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Review

Potential Health Functions of Collagen Bioactive Peptides: A Review

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Abstract: Collagen is the most abundant protein in nature and widely exits in animals. However collagen usage is not good enough, which is a waste of natural resources and an environmental burden. Collagen peptides have some special characters and functions. These functions can decide their usage. Therefore producing, discovering and using new functional collagen peptides are promising way to deal with this issue. Collagen peptides are produced by hydrolysing collagen or gelatin via chemical or biochemical methods. Biochemical hydrolysation is more proper to produce functional collagen peptides than chemical hydrolysation. The collagen hydrolysates or peptides have four levels of functions: Nutrition, basic functions, special biological functions and other useful properties. The nutrition is providing amino acids and small peptides. The basic functions are improving the state of connective tissues, such as skin and bone. The special biology functions include antioxidant, antihypertensive and other bioactive benefits. Other useful functions include antifreeze, metal chelation and forming edible film. All these functions decide that they can be used in food as well as other fields. The present work is a compilation of current information on the preparations and functions of collagen peptides. At last, recommendation for the further research of collagen peptides is proposed.

Keywords: Collagen Peptides, Bioactive Peptides, Skin, Antioxidant, Antibacterial

Introduction

The energy crisis, food crisis and environmental problems have become major global issues. It is imperative to explore new resources and increase the utilization of existing resources. The development and utilization of both biomaterials and bio-energy are methods to resolve these problems. Collagen, which exists in the connective tissue of animals, is the most abundant protein in various vertebrates and invertebrates (Dybka and Walczak, 2009). Almost one-third of mammalian proteins are collagens. Nowadays we should take full use of our resources and reduce the use of unsustainable polluted petrochemical resource. So it is meaningful to develop the usage of collagen.

Collagen is a promising resource as renewable materials, like macromolecule cellulose. It can be used as medical materials, food resources, chemical materials, etc. However the collagen utilization ratio is still very low nowadays, because large quantities of collagen are discarded as waste or underutilized to full advantage in the form of by-products (Pal and Suresh, 2016). Fish bone, skin, scale and animal husbandry contain a large number of collagen and are generally discarded as by-products (Blanco *et al*., 2017). In addition, it's not a good protein for human nutrition although the collagen is a very cheap and abundant protein. Collagen's amino acid composition proportion doesn't match human's need and collagen is hard to be digested in gastrointestinal tract and also its bioavailability is low. All of these limit the usage of collagen which is a waste of resources.

Research and development of collagen's new usages, as well as take full use of collagen, are important for us. Collagen can be hydrolyzed to peptides by chemical or biological methods. These collagen peptides may overcome the shortage of collagen including low bioavailability. The direct consumption of collagen peptides would improve collagen's bioavailability greatly. These collagen-rich by-products are often then inedible part of animals. However, collagen peptides can be achieved from these inedible portions hydrolyzing collagen. Then collagen peptides are also a promising form of take full use of our collagen sources.

At the same time, collagen derived peptides are an abundant source of functional chemicals. Collagen peptides or hydrolysates have been proven to have lots of special functions, such as benefits for skin and bone (Zdzieblik *et al*., 2017), antioxidant (Ketnawa *et al*., 2016), antimicrobial (Ennaas *et al*., 2016), antifreeze (Cao *et al*., 2016). These special functions of collagen peptides mean that collagen peptides can be used as functional foods, medicine, cosmetics, food additives and so on. In conclusion, collagen peptides have a bright future in lots of fields.

In this review we focus on the productions and the functions of collagen peptides and also some suggestions about collagen peptides research and development in the future are advised. Hope this article can provide some ideas to the other researchers and engineers about the usage of collagen peptides. This can improve the usage of collagen and stimulate the development of collagen industries.

Preparations of Collagen Peptides

Collagen and Gelatin

Collagen is the most abundant organic macromolecules in humans and animals. The collagen is a family of extracellular matrix protein and has at least 28 different types. The collagen contains helical trimeric *α* chains, which are composed of repeating peptide triplets of glycine-Xaa-Yaa (Xaa and Yaa are often proline and hydroxyproline, respectively). It also has two very short coil fragments at the N- and C-terminal regions, telo-peptides, which are largely made up of lysine and its derivatives (Gómez-Guillén *et al*., 2011).

Gelatin, which is derived from collagen, is a partial hydrolysate of collagen. The hydrogen and covalent bonds in collagen cleave and the α-helix becomes a random coil and then insoluble collagen becomes soluble gelatin (Gómez-Guillén *et al*., 2011). Gelatin is obtained from heating collagen above the transition temperature of collagen's triple-helix structure (Folk, 2015). In addition, chemicals can also change collagen into gelatin during collagen's extraction.

