

Marine bioresources produce a great variety of specific and potent bioactive molecules including natural organic compounds such as polysaccharides, peptides, proteins, enzymes and fatty acids etc. The protein collagen is widely used for biomedical and pharmaceutical applications owing to its cell attachment capabilities, excellent biocompatibility, biodegradability and weak antigenicity. The conventional source of collagen is from bovine and pig. However, the outbreak of prion diseases, such as bovine spongiform encephalopathy, has resulted in anxiety among users of collagen derived from these land animals. Thus, there is need to find an alternative source of collagen. Fish is one of the best candidates as alternative source because fish is unlikely to be associated with any side effects.

The overall objective of the work is to test if and how to use fish skin as an alternative source of collagen. In the present study, a considerable quantity of ASC and PDC were successfully extracted and characterized from skin of five species of fishes. Pepsin aided extraction proved to be a tool for obtaining the greater yield without a marked effect on the triple-helical structure. Further characterization studies with SDS PAGE, HPLC, FTIR and UV spectral analysis suggest that the extraction process yields collagen in pure form. The extracted collagen could be used for wide applications in food, pharmaceuticals, cosmetics and biomaterials. The following are some of the salient findings of this study.

Fish Collagen Hydrogel

Wound healing efficacy of the fish collagen hydrogel was evaluated in subcutaneous circular incision wound model on albino rats. The study demonstrated that within 2 weeks, the wound covered

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with gel were completely filled with new epithelium without any significant adverse reactions.

- ✓ The results clearly suggest that the hydrogel enhances re-epithelialization rather than a repair which was clear from the histopathological and biochemical analyses.
- ✓ There is significant increase in angiogenesis, collagen deposition, hexosamine content, epithelialization and wound contraction in hydrogel treated rats without inflammatory cells compared to the control group, indicating the tissue regeneration potential of fish collagen.
- ✓ The wound healing effects of fish collagen may be attributed to its capability on tissue remodeling process associated with collagen synthesis at the wound site, which is evident from increased tensile strength of skin.
- ✓ Furthermore, increased collagen deposition within the wound bed is effectively potentiated the vascularization process in wound healing. Also the angiogenesis in granulation tissues possibly improved blood supplementation to the wound site, thus providing nutrients and oxygen essential for the healing process.
- ✓ The topical administration of collagen hydrogel renders moist environment to facilitate the smoothness essentially required for fast healing process.

In conclusion, the overall research findings of the present study indicated that the topical application of fish collagen hydrogel increased collagen deposition and enhanced angiogenesis by reducing the duration of the inflammatory phase, which is an important factor not only in the re-epithelialization process, but also in wound contraction. It is concluded that the hydrogel can be effectively used as an active ingredient in the formulation of wound healing ointments.

Fish Collagen Peptide Supplement

Generally, the bioactive properties of collagen-derived peptides especially their resistance to proteolytic process make them potential ingredients in the formulations of health care food supplements. Recently enzymatic hydrolysis has become a valuable tool for modifying the functionality of proteins. In this study, an attempt has been made to prepare enzymatically liberated physiologically active peptides from fish collagen extract.

In order to obtain bioactive peptides, the grouper fish skin collagen was hydrolysed enzymatically using three different enzymes; pepsin, papain and protease consecutively. Since degree of hydrolysis depends on the bioactivity of peptides, the hydrolysis parameters were optimised for each enzyme using RSM in order to get maximum degree of hydrolysis.

- ✓ The optimum hydrolysis conditions were: 2.1 of pH; 36.62°C of temperature; 3.6% of E/S ratio and 5.47 h of time for pepsin.
- ✓ The optimum hydrolysis conditions for papain were 6.38 of pH; 26.22°C of temperature; 4.5% of E/S ratio and 4.25 h of time.
- ✓ In case of protease, the optimum hydrolysis conditions were 6.3 of pH; 39.86°C of temperature; 1.8% of E/S ratio and 4.25 h of time.
- ✓ Under these conditions, the enzymes have exerted maximum activity for the preparation of hydrolysate and the method is also found to be very effective in the optimisation of hydrolysis.
- ✓ The lyophilized hydrolysate is 100% soluble in water.
- ✓ The molecular weight distribution of the hydrolysate carried out by SDS-PAGE combined with MALDI TOF also confirmed the formation of potentially active peptides from fish collagen.

In conclusion, the generation of collagen peptides through enzymatic hydrolysis is highly related to their molecular structure and weight, which are greatly affected by processing conditions such as pH, time, enzyme substrate concentrations and temperature. Depending on the specificity of the enzymes, conditions applied in hydrolysis process, and the extent of hydrolysis, it is possible to generate a wide variety of collagen peptides with varying functional properties. The resultant protein hydrolysate may contain particular desirable characteristics of the new peptides formed.

