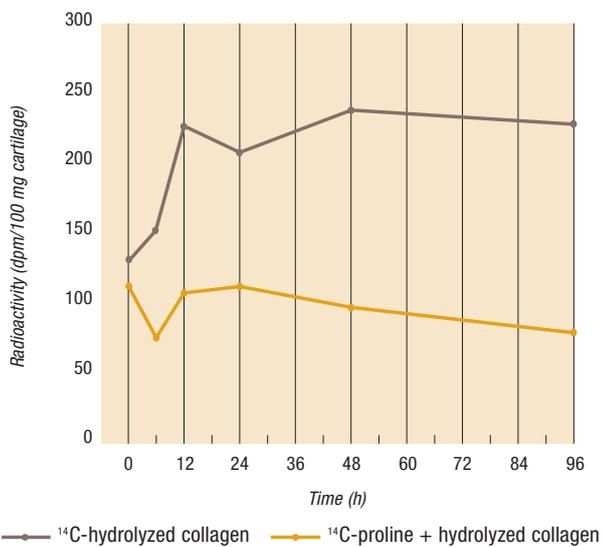
■ Pre-Clinical Studies

Oesser et al. (1999) investigated the time course of hydrolyzed collagen absorption as well as its subsequent distribution in various tissues and organs in mice [8]. Absorption of ¹⁴C-radiolabeled hydrolyzed collagen (¹⁴C-radiolabeled proline peptides, approximately 3 kDa MW) was compared to that in a control group (¹⁴C-radiolabeled



proline, as collagen-specific free amino acid, plus unlabeled hydrolyzed collagen). Ninety-five percent of orally administered hydrolyzed collagen was absorbed within the first 12 hours. Hydrolyzed collagen was not only taken up in the form of free amino acids, but also as peptides of 2.5-15 kDa MW-differentiating it from free amino acid sources. The distribution of labeled hydrolyzed collagen in the various tissues was similar to that in the control group (^{14}C -proline plus hydrolyzed collagen) except that there was a pronounced and continuing accumulation of ^{14}C -hydrolyzed collagen in cartilage. The measured radioactivity was more than twice as high in cartilage following ^{14}C -hydrolyzed collagen administration compared to the control group (^{14}C -proline plus hydrolyzed collagen) indicating that hydrolyzed collagen preferentially accumulated in cartilage tissue (See Figure 3).

Fig. 3 - Time course of ^{14}C -radiolabeled hydrolyzed collagen and ^{14}C -proline in cartilage after oral administration to mice

