Applications of Collagen and Collagen Peptides in Human Nutrition: A Review

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Globally, the current scenario of increased prevalence of non-communicable diseases (NCD) is as a result of life style changes such as sedentary occupation, increased mechanisation, physical inactivity and fast-food habits. According to a 'Times of India' report published in 2015, one out of four Indians suffers from diabetes, cardiovascular diseases and cancer with the growing incidence of lifestyle diseases. Reports suggest that by the end of 2020, cardiovascular diseases will become the major cause of death in India (WHO, 2009). As a repercussion, the occurrence age of non-communicable diseases will be 5-10 years lower than that in Western countries which will lead to early aging, and thereby result in considerable decline in the national productivity.

There is a famous quote by Hippocrates, the father of modern medicine, 'Let food be thy medicine and medicine be thy food'. He

believed in the healing power of nature. Today there is an increasing awareness among the scientists and researchers about the importance of healthy diet and hence extensive research is in progress across the globe for formulation of cost-effective and beneficial nutraceuticals which can prevent or delay the incidence of NCD and aging. Formulation of a diet rich in bioactive compounds with amazing healing properties can naturally eliminate the environment and food-induced health problems. Research on functional bioactive compounds from different sources such as plants, animals, micro-organisms and marine sources have been increased because of their health promoting roles above the nutritional value.

Collagen, the most abundant fibrous protein present in the connective tissues of vertebrates, is one of the most widely used biopolymer for the formulation of nutraceuticals and pharmaceuticals (Ogawa *et al.*, 2004). The major sources of collagen are bovine and porcine skin and bone. Collagen and collagen peptides possess excellent biocompatibility (Osser *et al.*, 1999), biodegradability, weak antigenicity, potential antioxidant properties (Lin *et al.*, 2012) and anti-inflammatory properties (Postlethwaite *et al.*, 1978). Moreover, collagen peptides formed by the partial hydrolysis of collagen possess a myriad of bioactive properties and known to modulate vascular endothelial function. The oral intake of hydrolysed collagen is involved in the maintenance of tissue homeostasis, and is capable of preventing diabetes, cardiovascular disease, peptic ulcer, arthritis etc. Being an important component of extracellular matrix (ECM), collagen plays an important role in the formation and remodelling of functional tissues. These exceptional properties of collagen and collagen peptides make them ideal for tissue engineering and regenerative medicine applications.

Structural hierarchy of collagen fibers

Collagen is the major framework component of ECM which provides structural integrity to tissues. Apart from their structural

role, they are also involved in cellular activities such as cell-cell communication, cellular mobility, cell division etc. It mediates the interaction between cell interior with ECM and thereby maintain the homeostasis in a complex hierarchy of multicellular organisms.

The unique triple helical structure of collagen is mainly composed of a repeating sequence of three amino acids 'Xaa-Yaa-Gly'. Every third amino acid is glycine. The second most occurring amino acid in collagen structure is the imino acid proline. About one-third of Xaa and Yaa positions are occupied by proline (Pro) and hydroxyproline (Hyp) residues. The presence of Hyp enables collagen molecules to interact with water molecules to form hydrogen bonds. Amino acid composition differs in different types of collagen and exhibits various functions in tissues. High imino acid content at both Xaa and Yaa positions is responsible for the stability of triple helix. The imino acids can be replaced by any of the aliphatic amino acids whereas the presence of non-polar side chain blocks the neighbouring amino acids from interaction with water molecule. Three α-helical structures are wound around each other tightly forming triple helical collagen fibrils. This is stabilized by the formation of hydrogen bond between the amino acid side chains. Parallel arrangement of the fibrils into bundles form collagen fibers (Battacharjee and Bansal, 2005).

Collagen formation in tissues

The ECM is being continuously degraded and reconstructed in a healthy tissue. This process is majorly carried out by the dermal tissue fibroblasts in special class of connective tissue, which are responsible for the reformation and organization of collagen in ECM. Various conditions such as aging, stress, auto-immune disorders, changes in diet etc. can alter the functioning of fibroblast and hence affect the quality and quantity of collagen formed. Collagen fibers become thicker and shorter, thus lose their elasticity. The exposure to reactive oxygen species (ROS) deteriorates the function of dermal tissues and affects the replenishment of collagen by fibroblasts and thereby loses

the structural integrity of dermal tissue (Sibilla *et al.*, 2015).