Hydrolyzation of Collagen and Gelatin

Low-molecular-weight collagen hydrolysates are generally thought to exert better bioactivities than their larger counterparts (Hong *et al*., 2019). Therefore, collagen peptides have more important applications after hydrolysis. Like other proteins, collagen and gelatin can also be hydrolyzed via chemicals and enzymes. These methods result in different products with different molecular sizes and amino acid sequences. The characteristics of collagen peptides are decided by the hydrolyzation methods' cleavage sites and action time. In general, chemicals can cleave all the peptide amide bonds. Enzymes are sensitive to some special sites in collagen or gelatin and these sites are decided by the enzyme types.

The products of chemical hydrolysis are often amino acids and small peptides that differ based on the action time, while the products of biochemical hydrolysis are often peptides with different sizes and part of amino acids that also differ based on the types of enzymes and the action time. In Fig. 1, we show the collagen products from different types of hydrolysis.

Chemical Hydrolysis

A high concentration of both acids and bases can cleave the peptide bonds of collagen. Chemical hydrolysis is cheap and simple to produce collagen hydrolysates or peptides. Acid-alkaline hydrolysis methods have short hydrolysis time and are applicable to industrial processes for they are cost effective and simple operative (Pal and Suresh, 2016). However, acid-alkaline hydrolysis is hard to control and the products are unpredictable. In addition, both acids and bases damage the amino acids.

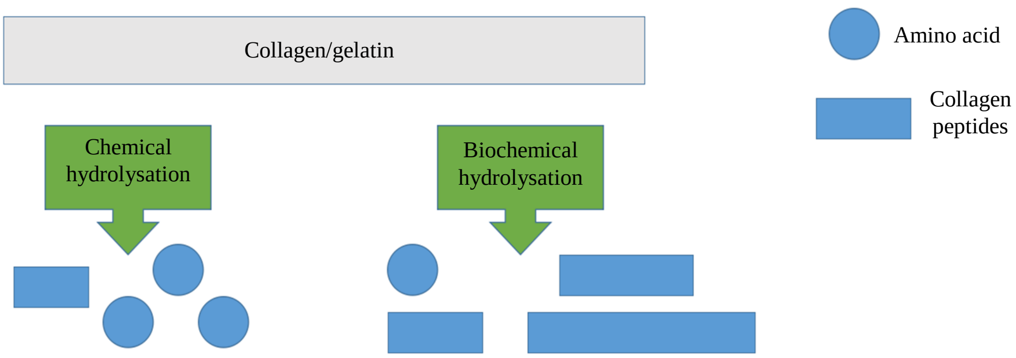

Fig. 1: The products of collagen or gelatin hydrolysates. The chemical hydrolysation of them produces amino acids and small peptides and the products of biochemical hydrolysation are different size of peptides and part of amino acids

Alkaline conditions can destroy amide nitrogen, transform arginine to ornithine and transform L-amino acids to D-amino acids (Liu *et al*., 2011). Acids can destroy the R groups of glutamine, asparagine, tryptophan and others. Then the preparation of collagen peptides via chemical hydrolysis is not very common. What's more, the uses of strong acids in the chemical hydrolysis cause some environment problems (Liu *et al*., 2011). In addition, collagen is often extracted via the assistance of acid or base. During the extraction, the collagen may be broken by solvent. The acidic collagen extracts acquired from the skin of the silver carp contain considerable quantities of small peptides (Wojtkowiak *et al*., 2016).

Biochemical Hydrolysis

Biochemical hydrolysis is another way to produce collagen hydrolysates or peptides. Collagen peptides/hydrolysate can be gained from hydrolysis of collagen by using exogenous and/or endogenous enzymes such as fermentation, gastrointestinal digestion and enzymatic hydrolysis (Pal and Suresh, 2016). Of all these methods, enzymatic hydrolysis is used most commonly. Compared with chemical hydrolysis, enzymolysis is more stable and mild. In addition, the products, e.g., peptides or hydrolysates, can be controlled and predicted.

The hydrolysates of collagen have different amino acid sequences and properties. These differences base on the different enzymes and hydrolysis condition. The enzymes can be non-specific proteinases and/or a specific collagenase or gelatinase. Non-specific proteinases can be trypsin, pancreatin, acidic proteases, neutral proteases, alkaline proteases and a combination of them. Collagenase or gelatinase derived from microorganisms and animals has good specifics and efficiency for collagen, such as the bacterial collagenase obtained from *Alcaligenesodorans* (Banerjee and Shanthi, 2012). Collagen from pig skin, cattle skin, fish scales and chicken feet are reported hydrolysing by collagenase (Hatanaka *et al*., 2014). Compared with animal-derived enzymes, enzymes from microorganisms have several advantages, including a wide variety of available catalytic activities and greater pH values and temperature stabilities (Ovissipour *et al*., 2012). Therefore, we often use collagenase or gelatinase that come from microorganisms.

