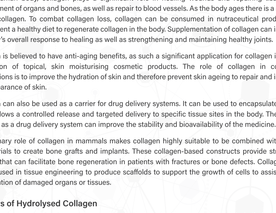
ENZYMATIC HYDROLYSIS FOR THE PRODUCTION OF COLLAGEN PEPTIDES

Collagen is produced naturally by the human body and is the most abundant protein in all mammals, providing the super structure that holds the body together. It is the key protein of all the connective tissues in the body including cartilage, bone, tendon and skin. Made up of fibrous structures, collagen provides both a strong and flexible structure to the body. Unlike other proteins, collagen has a unique combination of amino acids containing hydroxyproline, which is not present in other proteins, as well as high levels of glycine, proline, and arginine. Three main types of collagen (Type I, Type II and Type III), each composed of different amino acids, make up 90% of the collagen in the human body.

The key sources of collagen for use in industrial applications are bovine, porcine and marine/fish products. The origins of collagen have different advantages and disadvantages depending on their application and end product requirement. Bovine collagen extracted from cartilage, tendons and cow hides, are most similar in terms of collagen "Type" to the collagen found in the human body. Porcine derived collagen is often sourced from porcine skin. Although there are cultural barriers with porcine collagen not suitable for Kosher and Halal products and the potential impact on supply from common animal diseases such as swine flu, porcine collagen is also unique in that it often has a lower molecular weight than other sources of collagen. Marine collagen is derived from the by-products of the fish processing industry, such as fish viscera. Fish collagen has a higher bioavailability and absorption than bovine and porcine-derived collagen and therefore the health and nutritional benefits can be more easily absorbed.

Applications of Collagen



Collagen is an extremely functional and valuable protein, with many perceived health benefits. In its abundance from animal sources, collagen is widely used to add value across a breadth of different applications.

The functionality of collagen makes this an increasingly high value additive in the food and beverage industry to processed foods collagen can be used to replace the addition of animal fat. Its water holding capacity can improve the stability and consistency of food ingredients when heated as part of the cooking process. Collagen fibres can also be used to improve the emulsification of beverages.

In the human body, collagen plays a significant role in healing wounds and repairing tissue, the development of organs and bones, as well as repair to blood vessels. As the body ages there is a notable loss of collagen. To combat collagen loss, collagen can be consumed in nutraceutical products to supplement a healthy diet to regenerate collagen in the body. Supplementation of collagen can improve the body's overall response to healing as well as strengthening and maintaining healthy joints.

Collagen is believed to have anti-aging benefits, as such a significant application for collagen is in the production of topical, skin moisturising cosmetic products. The role of collagen in cosmetic preparations is to improve the hydration of skin and therefore prevent skin ageing to repair and improve the appearance of skin.

Collagen can also be used as a carrier for drug delivery systems. It can be used to encapsulate drugs, which allows a controlled release and targeted delivery to specific tissue sites in the body. The use of collagen as a drug delivery system can improve the stability and bioavailability of the medicine.

The primary role of collagen in mammals makes collagen highly suitable to be combined with other biomaterials to create bone grafts and implants. These collagen-based constructs provide structural support that can facilitate bone regeneration in patients with fractures or bone defects. Collagen can also be used in tissue engineering to produce scaffolds to support the growth of cells to assist in the regeneration of damaged organs or tissues.

Benefits of Hydrolysed Collagen

Collagen is a complex protein made up of three chains of amino acids. This triple helix is the main structure of collagen giving it it's fibrous quality for specific binding in its natural form. Native collagen is insoluble and so is limiting in its use in applications. Collagen extracted from skin and bones, known as gelatin or extracted collagen, has a much more versatile functionality and therefore greater suitability in food and pharmaceutical applications. The functionality of gelatin can be further enhanced through hydrolysis. Hydrolysing gelatin, or extracted collagen, is the process of breaking down this larger complex protein structure into smaller peptides and can be achieved by the action of proteolytic enzymes. The degree of hydrolysis achieved by the proteolytic enzymes will impact the size and hence, the functionality of the peptides generated.

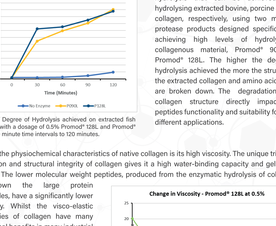


Figure 1: Enzymatic hydrolysis breaking down collagen structure into lower molecular weight peptides.

Hydrolysis not only impacts the size of the collagen peptides but also the physicochemical and biological properties. Extracted collagen can be hydrolysed by either chemical hydrolysis or enzymatic hydrolysis. The specificity of enzymes allows greater control over the hydrolysis process compared to chemical hydrolysis. Hydrolysis of collagen can be achieved using proteolytic enzymes, that enzymatically split the peptide bond between two amino acids.

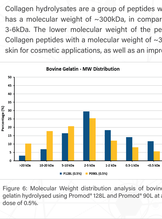


Figure 2: Degree of Hydrolysis achieved on extracted bovine collagen with a dosage of 0.5% Promod® 128L and Promod® 90L at 30 minute time intervals to 120 minutes.

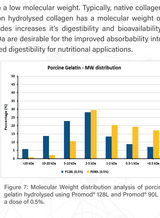


Figure 3: Degree of Hydrolysis achieved on extracted porcine collagen with a dosage of 0.5% Promod® 128L and Promod® 90L at 30 minute time intervals to 120 minutes.