Collagen in human nutrition

Bio-availability

According to Richelle *et al.* (2006) bio-availability of bioactive dietary component is determined by evaluating their capacity to cross the intestinal barrier membrane, and reaches the site of action through blood stream. The fibrous collagen is digested by several proteolytic enzymes in gastric track, gets fragmented into small di- or tri-peptides. These peptides enter the blood stream crossing the intestinal wall. Oesser *et al.* (1999) investigated the tissue distribution of gelatin by oral administration of ^{14}C labelled gelatin hydrolysate in mice. The absorption rate during a time period was measured along with their ensuing distribution in various tissues. The results showed that around 90% of the ingested gelatin hydrolysates were absorbed within 12 h after administration and mainly accumulated in cartilage. Watanabe-Kamiyama *et al.* (2010) investigated the absorption mechanism of low molecular weight collagen hydrolysate and its effect on skin and in prevention of osteoporosis in ovariectomized rats. Results demonstrated that low molecular weight collagen was retained in the skin for 14 days and exerted beneficial effect on osteoporosis by increasing bone density.

The oral intake of collagen hydrolysate was found to elevate the levels of sequentially similar bioactive compounds in plasma (Moskowitz, 2000). The experimental evidence was provided by Martinez-Alvarez *et al.* (2012), that the oral administration of siki collagen hydrolysate had increased the level of calcitonin gene-related peptide (CGRP). The siki hydrolysate-fed rats exhibited approximately 70% increment in plasma CGRP levels than that in control rats. Increased levels of collagen derived peptides were observed in the blood stream after oral intake (Iwai *et al.*, 2005; Hausch *et al.*, 2002). Clinical trials were conducted by oral administration of gelatin hydrolysate after 12 h fasting. Significant increase was observed in the

levels of hydroxyproline containing peptides after 1-2 h of ingestion, while negligible amount were present in blood before ingestion. Their presence has been identified as Pro-Hyp content in plasma and serum, as well as significant amount of Ala-Hyp, Ala-Hyp-Gly, Pro-Hyp-Gly, Leu-Hyp, Ile-Hyp, and Phe-Hyp were also identified as food-derived peptides in blood. Among this proportion, around 10% of the collagen-derived high molecular weight peptides reach cardiovascular system (Moskowitz, 2000) and they are resistant to plasma peptidases (Iwai *et al.*, 2005; Watanabe-Kamiyama *et al.*, 2010). After the dietary supplementation of hydroxyproline, expression levels of prolyl-4 hydroxylase α (I) were down stream-regulated in muscles along with an increased collagen synthesis, whereas no considerable change in the gene expression was observed in hepatic tissue (Zhang *et al.*, 2013).

Shigemura *et al.* (2014) have studied the dose-dependent changes in the levels of hydroxyproline both in free form and in peptides. The experiment illustrated that high dose are to be ingested for exhibiting significant level of bioactivities. Results showed that the intake of 334.6 mg of collagen hydrolysate did not attain the absorption maxima and at least 30 mg of collagen hydrolysate should be taken for exerting health benefits. Aito-Inoue *et al.* (2007) has established the role of PEPT1 proton-dependent transporter involved in the transport of Pro-Hyp across the intestinal barrier.

Role of collagen in maintaining skin health

Changed lifestyle and exposure to toxic pollutants causes deterioration of skin which leads to the formation of wrinkles and increases the susceptibility to dermatological diseases. Moreover, aging slows down the collagen replenishment process. Collagen fibres become thicker and shorter, leading to consequential loss of Type I collagen, and altering the ratio of collagen types in skin (Sibilla *et al.*, 2015).

Borumand and Sibilla (2015) have demonstrated that administration of hydrolyzed collagen as a dietary supplement improved the elasticity

and hydration of skin even in old age as well as reduced the formation of wrinkles. The study was conducted in 45-64 year old healthy non-smoking females who were instructed to take collagen hydrolysate for 12 weeks before breakfast and after which the skin elasticity and hydration were assessed.

The chemo-attractant collagen peptides transduce signal to dermal fibroblast tissues and replenishes new cells for repairing damage (Postlethwaite *et al.*, 1978). The supplementation of hydroxyproline through diet enhances the collagen biosynthesis in deep dermal tissues. Collagen peptides were also found to exhibit a protective action against UV radiation-induced skin damage (Hu *et al.*, 2009; Zhuang *et al.*, 2009). Experimental evidence has been provided by Sumida *et al.* (2004) and Matsumoto *et al.* (2006) that the daily intake of collagen hydrolysate improved skin hydration.