Recently, a new green technology called Subcritical Water Hydrolysis (SWH) was used for production of peptides from diverse proteins, including collagen (Ahmed and Chun, 2018). SWH may become one of the most promising methods for producing bioactive peptides from protein; because it only uses water instead of any hazardous solvent and also the hydrolysis processing is very fast, cost-effective and highly-efficient. However, the process needs high pressure and high temperature, which may be one of the weaknesses.

Collagen Hydrolysates and Collagen Peptides

Collagen is hydrolyzed by different methods to obtain peptides with bioactivities or with smaller sizes. Collagen hydrolysates have been studied as compounds of peptides and amino acids. Collagen hydrolysates are hard to be described and qualified. The entire collagen hydrolysate varies for different species, collagen types and the used enzyme types. Then they are often defined and described by the factors, for example: The hydrolysates of giant catfish skin using porcine trypsin (Ketnawa *et al*., 2016), hydrolysates of cobia gelatin using pancreatin (Yang *et al*., 2008) and hydrolysates of thornback ray skin using an enzyme preparation from *Bacillus subtilis* A26 (Lassoued *et al*., 2015a). Some collagen hydrolysates will be separated crudely. The separation methods include molecular size (Guillén *et al*., 2010), mental chelate ability (Guo *et al*., 2015), graded salting out, ion-exchange chromatography and hydrophobic chromatography.

Among the various collagen hydrolysates, some collagen peptides have been identified and studied. These collagen peptides have been studied carefully and precisely using synthetic peptides. Those collagen peptides were reported to have special functions, such as benefits for skin and bone, antioxidant activity, ACE-inhibitory activity and other functions. GHK (glycyl-L-histidyl-L-lysine, the tri-peptide from osteonectin) is one of the mostly studied collagen peptides. GHK and its copper complex can accelerate wound healing and skin repairing, remodel tissue, etc. (Fu *et al*., 2015). The collagen tri-peptide fragment GER and its multiple repeats affect the adhesion and spreading of mouse embryonic fibroblasts to different substrates (Ivanova *et al*., 2012). In addition, a lot of other collagen peptides have also been reported or separated from the collagen hydrolysates, such as DGAR (Sila and Bougatef, 2016), GAOGLOGP (Saiga *et al*., 2008) and GFOGP (Ichimura *et al*., 2009). We classified these collagen peptides by their functions in Table 1.

Functions of Collagen Peptides

Protein's functional and nutritional properties can be improved by hydrolysis (Pal and Suresh, 2016). Peptides from collagen also have various functions. First, as a type of protein fragment, collagen peptides can be digested and absorbed by creatures and used to provide materials and energy for our bodies. Collagen peptides are also fragments of a special protein, collagen, which is the basic of the connective tissue. Then collagen peptides will contribute to collagen growth and the structure of relative tissue. Some collagen peptides with special structures may have special functions, such as antioxidation and anti-hypertensive activities. Beside these bio-functions, collagen peptides also have other properties that can be

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used, e.g., antifreeze and antimicrobial activities and used as an emulsifier and de-foaming agent. In the Fig. 2, we show the functions of collagen peptides.

Nutrition

Collagens are the major fibrous component of the animal extracellular matrix and the most abundant protein in nature. As one of biological resources, collagen is regeneration and abundant. These collagens are a promising resource for foods just like the grain, so do the peptides derived from collagens. What's more collagen peptides are safe and low allergenicity. Some collagen peptides have been designated as Generally Recognized As Safe (GRAS) food products or food additives in USA (Dybka and Walczak, 2009). Then collagen peptides are potential and functional food resources.