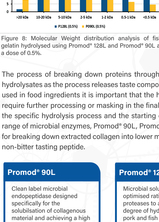


Figure 4: Degree of Hydrolysis achieved on extracted fish collagen with a dosage of 0.5% Promod® 128L and Promod® 90L at 30 minute time intervals to 120 minutes.

Figures 2, 3 and 4 show the degree of hydrolysis that can be achieved when hydrolysing extracted bovine, porcine and fish collagen, respectively, using two microbial protease products designed specifically for achieving high levels of hydrolysis of collagenous material, Promod® 90L and Promod® 128L. The higher the degree of hydrolysis achieved the more the structure of the extracted collagen and amino acid chains are broken down. The degradation of the collagen structure directly impacts the peptides functionality and suitability for use in different applications.

One of the physiochemical characteristics of native collagen is its high viscosity. The unique triple helix formation and structural integrity of collagen gives it a high water-binding capacity and gel-forming ability. The lower molecular weight peptides, produced from the enzymatic hydrolysis of collagen to breakdown the large protein molecules, have a significantly lower viscosity. Whilst the visco-elastic properties of collagen have many functional benefits in many industrial applications, reducing the viscosity of collagen through hydrolysis can be advantageous in improving the ease of processing and formulating the collagen into final products. A lower viscosity improves the collagens solubility in aqueous solutions. A material with high viscosity can be inherently difficult for many manufacturers.

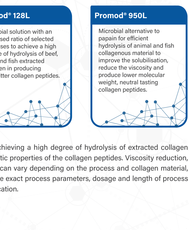


Figure 5: Viscosity reduction of extracted bovine collagen achieved with a dosage of 0.5% Promod® 128L at 30 minute time intervals to 120 minutes.

Collagen hydrolysates are a group of peptides with a low molecular weight. Typically, native collagen has a molecular weight of ~300kDa, in comparison hydrolysed collagen has a molecular weight of 3-6kDa. The lower molecular weight of the peptides increases it's digestibility and bioavailability. Collagen peptides with a molecular weight of ~3kDa are desirable for the improved absorbability into skin for cosmetic applications, as well as improved digestibility for nutritional applications.

Figure 6: Molecular Weight distribution analysis of bovine gelatin hydrolysed using Promod® 128L and Promod® 90L at a dose of 0.5%.

Figure 7: Molecular Weight distribution analysis of porcine gelatin hydrolysed using Promod® 128L and Promod® 90L at a dose of 0.5%.

Figure 8: Molecular Weight distribution analysis of fish gelatin hydrolysed using Promod® 128L and Promod® 90L at a dose of 0.5%.

Figures 6, 7 and 8 demonstrate the molecular weight distribution analysis of bovine, porcine and fish gelatin when hydrolysed using Promod® 90L or Promod® 128L. Gelatin is a combination of different length protein chains, and therefore the molecular weight distribution of different gelatin sources characterise its suitability in different applications. The unique combinations of proteolytic activities in Promod® 90L or Promod® 128L achieve a different molecular weight distribution following hydrolysis of the different gelatin substrates.

The process of breaking down proteins through enzymatic hydrolysis can impact the flavour of the hydrolysates as the process releases taste compounds from the protein. Where protein hydrolysates are used in food ingredients it is important that the hydrolysis process doesn't contribute off-flavours that require further processing or masking in the final food product. Changes in flavour vary depending on the specific hydrolysis process and the starting collagen material. Biocatalysts Ltd have developed a range of microbial enzymes, Promod® 90L, Promod® 128L and Promod® 950L, with proteolytic activities for breaking down extracted collagen into lower molecular weight peptides whilst maintaining a neutral, non-bitter tasting peptide.

Promod® 90L

Clean label microbial endopeptidase designed specifically for the solubilisation of collagenous material and achieving a high degree of hydrolysis.

Promod® 128L

Microbial solution with an optimised ratio of selected proteases to achieve a high degree of hydrolysis of beef, pork and fish extracted collagen in producing non-bitter collagen peptides.

Promod® 950L

Microbial alternative to papain for efficient hydrolysis of animal and fish collagenous material to improve the solubilisation, reduce the viscosity and produce lower molecular weight, neutral tasting collagen peptides.

These enzyme products are suitable for achieving a high degree of hydrolysis of extracted collagen without negatively impacting the organoleptic properties of the collagen peptides. Viscosity reduction, levels of hydrolysis and taste performance can vary depending on the process and collagen material, therefore trials are required to determine the exact process parameters, dosage and length of process to achieve the desired results in each application.

CONCLUSION

The information in this paper demonstrates the value that collagen can bring to a wide number of industrial applications. Further enzymatic processing of extracted collagen can produce higher value and quality collagen peptides that allow the health and nutritional benefits of collagen to be more easily absorbed or digested from the final product. Different sources of collagen will require different processing conditions for achieving unique collagen peptides with beneficial functionality.

If you could benefit from enzymatic hydrolysis of extracted collagen, contact Biocatalysts Ltd for a free sample of our Promod® enzymes designed specifically for hydrolysing collagen and collagenous material, or to speak to one of our enzyme specialists to identify the best solution for your product.