

MiniReview

Collagen in food and beverage industries

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Article history

Received: 5 May 2014
Received in revised form:
30 June 2014
Accepted: 8 July 2014

Keywords

Collagen
Food
Beverage
Nutrition
Health

Abstract

This paper reviews the structure, function and applications of collagens in food industry. Collagen is the most abundant protein in animal origin. It helps maintaining the structure of various tissues and organs. It is a modern foodstuff and widely used in food and beverage industries to improve the elasticity, consistency and stability of products. Furthermore, it also enhances the quality, nutritional and health value of the products. Collagen has been applied as protein dietary supplements, carriers, food additive, edible film and coatings. Therefore, this paper will review the functions and applications of collagen in the food and beverage industries. The structure and composition of collagen are also included.

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Introduction

Collagen is the most abundant and ubiquitous protein in animal origin, which comprising approximately 30% of total protein. Collagen is mainly presents in all connective tissues, including animal skin, bone, cartilage, tendon and blood vessels (Muyonga *et al.*, 2004; Pataridis *et al.*, 2008; Cheng *et al.*, 2009; Huo and Zhao, 2009; Aberoumand, 2012; Liu *et al.*, 2012). It is involved in the formation of fibrillar and microfibrillar networks of the extracellular matrix and basement membranes. The fibrillar protein is composing the major protein component of bone, cartilage, tendon, skin and other forms of connective tissues (Gelse, 2003; Huo and Zhao, 2009; Mocan *et al.*, 2011; Liu *et al.*, 2012). Collagen forms great tensile strength and stable insoluble fibrils, contributing to the stability and structural integrity of tissues and organs (Gelse *et al.*, 2003; Li *et al.*, 2009; Mocan *et al.*, 2011).

According to Gomez-Guillen *et al.* (2011), the properties of collagen can be classified into two groups. First, the properties related with their gelling behavior such as texturizing, thickening, gel formation, and water binding capacity. Second, the properties related to their surface behaviour, which include emulsion, foam formation, stabilization, adhesion and cohesion, protective colloid function and film-forming capacity. In addition, collagen is a good surface-active agent and demonstrates its ability to penetrate a lipid-free interface (Lee *et al.*, 2001).

As a result of its excellent biological compatibility

and degradability, with weak antigenicity, it has been widely applied in the food industries, pharmaceutical, cosmaceutical, biomedical, tissue engineering and film industries (Bae *et al.*, 2008; Nalinanon *et al.*, 2008; Matmaroh *et al.*, 2011; Liu *et al.*, 2012). It can form firm and stable fibers by self-aggregation and cross-linking, which makes it valuable in drug delivery system (Wang *et al.*, 2008). The wide application of collagen in medicine and pharmacology is also related to its natural properties, including hemostatic activity, biodegradability, low allergenicity with high antigenicity and biocompatibility (Helena *et al.*, 2013).

Collagen polypeptide chain and cross-linkages can be breakdown by partial thermal hydrolysis to form gelatin (Djagny *et al.*, 2001; Gómez-Guillén *et al.*, 2011). In another words, gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen. Gelatin was denatured form of native collagen and adopted a wide distributions and lower molecular weights than collagen. Collagen exhibited superior and distinct properties from gelatin such as higher enthalpy, greater network structure of fibril, basic isoelectric point and high resistance to protease hydrolysis (Zhang *et al.*, 2005). The native triple helices and fibril networks in the collagen membrane were more rigid and firm than the gelatin membrane (Zhang *et al.*, 2005). Collagen has great mechanical strength and reversible extensibility (Pauling and Corey, 1951) while gelatin is remarkably described on its unique rheological properties which are gel strength, thermal stability and viscoelastic properties

(Gómez-Guillén *et al.*, 2011).

In recent times, acid extraction with pepsin hydrolysis was a common method used to extract the collagen. Specifically, acetic acid has often been used as a solvent for the extraction of collagen (Cheng *et al.*, 2009). The pepsin activity can be increased at low pH and body temperature conditions. Since extreme conditions will damage the integrity of collagen structure, most methods on collagen extraction have been focused to low temperature and short time exposure (Lin and Liu, 2006).