Table 1: The collagen peptides with special biological functions or properties

Function	Peptides sequence	Origin	Reference
Antioxidative	GETGPAGPAGPIGPVGARGPAGPQGPRGDKGETGEQ	Bovine tendon	Banerjee *et al*. (2012)
	DGAR	Porcine skin	Sila and Bougatef (2016)
	LEELEEELEGCE	Bullfrog skin	Qian *et al*. (2008)
	GPLGLLGFLGPLGLS, FDSG- PAGVL, NGPLQAGQPGER	Squid tunic (*Dosidicus gigas*)	Alemán *et al*., (2011a; 2011b; Mendis *et al*., 2005)
	DPALATEPDPMPF	Nile Tilapia (*Oreochromis niloticus*)	Ngo *et al*. (2010)
	PMRGGGGYHY	Salmon skin	Wu *et al*. (2018)
ACE Inhibitory	AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP, PAGNPGADGQPGAKGANGAP	Bovine tendon	Banerjee *et al*. (2012)
	TCSP, TGGGNV, LLMLDNDLPP	Pacific cod skin (*Gadus macrocephalus*)	Ngo *et al*. (2011; Himaya *et al*., 2012)
	SPGPMGPR, GFPGPDGPPGPR	Thornback ray gelatin	Lassoued *et al*. (2015a; 2015b)
	GAOGLOGP	Chicken leg	Saiga *et al*. (2008)
	AP, VR	Atlantic salmon skin	Gu *et al*. (2011)
	GFOGP	Porcine skin	Ichimura *et al*. (2009)
	YYRA	Chicken bone	Nakade *et al*. (2008)
	GPLGLLGFLGPLGLS	Squid tunic (*Dosidicus gigas*)	Alemán *et al*. (2011a)
	DPALATEPDPMPF	Nile Tilapia (*Oreochromis niloticus*)	Vo *et al*. (2011)
Anti-freeze	GLLGPLGPRGLL	Pig skin	Cao *et al*. (2016)
	GAIGPAGPLGP	Shark	Wang *et al*. (2014)
	GSPGADGPIGAHypGTHypGPQGIAGQR, GNDGAHypGAAGPHypGPTG-PAGPHypGFHypGAAGAK	Chicken collagen	Du and Betti (2016)
Affect the adhesion	GER, (GER)n	Synthesized	Ivanova *et al*. (2008; 2012)
Wound healing	GETGPAGPAGPIGPVGARGPAGPQGPRGDKGETGEQ	Bovine tendon	Banerjee *et al*. (2015)
Antimicrobial	$(PR(GPR)_2(GPO)_4GPCCY\text{-amide})_3$	Designed	Masuda *et al*. (2016)

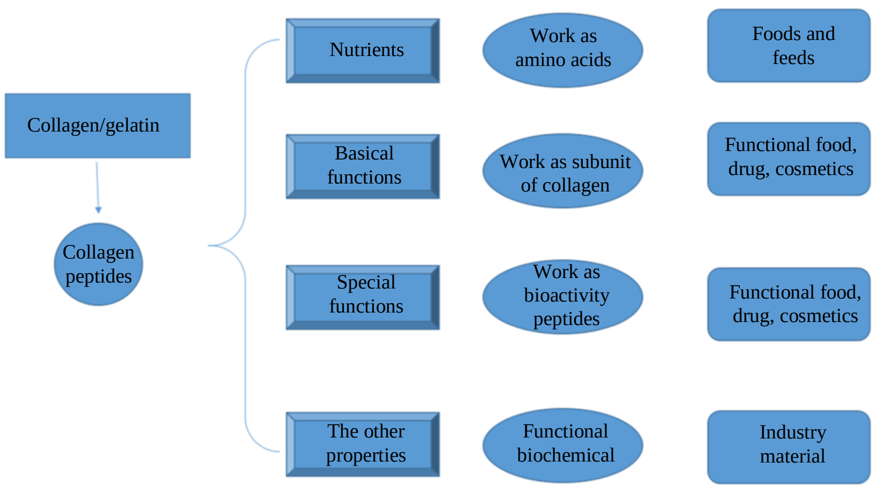

Fig. 2: The function of collagen peptides. The peptides derived from collagen have four levels of functions and all of these functions have their usage

Collagen and its hydrolysates do not have the proper amino acid composition for human because they do not contain sufficient amounts of *Y*, *W* and *F* residues. However, they still contain almost all of the natural amino acids and are rich in glycine, praline and so forth. Collagen peptides or hydrolysates provide amino acids for people with anorexia, anaemia and for vegetarians whose diet is lack of meat (Dybka and Walczak, 2009) and collagen peptides can be used in combination with other amino acids, such as tryptophan to overcome its low content of some essential amino acids. Due to presented, collagen peptides are still a good source of our foods.

Additionally, collagen peptides have a smaller size than collagen and this can help overcome the shortfalls of collagen, e.g., difficult digestion and low bioavailability. Collagen peptides can be digested into small molecules more easily than collagen. What's more, some collagen peptides, such as di-peptides and tri-peptides, can be directly absorbed (Freeman, 2015). Collagenase hydrolyzes collagen or gelatin into tri-peptides start with the G, such as GPHyp, GPA and GPHyp. Collagen tri-peptides are absorbed more efficiently than other collagen peptides in humans (Yamamoto *et al*., 2016). Then collagen peptides are better foods or foods additives than collagen.

Basic Functions

Collagen is a major material in connective tissues, e.g., skin, bone, cartilage and so on. Permanent collagens exchange processes in our body takes place during the whole human life and old fibrils are replaced by new one all the time (Dybka and Walczak, 2009). Collagen peptides, a subunit of collagen, will contribute to the formation of collagen and the statue of connective tissues. Then the properties of connective tissue would be affected by collagen peptides intake. What's more, the collagen hydrolysates/peptides can perfect the symptoms of some connective tissues diseases or disorders. Skin and cartilage are two categories containing lots of collagen and the benefit effects of collagen peptides are studied most. Besides, tendons, blood vessels, intestines also contain large quantities of collagen. Then collagen peptides may also contribute to these organs' functions or situation. For example, collagen peptides can increase blood vessel's elasticity and be used for high blood pressure adjuvant therapy. Also, the collagen peptides administration prevents intestinal barrier disruption following burn injury (Chen *et al*., 2019).