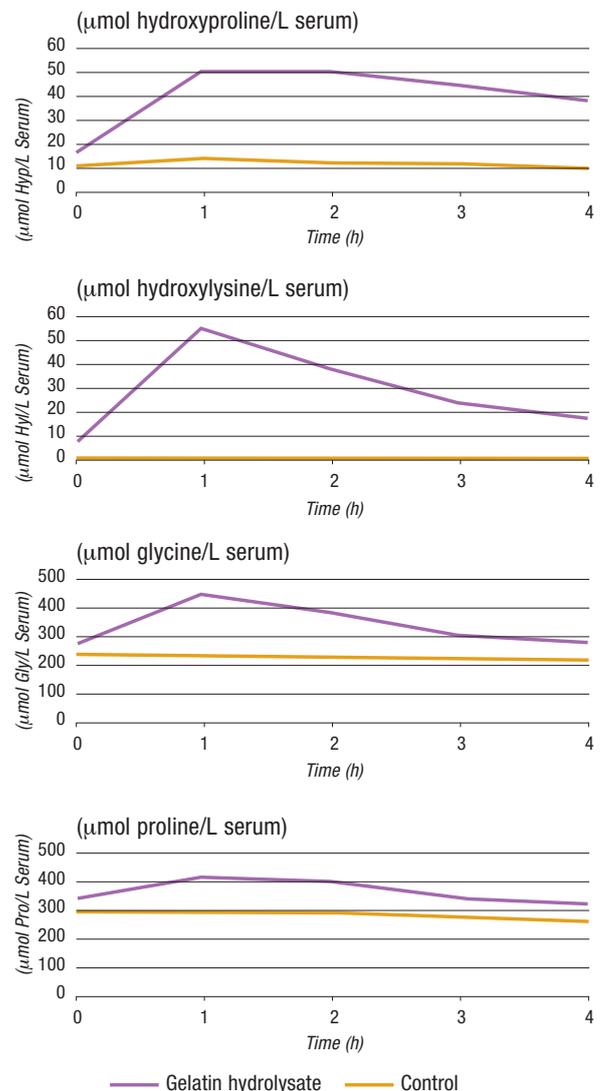


In addition, the lack of difference in accumulation of radioactivity in the liver (the main organ of metabolism) between ^{14}C -hydrolyzed collagen compared to ^{14}C -proline led to the conclusion that differences in metabolism could not explain the preferential accumulation in the cartilage. This study suggests that collagen hydrolysate accumulates in cartilage and may be involved in a feedback loop that helps to regulate chondrocyte metabolism. This may be relevant to the human body when joint cartilage homeostasis not optimal. Additional research is needed to identify the exact mechanisms of the preferential accumulation.

Clinical Studies

Hydroxyproline is one of the main components of ArthredTM hydrolyzed collagen and is therefore a good marker whose uptake can be quantified in blood serum [10]. The absorption of short-term orally-administered hydrolyzed collagen was measured by a single supplementation of 10g ArthredTM hydrolyzed collagen. When compared to a standard diet control without hydrolyzed collagen, supplementation resulted in a significant increase in collagen-characteristic amino acids like hydroxyproline, hydroxylysine, glycine, and proline in blood serum [9] (See Figure 4).

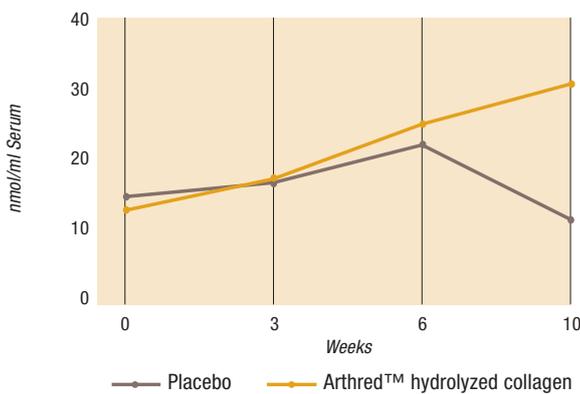
Fig. 4 - Amino acid concentration in serum after oral administration of gelatin hydrolysate





Beuker et al. (1993) conducted a double-blind, placebo-controlled clinical trial involving 52 student athletes (aged 21 to 27 years) to investigate amino acid concentrations in serum following long-term oral administration of Arthred™ hydrolyzed collagen or a standard diet without hydrolyzed collagen (placebo) during exercise [10]. The athletes performed one hour of physical training three times a week during a period of four months. The collagen-specific amino acid hydroxyproline concentration in plasma was continuously and significantly elevated during supplementation with hydrolyzed collagen providing evidence that collagen was absorbed into the bloodstream. The placebo had no effect on hydroxyproline levels in the blood (See Figure 5).

Fig. 5 - Hydroxyproline concentration during supplementation with Arthred™ hydrolyzed collagen in humans



Hydrolyzed Collagen and Joint Health

Potential benefits of gelatin (hydrolyzed collagen) supplementation for joint health have been envisaged since medieval times. Hildegard von Bingen (1098-1179) provided the basis for using collagen-containing preparations [4].

“He who has stabbing pain in his limbs and joints as well as stomach and intestinal pain should frequently eat plenty of well-cooked beef trotters, including fat and calluses. That soon gets rid of the pain.” [4]