The source of commercial collagen

Collagen has been extracted from the skin and bones of some vertebrate species, mainly bovine and swine. After the outbreaks of bovine spongiform encephalopathy, foot and mouth disease, autoimmune and allergic reactions, there were some restrictions on collagen from these sources (Cliché *et al.*, 2003; Liu *et al.*, 2007; Liu *et al.*, 2012).

Furthermore, collagens and gelatins halal status depends on the origin of raw materials used in its manufacture. Porcine based collagens are strictly prohibited (haram) for Muslims while cattles are permitted after being halal-slaughtered. Recently, halal authenticity is an issue of major concern in the food industry. Nonspecific collagen is highly suspected of containing porcine elements, and very strongly discouraged for use by the Muslims. However, fish based collagen are halal to Muslims (Riaz and Chaudry, 2004; Fadzilillah *et al.*, 2011). Therefore, collagens from marine life origin were widely used in the industry, which can be extracted from fish, sponges and jellyfish (Liu *et al.*, 2007; Parenteau-Bareil *et al.*, 2010).

Marine collagen has raised great interest for its potential application mainly in food manufacturing. Thus, extraction and characterization of collagen from different fish species has been reported, such as, pacific cod (*Gadus macrocephalus*) (Wang *et al.*, 2013), baltic cod (*Gadus morhua*) (Skierka and Sadowska, 2007), barramundi (*Lateolabrax niloticus*) and red tilapia (*Oreochromis niloticus*) (Jamilah *et al.*, 2012), hake (*Merluccius hubbsi*) (Ciarlo *et al.*, 1997), ocellate puffer fish (*Takifugus rubripes*) (Nagai *et al.*, 2002) surf smelt (*Hypomesus pretiosus japonicus Brevoort*) (Nagai *et al.*, 2010), bighead carp (*Hypophthalmichthys nobilis*) (Liu *et al.*, 2012), rainbow trout (*Onchorhynchus mykiss*) (Tabarestani *et al.*, 2012), albacore tuna (*Thunnus alalunga*), Dog Shark (*Scoliodon sorrakowah*) and Rohu (*Labeo rohita*) (Hema *et al.*, 2013), black drum and sheepshead seabream (Ogawa *et al.*, 2003), golden goatfish (*Parupeneus heptacanthus*) (Matmaroh *et al.*,

2011), logbarbel catfish (*Mystus macropterus*) (Zhang *et al.*, 2009) and Jumbo squid (*Dosidicus gigas*) mantle collagen (Uriarte-Montoya *et al.*, 2010).

The waste from surimi processing could also be the primary material for extracting collagen from underutilized fish resources. Several studies were reported on collagen extraction from Pacific whiting surimi processing byproducts (Kim and Park, 2004), skins of underutilized fishes (Bae *et al.*, 2008) and fish waste materials (Nagai and Suzuki, 2000; Aberoumand, 2010). Besides, alligator bones were also investigated as an alternative supply of highly thermo stable type I collagen. In addition, type I collagen isolated from the skin of giant red sea cucumber has shown as potential collagen source for nutraceutical application (Gómez-Guillén *et al.*, 2011).

Eggshell membrane collagen has been proven to be very low in autoimmune and allergic reactions as well as high in biosafety (King'ori, 2011). Previous works on the extraction and characterization of collagen from by-products of poultry animals have been reported. There were studies on chicken feet (Liu *et al.*, 2001; Prayitno, 2007; Almeida *et al.*, 2012), chicken skin (Cliche *et al.*, 2003), chicken bone (Omokanwaye *et al.*, 2010), bird feet (Lin and Liu, 2006), silky fowl feet (Cheng *et al.*, 2009), turkey skins (Nnanna *et al.*, 2006), avian bone (Knott and Bailey, 1999) and duck feet collagen (Huda *et al.*, 2013). Lin and Liu (2006) demonstrated that the bird feet contain abundant of collagen and may be a good source to replace mammalian collagen.

