Fish gelatin nanoparticles and their food applications: a review

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Abstract

Considerable attention has been directed to nanoparticles based on gelatin biopolymer due to its numerous available active group sites for attaching target molecules and acting as a drug or nutraceutical delivery system aiming to improve the therapeutic effects and also to reduce the side effects of formulated drugs as gelatin is a natural biodegradable biocompatible polymer, nontoxic, readily available, cheap and is used in parental formulations. With mammalian gelatin (pig and cow) as the major source of gelatin production, alternatives are required due to socio-cultural and health concerns to maintain halal status. This paper aims at reviewing fish skin gelatin from warm water species which can provide a potential alternative source of gelatin with almost the same rheological properties as mammalian gelatin and is a beneficial way to use fish waste such as skin, bones and fin which is generally discarded. The study also entails a lot of research being done in the field of nanoencapsulation of gelatin with various nutraceuticals as well as drug and gene therapy. There is an especially increasing interest in encapsulating biopeptides within gelatin nanoparticles in the functional food industry due to their role in preventing or delaying the onset of various diseases, food fortification, improvement of food quality, increase in shelf life, targeted peptide delivery and hence can be used as additives in food products. This review also attempts to provide an overview of the application of gelatin nanoparticles in nanoencapsulation in the food industry.

Introduction

Gelatin is a hydrocolloid polymer and is a derivative of collagen extracted from the skin, bone, and connective tissues of various animal kinds. On partial hydrolysis of collagen, it becomes a denaturalized protein and has found various applications as an important alternative source of protein in the field of food, materials, cosmetic, pharmaceutical and photographic industries (Jelloui et al., 2011) depending upon its rheological properties. While various physicochemical properties of gelatin such as the molecular composition, the color, taste and odor of gelatin, solubility, transparency are determining factors for its usability, gel strength, viscosity and thermal stability (gelling and melting temperatures) are the important criteria for establishing its overall commercial applicability. Therefore, gelatin is extremely versatile in its application if the food industry as an emulsifier, foaming agent, colloids stabilizer, fining agent, biodegradable packaging material and micro-encapsulating agent (Gómez-Guillén et al., 2011). Moreover, its shows promising biomedical utility including plasma expander, stabilizer in a number of protein formulations, vaccines and gelatin sponge (Gelfoam®). Having proved as a safe food supplement which is also documented by the classification as “Generally Recognized as Safe” (GRAS) by the US Food and Drug Administration (FDA).

Pig skin (46%), bovine hide (29.4%) and pork and cattle bones (23.1%) account for the world’s major gelatin production whereas fish gelatin, in the year 2010 has accounted for less than 1.5% of total gelatin production. However, the current production of gelatin has been doubled since 2002, suggesting that alternative non-mammalian sources have grown in importance (Gómez-Guillén et al., 2002). Gelatin from mammalian sources raises the sociocultural issue of not being halal and acceptable by various religious groups all over the world as well as the sanitary aspects regarding the animals such as the...
outbreak of mad cow disease in cows. Hence, an interest has risen in utilizing by-products from the fish industry to better use as gelatin sources and why exploring different species and optimizing the extraction of fish gelatin has attracted the attention of researchers in the last decade (Gómez-Guillén et al., 2002; Karim and Bhat, 2009). While 78% of the fish catch in both developed and developing countries accounts for human consumption, about 21% is left wasted for non-food usage (Vannuccini, 2004). According to Kelleher (2005), fish industry and processing generate a large biomass of fish waste in the form of skin, bones and fins which accounts for 7.3 million tons/year and is discarded into the environment. This has led to the establishment of fish waste as a potential halal gelatin source which confirms with many socio-cultural norms.

Gelatin is cheap and readily available. It offers the great advantages for its biocompatibility, biodegradability and low antigenicity (Elzoghby, 2013). Being derived from collagen, the most abundant protein source in animals, it is natural and does not produce any harmful by-products on degradation. However, a major drawback associated with gelatin is its heterogeneity in molecular weight distribution, which arises issues producing stable nanoparticles from it. Nonetheless, a lot of successful research has been done on the preparation of nanoparticles from gelatin and its derivatives and have been employed in a range of biomedical applications such as encapsulation of bioactive compounds, targeted drug delivery and sustained release inside the human body (Dwivedi et al., 2012).