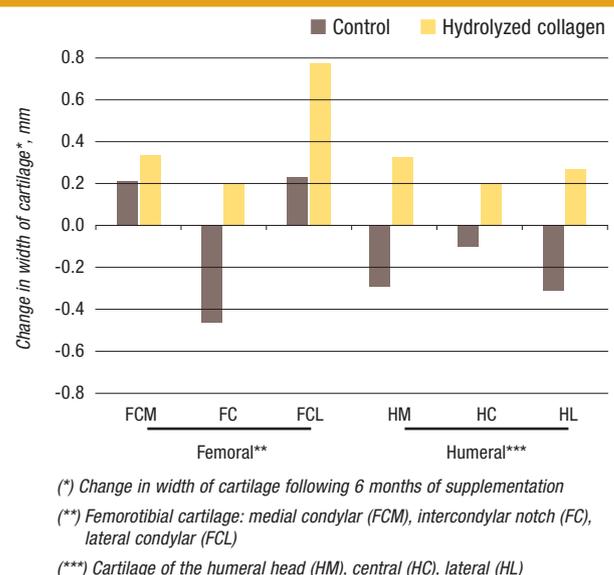
In the more recent past, several clinical trials have suggested that supplementation with hydrolyzed collagen may be helpful in reducing pain and improving functional ability in joints among people with unhealthy, arthritic, or degenerative joints. However, these studies are outside the scope of this review as they were conducted in populations that can not be regarded as generally healthy due to confirmed diagnosis of degenerative joint diseases.

The most recent clinical studies provide the first indication of the ability of hydrolyzed collagen to support more normal joint function and cartilage homeostasis. This clinical research has been conducted among generally healthy athletes providing preliminary evidence that hydrolyzed collagen may help in the maintenance of healthy cartilage homeostasis.

Clinical Studies

Among healthy, competitive Spanish male mountain bikers (n=16) and female basketball players (n=10), Fernandez and Perez (1998) examined the effect of hydrolyzed collagen (also called hydrolyzed gelatin) on shoulder and knee joints. Subjects consumed hydrolyzed collagen (10g/day) enriched with magnesium, vitamins B1, B2, B6 and pantothenic acid for a six-month period [11]. After supplementation, biometric ultrasound values of the cartilage showed statistically significant increases of cartilage thickness in supplemented athletes while control subjects exhibited either no change or decreasing cartilage thickness (See Figure 6). This indicates that enriched hydrolyzed collagen may help maintain cartilage homeostasis and joint health among athletes whose joints have been stressed through competitive athletic performance.

Fig. 6 - Effect of hydrolyzed collagen on cartilage thickness



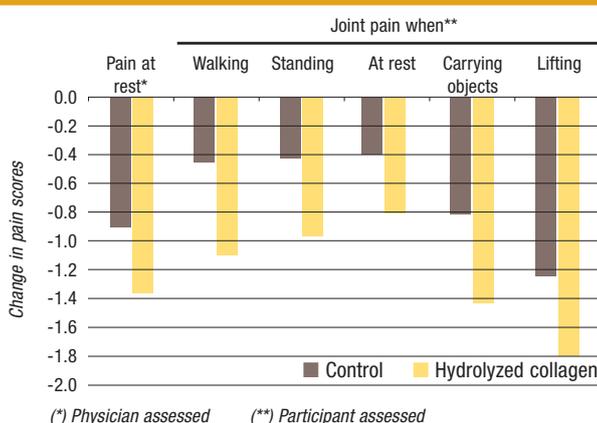
In another study, Flechsenhar (2005) examined 84 German athletes (average age 41 years) who consumed 10g collagen hydrolysate per day for 12 weeks [12]. The study population consisted of athletes who were generally healthy, participated in a variety of performance



classes, and had painful joints (knee, hip, shoulder). Excluded from the study population were athletes 1) with an acute injury or inflammation change, 2) consuming chondroitin or glucosamine, 3) expecting a change in analgesic or anti-inflammatory therapy, or 4) suffering from diagnosed disease. After 4 to 6 weeks and at the end of the study period (12 weeks), measurements of pain, restriction of movement, and inflammation were measured. In most instances, knee, hip, and shoulder pain at rest and during test exercises was reduced with hydrolyzed collagen supplementation [12].

Clark et al. (2008) investigated the effects of collagen hydrolysate on generally healthy and physically active subjects (n=97) competing in varsity or club sports and complaining from joint pain or discomfort [13]. Athletes were excluded from the study population if they 1) did not have joint pain or discomfort, 2) had an acute injury or inflammatory process, 3) consumed chondroitin, glucosamine, or other dietary supplements indicated for joint health, or 4) were likely to increase their dose of analgesics due to severe symptoms of arthralgia. In this 24-week, randomized, double-blind, placebo controlled study, subjects received either 10g collagen hydrolysate per day in a 25 ml liquid formulation or a placebo that contained xanthan. Physician- and participant-assessed joint pain measures assessed by visual analogue scales were improved (clinically and statistically significant) with supplementation of collagen hydrolysate (See Figure 7). The improvement in pain was more pronounced in a sub-group of subjects with knee pain. These results from clinical studies in generally healthy and physically active subjects provide preliminary indication that collagen hydrolysate may promote the healthy functioning of joints under conditions that may be associated with increased catabolic or degenerative processes in cartilage homeostasis.