Skin Benefits

Skin contains large quantities of collagen. Collagen and its hydrolysates can be used to construct skin and improve skin's properties. Then oral administration and external application of collagen peptides would benefit the skin and improve the skin's properties. Daily oral supplementation with collagen peptides improves skin hydration, elasticity and slows down skin aging and has a protective effect on joint health (Choi *et al*., 2014; Pyun *et al*., 2012; Czajka *et al*., 2018).

A collagen tri-peptide significantly improved both the moisture levels and skin colour (Berardesca *et al*., 2009). Collagen peptides can also help our skin cape with some bad situation. Ingestion of collagen peptides improves the skin properties of women during winter (Matsumoto, 2006). The skin benefit effects are also found in the skin of pigs (Matsuda *et al*., 2006), mice (Pyun *et al*., 2012; Okawa *et al*., 2012) and others, with the phenomenon of increased fibroblast density. Zague *et al*. (2018) found collagen hydrolysates treatment notably modulated cell metabolism and increased the content of procollagen I in monolayer model and the human dermal equivalent model.

At the same time collagen bioactive peptides can also protect skin from damage and heal all kinds of damage. These damages include UVB radiation, photothermolysis treatments, dry skin, sensitive atopic skin and cutaneous wounds. Collagen peptides from bovine tendon, chum salmon (*Oncorhynchusketa*) and jellyfish accelerate the wound healing process in rats (Wang *et al*., 2015a; Banerjee *et al*., 2015; Choi *et al*., 2014; Felician *et al*., 2019). Rapid wound closure activity of collagen peptides from a bovine Achilles tendon are also reported in women (Banerjee *et al*., 2014). Collagen peptides are also good to all kinds of slight injury. Collagen peptides treatment appears to be an effective and conservative therapy for UVB radiation (Pyun *et al*., 2012), photothermolysis treatment (Choi *et al*., 2014) and UV-irradiation (Zhang *et al*., 2017) by decreasing the abnormal elastic fibre formation, suppressing epidermal hyperplasia, increasing skin hydration and soluble type-I collagen content, etc. Besides, oral administration of collagen peptides also can improves dry skin. Okawa *et al.* (2012) found that collagen peptides normalize axon-guidance factors in the epidermis and reduce pruritus in dry skin model mice induced by acetone.

Bone and Cartilage Benefits

Collagens are also the major material in bone and cartilage. Similarly, collagens and their hydrolysates can also benefit the bone and cartilage. Osteoporosis and osteoarthritis are two kinds of disorders or diseases relative to the bone and cartilage and their therapeutic affect nowadays are limited in many patients and there is not a perfect treatment for these two disorders (Alemán and Martinezalvarez, 2013). New and safer compounds have been used in treatment for they can repair the damaged articular cartilage or at least decelerate its progressive degradation (Kanshana *et al*., 2018). Beside the glucosamine, methyl-sulfonyl-methane, chondroitin sulphate, collagen hydrolysates are also considered as symptomatic pharmaceutical drugs (Alemán and Martinezalvarez, 2013).

Osteoporosis has lots of symptoms: Pain, bone fragility and easy to fracture for loss of calcium and other mineral elements. All these symptoms would affect our life. Collagen peptides have been reported that it can prevent osteoporosis or treat it. Collagen peptides extracted from fish scales prevent osteoporosis and assist in bone remodelling (Hu *et al*., 2016). Collagen peptides from deer sinew prevent bone loss in ovariectomized rats (Zhang *et al*., 2014). The intaking of collagen peptides increases bone mineral density and bone markers in postmenopausal women (König *et al*., 2018). Collagen peptides apparently stimulate the calcification of human osteoblastic cells in cultures and increase the production of type I collagen (Tsuruoka *et al*., 2007). The bovine bone collagen peptides may prevent age-related bone loss and improve bone microarchitecture by increasing collagen synthesis and inhibiting bone absorption (Song *et al*., 2019).

Osteoarthritis is a joint disease which involves the joint cartilage and its associated structures (Alemán and Martinezalvarez, 2013). A lot of literatures have reported that collagen peptides or hydrolysates can be benefit for some diseases. Collagen peptides isolated from pork skin and bovine bone are effective supplements to improve physical problems associated with osteoarthritis (Kumar *et al*., 2015). The supplementation of collagen peptides in young adults with functional knee problems led to an improvement in activity-related joint pain (Zdzieblik *et al*., 2017). Collagen peptides have the potential to exert a chondro-protective action on osteoarthritis by inhibiting matrix metalloproteinase-13 expression and type II collagen degeneration (Isaka *et al*., 2017). Periodic injections of collagen peptides delay cartilage degeneration in rabbit experimental osteoarthritis by promoting the synthesis of type II collagen and prevent proteoglycan loss in rabbits (Naraoka *et al*., 2013).