Structure and composition of collagen

The collagen is a protein forming triple helix of three polypeptide chains in the extracellular matrix. Every chain is composed of thousands amino acids based on the Gly-X-Y sequence (Figure 1). The X and Y positions are mostly found to be proline and hydroxyproline (Gelse, 2003; Cheng *et al.*, 2009; Parenteau-Bareil *et al.*, 2010; Liu *et al.*, 2012). An interchain hydrogen bonding between glycine and amide group in an adjacent chain is a key factor in stabilizing the collagen triple helix (Matmaroh *et al.*, 2011). It is a hydrophilic protein because of the greater content of acidic, basic and hydroxylated amino acid residues than the lipophilic residues (Greene, 2003). Collagen has a complex molecular structure and interacts with each other at different levels forming higher order structures with distinctive features (Friess, 1998).

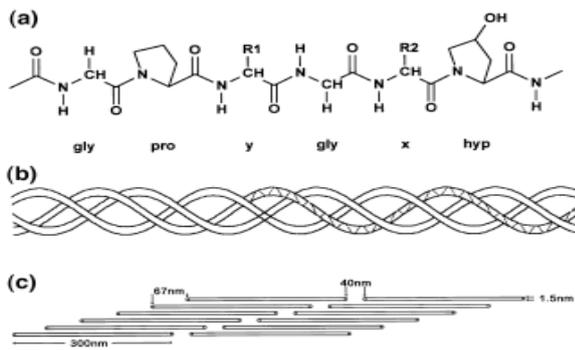


Figure 1. Chemical structure of collagen type I. (a) Primary amino acid sequence, (b) secondary left handed helix and tertiary right handed triple-helix structure and (c) staggered quaternary structure (Friess, 1998).

Collagen has been shown to vary in their amino acids composition, particularly proline and hydroxyproline. Glycine, proline and hydroxyproline are the most important amino acids in collagen, which accounts for 50% of the total protein content (Huo and Zhao, 2009; Matmaroh *et al.*, 2011). The proline and hydroxyproline content is particularly important for the gelling effect. Meanwhile, hydroxyproline is believed to play a singular role in the stabilization of the triple-stranded collagen helix due to its hydrogen-bonding ability through its -OH group. Moreover, it has also been observed that the total Gly-Pro-Hyp sequence content is one of the main factors affecting collagen thermostability (Gómez-Guillén *et al.*, 2011).

Generally, mammalian protein contain large amounts of hydroxyproline and hydroxylysine, and the total imino acid (proline and hydroxyproline) content is high (Karim and Bhat, 2009). Imino acid of poultry collagen may similar or slightly lower to that mammalian collagen (Lin and Liu, 2006). The fish collagens have less proline and hydroxyproline but higher in serine and threonine than mammalian collagens. Methionine is also present in greater amounts (Piez and Gross, 1960). Similar results were reported from the study of fish and squid skin with mammalian collagens (Nam *et al.*, 2008). There is a correlation between the imino acid values on the stability of the collagen fibers and influences the shrinkage and denaturation temperature as well (Piez and Gross, 1960; Pati *et al.*, 2010). The high imino acid content is extremely important because it affects the functional properties which are solubility, cross-linking ability and thermal stability of collagen (Gomez-Guillen *et al.*, 2002). Therefore, high imino acid collagen may have wider applications in food and beverage industry.

Type of collagen

There are 27 types of collagen reported by Canty and Kadler (2005). The collagen types were classified by their size, function and distribution which differ considerably in their amino acids composition (Gelse, 2003; Pataridis *et al.*, 2008; Cheng *et al.*, 2009; Liu *et al.*, 2012). Every collagen type has its special amino acid composition, and each performs a distinctive role in the tissue. Type I, II and III are the most abundant collagen which responsible for tissue strength, elasticity and water retention capacity (Cheng *et al.*, 2009).