The addition of various bioactive compounds showing promising health benefits such as vitamins, minerals, antioxidants, antimicrobial, biopeptides, probiotics, enzymes, polyphenols and even targeted drugs have become a growing trend in the contemporary food industry, thereby improving the functional and nutritional value of food. Nanoencapsulation of bioactive compounds is widely employed for achieving stabilized food composition and the nanocarrier food systems comprise of lipid or natural biodegradable polymer-based capsules such as albumin, gelatin, alginate, collagen, chitosan, and α-lactalbumin are most often utilized for encapsulation (Reis et al., 2006; Graveland-Bikker and De Kruif, 2006). Since the delivery of any bioactive compound to various sites within the body is directly affected by the particle size (Kawashima, 2001; Hughes, 2005) nanoparticles improve the bioavailability, delivery properties, and solubility of the nutraceuticals due to more surface area per unit volume and thus their biological activity improves and allows them to enter the bloodstream from the gut more easily. Nanoencapsulation also protect the bioactive compounds in the digestion stream from oral and rapid intestinal degradation until their release at targeted sites (Gouin, 2004).

Epidemiological studies have established bioactive peptides derived from major protein sources such as meat, milk, egg, soybeans, fish, nuts, legumes with numerous health benefits such as antihypertensive, antioxidant, anti-inflammatory, antimicrobial, immuno-modulatory and other biologically relevant activities (Sacks et al., 2006; Pan et al., 2009). Many of such bioactive peptides (biopeptides) are potent antioxidants and as oxidative stress is the main cause of the onset of various chronic diseases such as various forms of cancer, tumor, hypertension, arthritis, antioxidant biopeptides could be a potential remedy to prevent or delay the onset of such diseases. Nano-encapsulated antioxidant biopeptides are highly permeable through the human intestines where fast degradation and better uptake of peptides into the bloodstream takes place and therefore incorporation into food systems can provide with many health benefits.

The aim of this paper is to review the latest development of nanoparticle production from fish and its application in food, which include physicochemical properties of the nanoparticles, the production optimization, and implication of using fish nanoparticles for encapsulating of biopeptides.

**Gelatin: structure and chemical composition**

Gelatin, which is a readily available natural polymer, is obtained upon denaturation of the collagen molecule and has the same chemical composition to that of collagen. It’s a triple helical structure due to the three α-chains as they provide an ideal environment for hydrogen bonding, each chain composed of several repetitions of amino acid sequences; Gly-X-Y sequence, where X is often proline, and Y is often hydroxyproline which is especially important for the gelling effect gelatin exhibits (Duan et al., 2011; Gómez-Guillén et al., 2011). The gelatin molecule is ~1.83% positively charged (lysine and arginine), ~12% negatively charged (glutamic and aspartic acid) and ~11% of the chain hydrophobic in nature (comprising leucine, isoleucine, methionine and valine). The two main factors which determine the application of gelatin in the commercial sector include the amino acid composition, which is species specific and the molecular weight distribution of the gelatin molecule, which is dependent on the processing conditions. The industrially manufactured gelatins are mixtures of different compounds known
as α-chains, β-chains and ε-chains (Karim and Bhat, 2009).

Commercially used gelatin is produced by partial alkaline or acidic hydrolysis of the collagen which undergoes pre-treatment normally done by heating in water at temperatures higher than 45°C which changes collagen into a soluble form. The native collagen molecule is cross-linked which is a determining factor for the pre-treatment processes which highly depends on certain factors as the species from which collagen is extracted, the age of the species, collagen type, animal tissue and so on. It is then followed by a chemical pre-treatment step which helps dissociate the non-covalent bonds, it helps swell the collagen molecule and aids in its solubilization for further treatment steps. Subsequent heat treatment cleaves the hydrogen and covalent bonds to destabilize the triple helix, resulting in helix-to-coil transition and conversion into soluble gelatin (Gómez-Guillén et al., 2002). Gelatin is available in two types, both used for industrial applications; type A, isoelectric point (pI) 7–9, prepared by an acid hydrolysis of pig skin type I collagen) or gelatin type B, pI 4.8–5, prepared by an alkaline hydrolysis of bovine collagen protein (Busch and Kniep, 2003; Mohanty et al., 2005; Wang et al., 2012). The degree of collagen conversion into gelatin is related to the severity of both the pre-treatment and the warm-water extraction process, as a function of pH, temperature, and extraction time.