Anti-arthritic actions of fish collagen peptides

The present research work also aimed at the scientific validation of the protective effect of Fish Collagen Peptide (FCP) against Complete Freund's Adjuvant-induced arthritis in rats. Complete Freund's adjuvant induced arthritis in rat model is the best and most widely used experimental model for testing the efficacy of anti-arthritic drugs as the clinical and laboratory features closely resembles the human rheumatoid disease.

In the present study, an attempt has been made to examine beneficial anti-arthritic potential of FCP on the pathophysiological and pharmacological control of inflammation process in rats. The following are some of the salient findings related to anti-arthritic actions of collagen peptide in attenuating the inflammatory process involved in arthritic condition.

- ✓ The morphological aberrations associated with chronic symptoms of arthritis in the level of paw oedema, changes in body weight, arthritic index values were brought back to near normalcy upon treatment with FCP.
- ✓ No significant changes were observed in biochemical parameters of ALP, ACP, and AST, ALT, blood urea, creatinine and total protein in the liver and kidney of experimental rats after 42 days of per se administration of FCP, showing its non-toxic nature.

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- ✓ The significant reduction was noted in level of anti CCP content, most reliable marker for detecting the prognosis of arthritic inflammation, in FCP treated group as compared to arthritic control group, indicating the extent of effectiveness of FCP in ameliorating arthritic disorders.
- ✓ Supplementation of FCP was found to attenuate the liberation of acid phosphatase, a potent lysosomal hydrolase involved in the degeneration process, clearly substantiating the anti-arthritic property of FCP in level comparison with standard anti-arthritic drugs.
- ✓ Interestingly, the supplementation of FCP was found to modulate the inflammatory responses of arthritis without modulating the expression of COX I, which is in generally adversely affected during NASIDs regimen.
- ✓ The evidences from X-ray radiographs, bone histopathology of synovial joints of the study further confirmed the anti-arthritic activity of FCP.

The results of the present research findings indicated that the FCP can be effectively utilized not only in attenuating symptoms of arthritis but also in the treatment of the diseases due to their tissue regeneration capability.

However, further research is necessary to elucidate the exact mechanism of action involved in the anti-arthritic activity of fish collagen hydrolysates. In this scenario, it is hypothesized that the antiarthritic effect of collagen peptide is related to the stimulating effect on collagen biosynthesis in arthritic joints.

In vitro collagen synthesis in cell lines supplemented with fish collagen peptide

An attempt has been made to study the effects of FCP on human osteoblast cell lines (HOS) in both time and dose dependent manner to confirm its role in the collagen biosynthesis.

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- ✓ Significant increase in the number of cells was observed upon FCP-treated culture after 6, 12 and 24 hours as compared to that of untreated cell culture.
- ✓ FCP was found to exert a dose dependent effect on modulating collagen synthesis in osteoblast cells.
- ✓ Quantification of collagen by chromogenic precipitation with Sirius red confirmed increased production of collagen in FCP-treated cells.
- ✓ Western blot and immunocytochemistry analyses further confirmed the FCP-mediated stimulatory action on collagen biosynthesis in cell culture.
- ✓ It is interesting to observe that collagen free protein hydrolysate treatments were unable to enhance the collagen production in osteoblast cells.

Conclusions

Collagen purified from marine sources proved as an effective bio molecule of pharmaceutical importance.

Wound healing studies confirms that topical application of collagen hydrogel increased collagen deposition and angiogenesis over the wound area leads to re-epithelialization rather than a repair of wounded tissue. So it is concluded that the hydrogel can be effectively used as an active ingredient in the formulation of wound healing ointments.

Second part of the study confirms that fish collagen hydrolysate is capable of generating bioactive peptides of therapeutic importance required for the treatment of joint disorders. The study optimised the process parameters for enzymatic hydrolysis of collagen for getting maximum degree of hydrolysis.

The study confirms that the developed collagen product stimulates the joint matrix to synthesise collagen and helping to maintain the structure of the joint and potentially aiding joint

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comfort. So it is concluded that the fish collagen peptide developed can make a formulation of pharmaceutical drug for joint regeneration. Thorough clinical studies are essential and mandatory to confirm the safety aspects of FCP before recommending it as a pharmaceutical supplement for human applications.

In the present society, aging is widely associated with joint inflammation and disability (conditions of osteoarthritis, osteoporosis and osteopenia). In all cases it is common that there is progressive degeneration of glycoproteins and associated collagens in bone and joint tissues. Current options to promote joint comfort are limited to medicines that can only reduce the symptoms like pain or inflammation and also have adverse health effects. The developed FCP is biocompatible, less immunogenic and can be used effectively for joint regenerative therapy.