Fig. 7 - Effect of hydrolyzed collagen on cartilage thickness

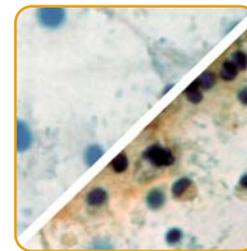


Mode of Action of Hydrolyzed Collagen

The functional integrity of cartilage is dependent on maintenance of the extracellular matrix, a process controlled by articular chondrocytes as previously described. Oesser and Seifert (2003) investigated the effect of hydrolyzed collagen on the formation of type II collagen by articular chondrocytes in a cell culture model [3]. Chondrocytes were cultivated over a period of eleven days. In the presence of collagen hydrolysate (0.5mg/mL) and type II collagen, the articular chondrocytes were able to stimulate type II collagen biosynthesis over the 11-day period. (See Figure 8, lower right).

Fig. 8 - Enhancement of type II collagen biosynthesis by hydrolyzed collagen

Without hydrolyzed collagen



With hydrolyzed collagen

The control, containing native type I and type II collagen in a collagen-free hydrolysate, failed to show any effect (See Figure 8, upper left). These results indicate a stimulatory effect of hydrolyzed collagen on type II collagen biosynthesis of chondrocytes and suggest the need for further testing to determine the mechanisms that regulate collagen biosynthesis.

This study and other work by Oesser (Bello and Oesser, 2006 [14]; Oesser et al., 1999 [8]) suggest that collagen hydrolysate is absorbed from the intestine in its high molecular weight form, preferentially accumulates in the cartilage, and is able to stimulate chondrocyte metabolism. Oesser and colleagues speculate that collagen hydrolysate may be useful as an oral supplement to activate collagen biosynthesis in human chondrocytes in conditions where cartilage is stressed and joints are painful. They further identify individuals who may experience joint stress, discomfort, or pain as including individuals age 50 and older, active and vigorous athletes, individuals engaging in repetitive motions, and individuals who are overweight, sedentary, or have a familial history of joint disease. These conditions correspond to those that are known to shift cartilage homeostasis to a more catabolic state.



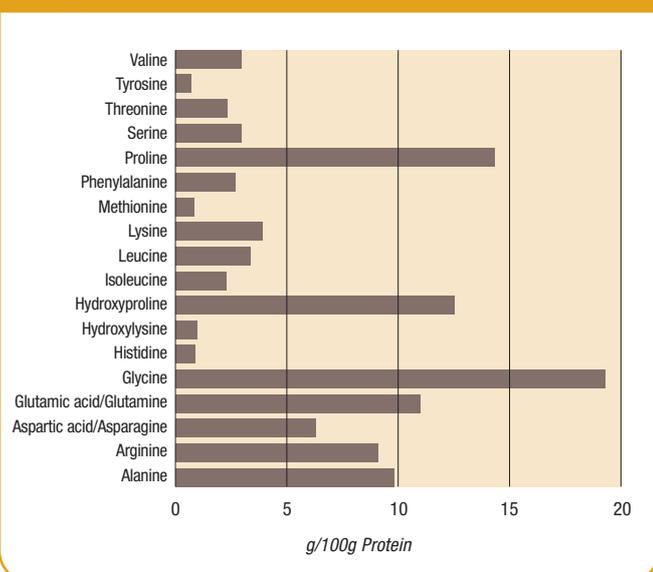
Suggested Intake

Ten grams of Arthred™ hydrolyzed collagen, such as in powder form, mixed into a favorite non-carbonated beverage, hot or cold, is the suggested daily intake. This amount has been shown to be well tolerated in preliminary clinical trials. Supplementation with Arthred™ hydrolyzed collagen daily may help to provide the body with nutritional support for joint function when joints are stressed in daily activities to maintain joint health in a population with healthy joints. However, more studies are needed to confirm these potential health benefits of hydrolyzed collagen among individuals with healthy joints.