Generally, Type I collagen has the highest percentage and extensively applied in industry. Type I collagen forms more than 90% of the organic mass of bone and is the most important collagen of tendons, skin, ligaments, cornea, and most interstitial connective tissues. It provides tensile stiffness for tendons and fascia in organs. In bones, it defines extensive biomechanical properties regarding load bearing, tensile strength, and torsional stiffness after calcification (Gelse *et al.*, 2003; Cheng *et al.*, 2009). Nonetheless, the major sources of type I collagen are mainly from mammalian tissues (Ikoma *et al.*, 2003).

Type II collagen is the main collagen in cartilage of mammals. These collagen types are essential for the synthesis and reconstruction of connective tissue all over the body. This collagen type is reportedly help in reducing the destruction of collagen within the body, may provide anti-inflammatory activity and may improve joint flexibility (Crowley *et al.*, 2009). However, type II collagens have the constraint for applications because of their low yield and complex sample pretreatment before extraction is required (Cao *et al.*, 2013).

Type III collagen is widely distributed in collagen type I containing tissues. It is an important component of reticular fibers in the interstitial tissue of the lungs, liver, dermis, spleen, and vessels. The molecule also often contributes to mixed fibrils with type I collagen and is also abundant in elastic tissues (Gelse *et al.*, 2003).

Applications of collagen in foods and beverages industries

Nowadays, collagen has become in demand ingredient towards the healthy foods development. Collagen productions in the body decrease with age and bad diet. As collagen injections are not a preference to most people, the next best alternative to gain collagen is through diet. Therefore, collagen has been blended together in variety of foods and beverages products.

Currently, there are many available commercial

collagen products from different sources marketed locally. Examples of food grade bovine collagen are Colageno manufactured by JBS, Brazil and Cosen by Jiangxi Cosen Biochemical, China. Meanwhile, Ovinex is type I and III food grade ovine collagen manufactured using enzymatic process by Hollista CollTech, Australia. In addition, Peptan by Rousselot SAS, France and Ni-Kollagen by Bionic Life Science, Malaysia are several marine collagen suggested for applications in dietary supplements, functional food, drink and beverage, confectionery and desserts.

Collagen Supplements

Collagen and health benefits linked have led to the establishment of collagen supplements industry. Due to the moisture absorption features, collagen and its fractions have shown a major function as valuable nutritive fibers and protein source in composing human diets (Neklyudov, 2003). As human grow older, collagen synthesis will decrease and the tissues get thinner, weaker and less supple. Collagen supplements are intended to uphold skin, hair, nails and body tissues of the users (Wong, 2010; King'ori, 2011). The metabolites of collagen assemble bone, skin and ligaments by attracting fibroblasts that generate the synthesis of new collagen. It develops the diameter of collagen fibrils in the dermis and cohesion of the dermal collagen fibers. Therefore, the thickness, suppleness and resilience, as well as hydration of the tissues will be enhanced (King'ori, 2011).

Nutricosmetics are usually offered in the form of liquids, pills or functional foods (Wong, 2010). Hence, the local snack manufacturer, Munchy's, has introduced 'Wheat Krunch Collagen' to promote the collagen goodness. The baked crackers were added with marine collagen which contains about 1200 mg collagen (Anonymous, 2011). The collagen may advance the function of skin dermis and epidermis by increasing the water absorption ability of the outermost skin layer. Hydration of skin tissue is directly allied to smoothness and reduces wrinkling (King'ori, 2011).

Collagen supplement can boost up lean muscle gain, decrease recovery time, reconstruct damaged joint structure and improve cardiovascular performances. This is achieved by collagen's promotion of natural creatine, an essential amino acid in new muscle growth following workouts. Arginine within the hydrolyzed collagen also promotes muscle mass (King'ori, 2011). Therefore, collagen is in demand within the sports nutrition field.