An experiment by Liang *et al.* (2010) revealed that prolonged consumption of marine collagen hydrolysate (MCH) from Chum Salmon skin prevented collagen loss and collagen fragmentation in chronologically aged skin. There is an increase in the expression level of collagen Type I and III through activating Smad signalling pathway with enhanced TGF-βRII expression level. The results were also shown to reduce the MMP-1 activity as well as oxidative stress.

Role in maintaining bone density and bone resorption

Collagen is the major structural component of bone which has an important role in differentiation of osteoclast and calcification in bone tissue (Takeuchi *et al.*, 1996; Takeuchi *et al.*, 1997). The studies revealed that the oral intake of collagen hydrolysate positively modulate the bone mass content and density in experimental rats and mice. The animals were fed calcium or protein deficient diet during the experiment (Wu *et al.*, 2004; Koyama *et al.*, 2001). Nomura *et al.* (2005) had studied the effect of oral ingestion of hydrolysed collagen in ovariectomized (OVX) rats. The results showed an increased deposition of Type I collagen and proteoglycan in the bone matrix.

Guillerminet *et al.* (2010) has conducted similar studies to investigate their effect in the treatment of osteoporosis and to elucidate its mechanism of action. Ovariectomized or sham-operated (SHAM) female mice were fed with collagen hydrolysate-incorporated diet for 12 weeks. A significant increase in the bone strength was observed in 25 g/kg body weight collagen hydrolysate-fed OVX mice. The collagen was found to improve the *in vitro* osteoblast activity and increase the external diameter of cortical areas of the femurs *in vivo*. The collagen fed OVX mice was found to have better bone mineral density gain than OVX control mice after 8th week of surgery.

Collagen or collagen hydrolysate are also considered as better therapeutic agent for preventing osteoarthritis (Bello and Oesser, 2006). Collagen hydrolysate-incorporated diet was demonstrated to boost up the preventive action of calcitonin against osteoporosis (Adam *et al.*, 1996). Collagen hydrolysates and gelatin from chicken and porcine sources were capable of improving mineral deposition in bones and joints, thereby reduces the risk of osteoporosis (Beynen *et al.*, 2010; Hays *et al.*, 2009; Watanabe-Kamiyama *et al.*, 2010). Oral intake of collagen peptides improved the homeostasis of bone metabolism in growing and mature rats especially under calcium deficient condition (Wu *et al.*, 2004).

Cuneo *et al.* (2010) has conducted randomized double blind placebo clinical trials in post-menopausal women (45-65 years) with low bone mineral density. The patients were assigned to intake 10g protein hydrolysate for 24 weeks and have been subjected to biochemical evaluation. Results revealed that the bovine collagen hydrolysate has no significant effect in the bone metabolism and remodelling in women after menopause.

The growing age and auto-immune diseases are the major reasons for arthritic difficulties. Collagen hydrolysates are considered as an effective nutraceutical for the prevention of inflammatory damage in cartilage. Oesser and Seifert (2003) have developed tissue engineered

cartilage of proteoglycan and collagen using articular chondrocytes *in vitro*. The ligand receptor interaction of collagen fragments with integrin domain might be the molecular mode of action of collagen hydrolysate in tissue reformation (Siebert *et al.*, 2010; Stotzel *et al.*, 2012).

Type 1 collagen induces the differentiation of bone marrow cells into osteoblast. Asp-Gly-Glu-Ala domain of the collagen Type 1 mediates the extracellular signals through $\alpha 2\beta 1$ integrin receptors on cell membrane and stimulates osteoblast related gene expression of bone marrow cells (Mizuno and Kuboki, 2001).

Antioxidant properties of hydrolysed collagen

Antioxidants are a group of biomolecules which are capable of preventing oxidation of cellular components and protect cells from free radical attack. The oxidative stress-induced cellular damage and subsequent diseases can be avoided by including antioxidants in diet. Research has indicated that the collagen peptides obtained from marine sources possess high antioxidant property (Pei *et al.*, 2010) and prevent skin ageing (Tanaka *et al.*, 2009), ulcer (Iu *et al.*, 2006), hypertension (Zhang *et al.*, 2009) etc. Marine collagen was also reported to improve lipid metabolism (Saito *et al.*, 2009).