Technical Data

Arthred™ hydrolyzed collagen is spray dried and agglomerated to fine granules. When added to mixtures, it has no effect on the sensory properties of the final product even when used in a highly concentrated form. Arthred™ hydrolyzed collagen contains no fat or carbohydrates, no purine or cholesterol, and is free from preservatives in most forms. Arthred™ hydrolyzed collagen contains 89 - 94 % protein, 4 - 9 % water, and not more than 2 % minerals. The average molecular weight of Arthred™ hydrolyzed collagen is approximately 3 kDa, which corresponds to a mean chain length of 25 - 30 amino acids with the typical amino-acid composition as shown in Figure 9.

Fig. 9 - Average amino acid composition of hydrolyzed collagen



Arthred™ hydrolyzed collagen is compatible with a wide variety of functional food applications due to its characteristics:

- Non-gelling - due to low mean MW (See Figure 10).
- Water soluble - contains fine-grained granules that dissolve rapidly in cold or warm water without forming clumps; forms a yellowish clear solution (between pH 5 and 6.5) (See Figure 11); works well for carbonated and instant beverage applications.
- Dust-free - can be poured and dispensed without generating dust
- Excellent flowability - no cohesion or clogging
- Neutral flavor- can easily be blended with sweet or savory flavor systems
- Stable - average shelf-life of five years, under cool and dry conditions; in aqueous solutions average shelf life of 1 year+ (after pasteurization, with preservatives (e.g. benzoic acid, sorbic acid))
- Good binding agent-binder for food components in energy bar or high-fiber bar applications

Fig. 10 - Viscosity of Arthred™ hydrolyzed collagen (25°C) in water

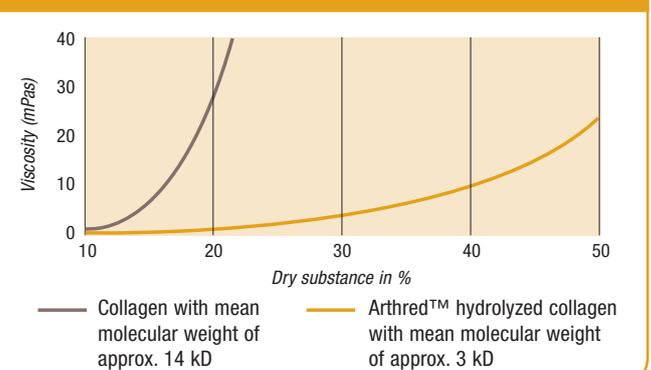


Fig. 11 - Time for complete dissolution of Arthred™ hydrolyzed collagen in water at different temperatures with stirring

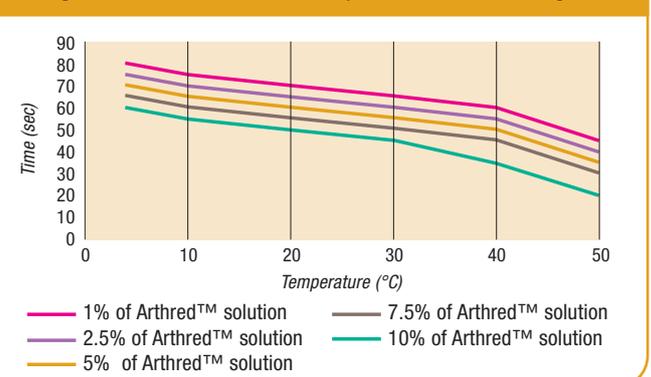
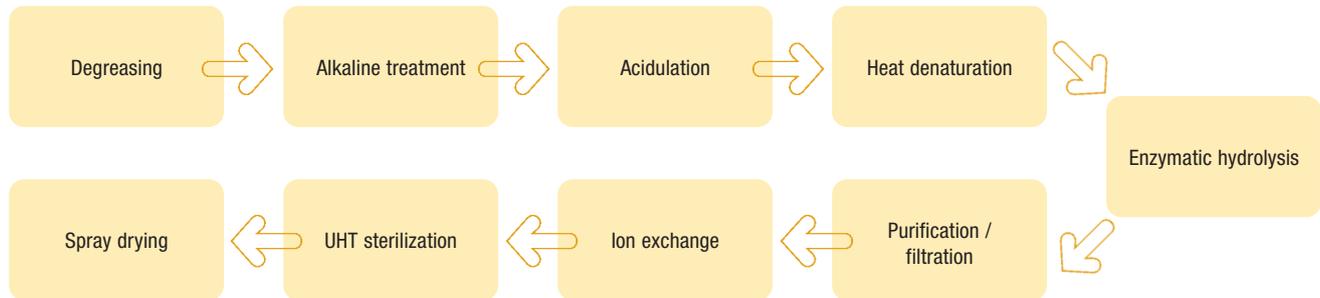