Collagen type II is efficient in the treatment of rheumatoid arthritis, a chronic inflammatory

sickness characterized by pain, swelling and stiffness of multiple joints (Zhang *et al.*, 2008). A controlled study using chicken type II collagen in rheumatoid arthritis patients verified that after the 6 months observation, there was a significant reduces in pain, morning stiffness, tender joint count and swollen joint count of the patients.

Collagen as food additives

Food additive refers to substance added into foods during processing to improve color, texture, flavor or qualities. The examples are antioxidants, emulsifiers, thickeners, preservatives and colorants. Collagens are used as food additives, which improve the rheological properties of sausages and frankfurters as well as assurance the presence of animal nutritive fibers in adequate amount (Neklyudov, 2003). Meat containing raw material added with collagen or its fractions could enhance its technological and rheological properties. Addition of collagen to liverwurst or paste improves the products' quality and reduces the occurrence rate of fat caps.

Santana *et al.* (2011) suggested that the heat treated collagen fiber has a good potential for use as emulsifier in the food application, especially in acidic products. The stability, microstructure and rheology of the oil-in-water emulsions were evaluated. The phase separation and droplet size of the emulsions prepared has decrease protein concentration and reduced pH value, allowing the production of electrostatically stable emulsions at acidic pH. The acid emulsions by high pressure homogenization showed droplets with lower dispersion and decrease six times than the primary emulsions in surface mean diameter. According to Gray (2011), heat stabilized collagen fiber may be a natural alternative to synthetic emulsifiers for use in acidic food and drink formulations. The heating process under acidic decreased the protein charge and increased protein solubility in water, which probably decreased the oil-protein interactions. As a consequence, the primary emulsions composed by heat treated collagen fiber have a higher creaming index and emulsion rate (Santana *et al.*, 2012).

Rao *et al.* (1981) reported the use of food grade collagen to replace lean meat in bologna formulations. Coarse bologna and the fine emulsion bologna were made with replacement of lean meat with fibrous collagen. No undesirable effects were found on shrinkage, volume changes, emulsion stability, free and total water or fat and protein content. The collagen replacement also did not significantly influence the pH, pressed fluid, cooking loss and color differences. As a result, they concluded that the

addition of bovine hide collagen in coarse bologna and fine emulsion bologna is feasible.

Duck feet collagen was added to threadfin bream and sardine surimi to study its effect on physicochemical properties. Addition of the collagen resulted a significant improvement in the properties of surimi. Besides improving the gel strength and gel hardness, the collagen was able to improve the folding test score and the colour lightness. The study recommended that collagen would be an alternative basis of protein additive for the development of the surimi quality (Huda *et al.*, 2013). Furthermore, the addition of chicken feet collagen for the jelly production was also reported to have good willingness and acceptance from the consumer (Almeida *et al.*, 2012).

Collagen as edible films and coatings

Edible films and coatings are edible materials applied on or within foods in thin layers by wrapping or immersing, brushing or spraying (Greene, 2003). The main application of collagen films is as barrier membrane to protect against the migration of oxygen, moistures and solutes, providing structural integrity and vapor permeability to the food products (Greene, 2003; Bourtoom, 2008; Dahm, 2011). Moreover, edible films in the food products have great prospective to prolong the shelf life of foods (Greene, 2003).

It is well known that collagen subjected to special treatment may be used for preparing sausage casings (Neklyudov, 2003). Production of collagen sausage casings from the regenerated corium layer of food grade beef hides is a well established technology (Gennadios *et al.*, 1997). Besides, an alternative method to preformed collagen casing has been developed where the collagen casing is coextruded around the sausage meat batter. The coextrusion process is continuous and well controlled than the conventional batch process where meat batter is stuffed into preformed casings (Gennadios *et al.*, 1997).