The collagen hydrolysates prepared from Jellyfish have been evaluated for its antioxidant activity by Ding *et al.* (2011). *In vivo* experiments were conducted in aging mice models. Administration of Jellyfish collagen hydrolysate in the aged mice was found to modulate the antioxidant system effectively. The results showed declined activity of Glutathione peroxidase in serum and Superoxide dismutase in liver tissue homogenate when compared with the control.

Lin *et al.* (2012) have investigated the protective action of Marine Collagen Peptides (MCP) against early alcoholic liver injury in rats. Alcoholic Liver Damage (ALD) is mainly caused by oxidative stress, followed by mitochondrial dysfunction and activation of inflammatory

mediators (Tilg and Diehl, 2000; Albano, 2008). The ALD was induced in experimental rats by oral administration of alcohol. The treatment group was administered with MCP intra-gastrically for four weeks. The effect was then studied by biochemical analysis and histopathological evaluation. The histopathological observations demonstrated the protective effect of MCP against oxidative damage.

Anti-inflammatory property

In 1978, Postlethwaite and his colleagues had demonstrated collagen peptides containing hydroxyproline as a chemo-tactic factor for fibroblasts. The collagen derived small peptides such as Pro-Hyp-Gly, Pro-Hyp and analogs exhibit chemotactic activity to fibroblasts, neutrophils (Postlethwaite *et al.*, 1978; Laskin and Pendino, 1986), and monocytes (Postlethwaite and Kang, 1976). Ala-Hyp and Gly-Pro-Val are potential inhibitors of angiotensin converting enzyme (Kim *et al.*, 2001; Oshima *et al.*, 1979) and Gly-Pro-Hyp could be involved in the process of platelet aggregation (Knight *et al.*, 1999).

Maintenance of nitrogen balance

Hays *et al.* (2009) has compared the nitrogen retention capacity of two proteins, whey protein concentrate and concentrated fortified collagen hydrolysate in healthy elder women who were fed with eucaloric diet. Whey protein consumption resulted in weight loss and no change in the body weight was observed in collagen hydrolysate-fed group. At the same time nitrogen excretion was comparatively lower in collagen hydrolysate treated group than whey protein treated group.

Hypolipidemic effect

Lin *et al.* (2012) has demonstrated that the administration of collagen peptides could negate the increased levels of cholesterol and triacyl glycerol in alcoholic liver damage induced rats. Saito and his colleagues (2009) have demonstrated the lipid lowering effect of chum salmon or rainbow trout collagen peptides. The peptide-fed group was found to exhibit lower levels of plasma lipids when compared to the

control group. The incorporation of peptides in diet might have altered the absorption and metabolism of lipids and transiently lowered the levels of triglycerides compared to that of control group.

Anti-diabetic properties

Marine collagen peptides were found to exhibit anti-diabetic activity in clinical trials. Their mode of action was different in patients with diabetes and diabetes with hypertension. Feng *et al.* (2010) demonstrated that the MCPs exert more profound protection in patients with diabetes alone. The MCP administration decreased the levels of fatty acids, high sensitive C-Reactive protein, resistin and prostacyclin significantly in diabetics. The levels of cytochrome P450, leptin and nitric oxide were decreased in diabetics with/without hypertension. Gelatin hydrolysates as inhibitor of dipeptidyl peptidase IV as well as stimulator of glucogon like peptide 1 were found to improve glycemic control in diabetic rats (Wang *et al.*, 2015).

Summary

Collagen and collagen peptides are being considered as a safe food ingredient by the society. Their hydrolysis can produce several biologically active peptides, which exhibit a wide range of bioactivities. The studies have established the various bio-properties expressed by collagen. Their properties make them a suitable compound for the formulation of nutraceuticals.

Orally administered hydrolysed collagen can be digested and absorbed as small peptides into the blood stream. Regular intake of collagen-incorporated diet is useful in maintaining healthy skin and bone; attenuate ROS-induced tissue damage and suppress inflammatory responses. They have been proved to possess anti-diabetic property and also prevent nitrogen imbalance in body. They can modulate the lipid metabolism and exert hypolipidemic activity. Thus incorporation of collagen-based nutraceuticals can alleviate age-related health issues and keep people active for longer periods.

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