Fig. 12 - Arthred™ hydrolyzed collagen is produced using a complex multi-step procedure



Safety and Regulatory Considerations

The raw material for Arthred™ hydrolyzed collagen is sourced from pigs or cattle. Raw materials used in the production of Arthred™ hydrolyzed collagen originate from healthy animals that have been approved for human consumption by the relevant regulatory authorities.

The manufacturing process for hydrolyzed collagen is well-controlled leading to a product that meets food grade specifications (See Figure 12). The FDA recognizes hydrolyzed collagen (gelatin) as GRAS (Generally Recognized as Safe) when used in accordance with Good Manufacturing Practices [15,16,17]. In addition, the Scientific Committee on Food of the EU (European Union) confirmed in 2002 that gelatin (hydrolyzed collagen) is safe for human consumption provided it is manufactured according to good hygiene practices [18]. Additionally, the European Food Safety Authority (EFSA) confirmed the safety of hydrolyzed collagen or gelatin from cattle [19].

Summary

- Arthred™ hydrolyzed collagen has short-chain peptides of low mean MW of approximately 3 kDa (mean chain length of 25 - 30 amino acids).
- Due to its source, the amino-acid composition of Arthred™ hydrolyzed collagen resembles that of collagen in articular cartilage which is characterized by a high proportion of hydroxyproline and hydroxylysine.
- Arthred™ hydrolyzed collagen is well-absorbed and the results of a study in mice suggests it accumulates in cartilage tissue.
- Preliminary results on the biometry of cartilage of competitive athletes indicate that hydrolyzed collagen, such as Arthred™ hydrolyzed collagen, might support joint maintenance in generally healthy, physically active individuals.
- *In vitro* studies indicate a possible stimulatory effect of Arthred™ hydrolyzed collagen on the type II collagen biosynthesis and secretion in chondrocytes.
- A 10 g intake of Arthred™ hydrolyzed collagen per day is suggested based on the available data.
- Arthred™ hydrolyzed collagen is stable and readily soluble by stirring into cold or warm water. It does not gel in concentrated solutions.
- Hydrolyzed collagen is confirmed as GRAS by the FDA and safe for human consumption by the Scientific Committee on Food of the European Union.

The statements in this brochure have not been evaluated by the Food and Drug Administration or EU regulatory authorities. This product is not intended to diagnose, treat, cure, or prevent any disease.



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This brochure is designed to provide you, a valued Cargill business customer, with an overview of the available scientific data on hydrolyzed collagen. The purpose of the brochure is to provide you with a sense of the current state of the research and the direction in which it is headed.

The brochure is not intended to serve as the basis for any claims that you may choose to make with respect to your products. It is important to note that in the EU and in the USA, claims that state, suggest, or imply that a relationship exists between a substance and health should meet stringent standards with regard to adequacy of supportive evidence. In addition prior approval by the EU or FDA may be needed before certain claims can be made, for instance, on the label or labeling of food products. Therefore, you may need to seek EU or FDA approval prior to making claims based on data discussed in the brochure. Moreover, any claim that a product is "intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals" would likely be viewed as a prohibited drug claim, unless being done within the context of a medicine/drug registration.

Regulations regarding claims are complicated and we strongly recommend that you seek the assistance of legal counsel prior to making any claims. In some instances, the manner in which a claim is worded can make the difference between an acceptable claim and a prohibited claim. In some cases, however, a claim may be prohibited regardless of the wording.

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collaborate > create > succeed™

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