An edible collagen film proposed for use on netted roasts, boneless hams, fish fillets, roast beef and meat pastes were able to reduce cook shrinkage, enhance product juiciness and ease the removal of elastic stretch netting after heat treatment (Gennadios *et al.*, 1997). Greene (2003) had evaluated the use of collagen coatings as flavor protection in dry pet food made with rendered poultry fat. During the study, collagen was extracted from chicken skins, soaked in an acidic solution, applied to dry cat food and dried to form a surface film. The collagen coatings function as a protective barrier against oxidation in the poultry

fat which may lead to the musty and moldy aromas. The potential of replacing plastic meat wrappings with collagen based edible films was studied by Gennadios *et al.* (1997). There were no significant different when beef cubes wrapped in collagen based films and freezed for 20 weeks compared to the plastic wrapped controls in terms of oxidation, color, microbial growth and sensory attributes (Gennadios *et al.*, 1997).

Collagen in drinks

Nowadays, collagen-infused drinks are another trend in global market. There are a lot of products released by the manufacturers such as soy collagen, cocoa collagen, cappuccino collagen, juice with collagen and bird nest drink with collagen. Tree (2012) suggested an energy drink infused with collagen to help promotes the body's natural capacity to generate the fatty tissues. Generally, the collagen drink claims to stimulate the collagen making mechanism in the body, which in turn will promote the body tissues and reduce the skin wrinkles and sagging.

In Malaysia, several organizations have carried research and development on collagen drinks. Malaysia Dairy Industries (MDI) has added collagen peptides in their nutritious probiotic drink (Soo and Tan, 2009). It contains prebiotic fibre and added with 500 mg of collagen peptides and 30 mg of vitamin C. The collagen peptides served as components required to synthesize collagen. In addition, vitamin C was added in the drink as an antioxidant and a vital coenzyme in the biosynthesis of collagen. As a result, "Vitagen Collagen" drink was created to stimulate the growth of beneficial gut bacteria and to radiate beauty from beyond skin deep. In addition, Avon has also formulated the Avon Life Marine Fish Peptide Collagen Drink, a revolutionary drink made from natural and high quality fish peptide collagen from Salmon fish skin, vitamin C and fructo-oligosaccharides (Najumudeen, 2012; Anonymous, 2012). According to Yacoubou (2011), Nestle Malaysia has also released a Nescafe Body Partner, Kacip Fatimah and Collagen Coffee that contained collagen from fish source.

The triple helix and rod like structure of collagen is thermally labile and play a significant function as a clarifying agent in the cloudy alcoholic beverages by aggregation of the yeast and other insoluble particles (Zhang *et al.*, 2005). Hickman *et al.* (2000) exposed that bovine collagen solutions were possible to be useful in refining beers and yeast preparations by chemical modification. Collagen has a distinctive caprylic taste. However, collagen containing food and drink taste can be significantly improved by blending

sucralose and stevia extract, desirably further blending with acesulfame potassium (Takemori *et al.*, 2005).

Collagen as carriers

Collagen in the form of films or coatings could function as carriers of active substances such as antioxidants, antimicrobials, colors and flavors (Han and Gennadios, 2005; Bourtoom, 2008). Collagen was able to be isolated into an aqueous solution and molded into various forms of delivery systems. The applications of collagen as delivery systems are mini pellets and tablets for protein delivery (Lee *et al.*, 2001).

Collagen preparations have shown good potential to be applied as carriers of rosemary extract in the production of processed meat, but the application are dependent on the preparations forms and properties of the extract. Referring to Waszkowiak and Dolata (2007), collagen in fiber form was a better carrier of rosemary extract than collagen hydrolysate. Furthermore, the introduction of rosemary extract to meat products through a collagen fiber preparation may enhance its antioxidant activity.

Conclusion

Collagen has shown to be an important ingredient in the food and beverage industries. It is mostly used in the form of collagen fiber. Collagen has been applied as protein dietary supplements, carriers in the meat processing, edible film and coatings of products, and food additive to improve products' quality. In addition, collagen may boost the health and nutritional value of the products.

Acknowledgement

The author thanks Universiti Putra Malaysia (UPM) for the continuous support in completing this manuscript.

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