Collagen peptides or hydrolysates can also regular gene expression related to osteogenetic differentiation (Wang *et al*., 2015b), promoting bone healing process (Hata *et al*., 2008). In conclusion collagen hydrolysates are promising therapeutic agent in the treatment of bone and cartilage disorders, such as: Osteoarthritis and osteoporosis (Wu *et al*., 2017).

Special Bio-Functions

Structure decides the function and collagen peptides with a variety of structures have a variety of functions. Some collagen hydrolysates or peptides are reported to have special bioactivities, such as antioxidant, anti-hypertensive and anti-cancer activities. These peptides are called cryptic peptides or cryptides (Banerjee and Shanthi, 2016). Collagen bioactive peptides are mainly obtained by enzymatic hydrolysis and are more popular for their safety.

Antioxidant

Many diseases and disorders, such as cancer, mutations, age, diabetes, cardiovascular disease, Alzheimer’s, are related to the fail of antioxidant system in our body. Antioxidants can deal with this problem and improve life expectancy. The antioxidant properties of peptides have been intensely studied for food proteins can inhibit lipid peroxidation (Banerjee and Shanthi, 2016). Lots of collagen peptides or hydrolysates have been proved having antioxidant activities. Peptides from enzyme hydrolysates of discarded fish processing wastes exhibit antioxidant property (Sila and Bougatef, 2016). Collagen hydrolysates in snakehead murrel (*Channastriata*) skins demonstrate significant antioxidant activities compared with that of butylated hydroxytoluene, vitamin C and E (Sinaga, 2015). Collagen peptides from three types of abalone foot muscles have antioxidant activities *in vitro* and *in vivo* (Guo *et al*., 2016). One bioactive peptide isolated from a bovine tendon collagen confers protection to cells against oxidative stress (Banerjee *et al*., 2014). The royal jelly-collagen peptide has also ability of protection against oxidative stress and anti-aging effect (Qiu *et al*., 2020). Collagen peptides are promising antioxidant which deserves further study.

ACE Inhibitory Activity

Angiotensin I-Converting Enzyme (ACE) inhibitory agents can inhibit the catalyze degradation of bradykinin. However ACE inhibitor used now have some side effects. Then ACE inhibitor form protein will be very promising for their safety. Some collagen peptides have been reported have anti-hypertensive and ACE-inhibitory activities. These ACE inhibitory peptides are thought containing 10-12 amino acids and having a hydrophobic C terminal. Collagen peptides from milk fish (*Chanoschanos*) skin possess ACE inhibitor activities (Baehaki *et al*., 2016). Two peptides from bovine collagen exert anti-hypertensive effects *in vivo* (Yu *et al*., 2016). ACE inhibitory peptides GP and GFHypGP, isolated from porcine skin collagen hydrolysates, also had an antihypertensive effect on spontaneously hypertensive rats (Masuda *et al*., 2018). Peptides from pig femoral bones have anti-hypertensive and ACE inhibitory activities (Liu *et al*., 2014).

Besides anti-hypertensive and ACE-inhibitory activities, some collagen peptides are also reported to have anti-atherosclerosis and anti-cholesterol activities. Peptides from turkey head collagen can bind cholic and deoxycholic acids and significantly inhibit plasma amine oxidase in a dose- and time-dependent manner (Khiari *et al*., 2014). Collagen peptides inhibit atherosclerosis development (Tang *et al*., 2015). Some collagen hydrolysates from snakehead murrel

(*Channastriata*) skin demonstrate anti-cholesterol activities comparable to that of pravastatin (Sinaga, 2015). Hypertension, atherosclerosis and cholesterol are three problems related to cardiovascular and cerebrovascular diseases. Peptides from collagen have been proven to have functions to release or cure these problems.

Antimicrobial Activity

Although there are lots of potent antimicrobial compounds nowadays, antimicrobial peptides are safer in the food safety and food protection as natural products. Antimicrobial peptides have a broad activity spectrum and a rapid microbe killed effect (Alemán and Martinezalvarez, 2013). There have some reports that collagen peptides or hydrolysates have antimicrobial activity (Gómez-Guillén *et al*., 2011). A designed peptide based on collagen-like (G-P-Yaa)-repeating sequences showed activity against gram-negative *Escherichia coli* and gram-positive *Bacillus subtilis* (Masuda *et al*., 2016). Collagen peptide derived from fish gelatin has antimicrobial activity because it contains hydrophobic motif of GPA triplets in structure (Zhang *et al*., 2017). Peptide fractions from tuna and squid skin gelatin within a range of 1-10 kDa and <1 kDa are reported having antimicrobial activity (Guillén *et al*., 2010).

Other Biofunctions

In addition to the two functions above, collagen peptides also have other biological functions. These biological functions include anti-cancer activity, opioid-like activity, etc.

Collagen-peptide ingestion suppresses allergic responses by skewing the balance of CD4+ T cells toward Th1 and Treg cells (Venkatesan *et al*., 2017). Collagen peptides demonstrated a comparable effect with glutamine in ameliorating post-burn inflammatory responses in mice with burns and the peptides could be considered as a potential immunonutritional supplement in external nutrition to improve post-burn outcomes in burn patients (Chen *et al*., 2017a).

In addition, collagen peptides are reported to have satiety, opioid-like activity, calciotropic activity (CGRP-like molecules) (Alemán and Martinezalvarez, 2013). Low molecular weight collagen peptides have strong activities for protecting the tight junction barrier function against TNF-α stimulation in Caco-2 cells (Chen *et al*., 2017b). Collagen peptides from milk fish (*Chanoschanos*) skin possess anti-cancer activity (Baehaki *et al*., 2016). Collagen peptides enhanced hyaluronic acid production in human dermal fibroblasts *in vitro* and in murine skin *in vivo* (Okawa *et al*., 2012).

Useful Properties

In addition to these bio-functions, collagen peptides also have various other properties. The properties of collagen peptides will contribute to their usage. Collagen peptides have been reported to have antifreeze activity, metal ion chelate abilities, film-form and promoting gel hardness.

Antifreeze

Antifreeze activity is a popular issue in industry. Antifreeze agent can preserve the structure, texture and quality of frozen foods. For example, antifreeze agent can inhibit the form the crystal and keep the texture delicate. Specific collagen peptides have been reported can inhibit ice crystal growth. Collagen peptides from pig skin have cryoprotective effects on conventional dough products (Chen *et al*., 2016). Collagen peptides from chicken inhibit ice crystal growth in sucrose model systems (Du and Betti, 2016). Collagen peptides from porcine greatly elevate the glass transition temperature of ice cream by binding with ice (Cao *et al*., 2016).

Beside the potential application in food preservation, collagen peptides with antifreeze and cryoprotection activity also take effect on cryo-protection of cells and tissues (Nguyen *et al*., 2018). Collagen peptides from pig skin have antifreeze effects on freeze-dried *Streptococcus* thermophiles, because they interact with cell membrane phospholipids during freeze drying (Wang *et al*., 2015c). A peptide derived from shark skin collagen hydrolysates also has hypothermia protective activity on *Lactobacillus bulgaricus* (Wang *et al*., 2014). The collagen hydrolysates can promise alternative antifreeze proteins. The most studied ones, fall into two groups: Antifreeze Proteins (AFP) and Antifreeze Glycoproteins (AFGP).

In addition to inhibiting ice nucleation, these proteins inhibit icecrystal growth and change the crystal habit of ice by binding to small ice crystals. One of the common features among these antifreeze proteins is that their ice binding face is flat and the distance between oxygen atoms on this face is about the same as that in ice nuclei, that is, about 4.52 Å (Kumar *et al*., 2015). Molecular modeling of model gelatin peptides revealed that they form an oxygen triad plane at the C-terminus with oxygen-oxygen distances similar to those found in ice nuclei. Binding of this oxygen triad plane to the prism face of ice nuclei via hydrogen bonding appears to be the mechanism by which gelatin hydrolysate might be inhibiting ice crystal growth in ice cream mix (Damodaran, 2007).

In nature, antifreeze proteins exist in a wide range of living organisms, such as Antarctic fish, insects, plants, bacteria and fungi, all thriving in a sub-zero environment. These antifreeze substances are synthesized in their bodies and are believed to play a role in protecting biological tissues from freezing damage during harsh winters or in cold surroundings, through noncolligatively depressing the freezing point without affecting the melting point (thermal hysteresis proteins) and/or inhibiting ice crystal growth (ice-structuring proteins) in their body fluids.

Mental Ion Chelate Ability

Chelateable peptides protect minerals from the inhibition effect of phytic acid or other inhibitors in the gastrointestinal tract (Li *et al*., 2017). Therefore, the mineral will have a higher bioavailability with the exit of chelateable collagen peptides. This property of collagen peptides can be used to promote mineral absorption for the mineral deficiencies would affect people's health. Some collagen peptides have been reported as mineral absorption promoters. Collagen peptide chelated mineral (Ca^{2+}, Iron, Zn^{2+}, Mg^{2+}) products can be used in foods, feeds and pharmaceuticals (Wu *et al*., 2019). Several peptides from Alaska Pollock skin collagen have a mineral (Ca^{2+}, Fe and Cu^{2+}) chelation ability. In addition, they have the potential to be used as functional ingredients to manage mineral deficiencies (Guo *et al*., 2015).

Calcium is an element that studied most often. Tilapia scale collagen hydrolysates can chelate with Ca^{2+} (Liao *et al*., 2020). In addition, these hydrolysates are potential functional foods with a calcium-supplement effect. Collagen peptides from deer sinew significantly increase the levels of serum calcium (Zhang *et al*., 2014). Besides, collagen peptides can also chelate with iron (Guo *et al*., 2013), copper (Li *et al*., 2015) and promote their absorption.

Other Properties

In addition, collagen peptides have lots of other properties. These properties determine their other usages, e.g., surfactant, gel-form and film-form. As a zwitter-ion, collagen peptides act as a surfactant. They can act as an emulsifier, foaming agent, de-foaming agent, etc. The gel-form and film-form are properties of the collagen or gelatin. Collagen peptides can also contribute to forming a gel or film. Some big collagen peptides even have the properties of gels or films. In addition, adding a certain concentration of collagen peptides can promote the hardness of agar gel (Yoshimura *et al*., 2007).

Recommendation for Further Research

The remarkable growth of bioactive peptide development in the past decade has led to a large number of market approvals and the market value is expected to hit $25 billion by 2018 (Daliri *et al*., 2018). As food derived bioactive peptides, collagen peptides have huge potential in functional foods, cosmetic and medicine industry. Collagen peptides will be interested for more and more researchers, manufacturer and consumers.

The applications of collagen peptides should be promoted. The benefits of collagen peptides will contribute to their development. If the commercialization of collagen peptides is successful, its development will be ensured. In addition, people will be motivated to research and develop the applications. We should also encourage researchers to develop new production methods for collagen peptides. Those products should be used to replace the production of petrochemical materials to help our fragile environment.

We should develop collagen peptides from marine environments. Marine ecosystems are huge reservoirs of collagen. However, most marine collagen is discarded or used as low value feed. Additionally, marine collagen is easy to denature, especially collagen that derived from deep sea or cold zone animals (Gómez-Guillén *et al*., 2011). Marine collagen can be used in gelatin form and marine gelatin (especially gelatin from cold water) has a weaker gel strength and lower gelling and melting temperatures than that of non-marine gelatine (Gómez-Guillén *et al*., 2011). These drawbacks limit their usage as gel-forming materials. Marine collagen can be used in its hydrolysis form to overcome the drawback that it is easily denatured and its usage will relieve the shortage of terrestrial collagen. Terrestrial collagen cannot meet the requirements for development due to the population, epidemic diseases in livestock and religion reasons.

The structure-activity relationship is the key point for collagen peptide use as an active ingredient in food. Structure determines the function. We should determine which structure characteristic determines a function and which factor affects the function. These rules will allow us to find or synthesis more effective collagen peptides. In addition, the mechanisms of collagen peptide functions are also important.

Try to get help from other disciplines, especially informatics. With the assistant of bioinformatics, finding functional collagen peptides is easy and we can predict that which food produce a special peptides during its digest. Besides we also need the assistant of bioengineering. We can produce a large number of target collagen peptide by fermentation of the engineering bacteria.

The toxicology of collagen peptides should be noted because they are used in foods, drugs and cosmetics. Although collagen and its hydrolysates are relatively safe and minimally allergenic, it is possible that some of them have a strong allergen city and some protein hydrolysates may contain prions. The collagen hydrolysates from cattle with mad cow disease may contain some prions.

Conclusion

Collagen or gelatin is a plentiful biomaterial. Collagens, gelatins and their hydrolysates can be used as foods, foods additives, functional foods, medicine and cosmetics. However, they are often used as low value materials or discarded. The full use of collagen or gelatin can be realized via hydrolysis methods.

Collagen or gelatin can be hydrolysed using different types of enzymes or chemicals to produce collagen peptides. Chemical hydrolysis is cheap but difficult to control. It often produces amino acids and small peptide fragments. In contrast, biochemical hydrolysis is predictable and stable. Hydrolysis with enzymes often results in different sizes of peptides that we can predict.

These hydrolysates or collagen peptides have a lot of functions. Collagen peptides can act as a provider of amino acids, a collagen component, a bioactive substance, or a functional material. These functions determine that they can be used in functional foods, cosmetic and medicine. In addition to these bio-functions, they can also be used as food additives for their antifreeze and antimicrobial activities or as a surfactant because of their special structures.

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Author's Contributions

Mengqian Chen and Yanan Li: Wrote the paper.

Gangrong Huang: Provides ideas and helped to revise the article.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

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