Nanotechnology

Nanotechnology has created a new revolution in the scientific fields recently especially in the food industry from production to processing, storage and development of innovative materials, products, and applications. Nanotechnology encompasses the production, processing, and application of materials with sizes less than 1,000 nm (Sanguansri and Augustin, 2006). Nanotechnology is unique as it deals with reduced size particles which have increased the surface to volume ratio. This helps in an increased reactivity in mechanical, electrical and optical properties which offer many unique and novel applications in various fields (Neethirajan and Jayas, 2010).

Nanotechnology in the food industry has caused macroscale change to food characteristics such as texture, taste, other sensory attributes, coloring strength, processability, and stability during shelf-life, leading to a great number of new products. Moreover, nanotechnology can also improve the water solubility, thermal stability, and oral bioavailability of bioactive compounds (McClements et al., 2009; Huang et al., 2011; Silva et al., 2012). Industries use nanocomposites in food packaging material for controlling diffusion and microbial protection, nanobiosensors for detection of contamination and quality deterioration, and nanoencapsulation or nanocarrier for controlled delivery of nutraceuticals.

Various base polymers are used for the preparation of biodegradable nanoparticles depending on the desired applications such as proteins, polysaccharides and synthetic biodegradable polymers. It is necessary that these biopolymers are compatible, show minimum toxicity, must be sterile and non-pyrogenic, and good capacity for accommodation of desired products and protect them from degradation. The preparation process depends on many factors such as 1) size of the desired nanoparticles, 2) properties of the drug (aqueous solubility, stability, etc.) to be encapsulated in the polymer, 3) surface characteristics and functionality, 4) degree of biodegradability and biocompatibility, and 5) drug release profile of the final product.

Nanoparticles are colloidal solutions with the size ranging between 10-1000 nm and can be classified as 1) nanocapsules, which have hollow interior cavities to accommodate the bioactive compound protected by a biopolymer membrane 2) nanospheres, consist of a matrix system where the bioactive compound is uniformly dispersed within the polymer. This method of encapsulating bioactive compounds within polymer matrixes helps protect the contained bioactive molecule such as polyphenols, micronutrients, and antioxidants, vitamins against the adverse environment and for a targeted release in the body.

Gelatin nanoparticles from fish collagen

With the current demand for gelatin, fish by-products represent a potential alternative source of highly soluble collagen. Skin gelatin from various fish species as shown in Table 1 have been extracted and characterized. The collagen from fish has a low concentration of inter and intra crosslinking, which makes it feasible to produce gelatin (type-A) at a mild acid pre-treatment with an iso-electric varying from 6.5 to 9 (Busch and Kniep, 2003; Mohanty et al., 2005; Wang et al., 2012). Increasing The acid treatment gives hydrogen ions to collagen molecules, thus enabling faster absorption of water which is held in by electrostatic forces between charged polar groups (electrostatic swelling) or by hydrogen bonding between uncharged polar groups and negative atoms (lyotropic hydration). Wang et al. (2012) established that the type and concentration of acid used will determine the type and molecular weight distribution of gelatin obtained, as the acid.
used will impel the swelling and solubilization of the collagen fibers.

The current most applied technique for preparation of fish gelatin nanoparticles is desolvation which can be used to produce both type-A and B gelatins, followed by techniques such as coacervation and water-in-oil emulsification. In desolvation, the desired protein polymer solution is mixed with solvents of varied polarity and hydrogen bonding. As a displacement reaction takes place between the water molecules and the protein surface, precipitates of protein polymers are obtained (Coester et al., 2000). The gelatin protein molecules in solutions are well distributed and with subsequent addition of various solvents such as ethanol, acetone or isopropanol. Their solubility in water decreases due to the high hydrogen bonding between the solvents which displace water molecules creating phase separation of the rolled-up gelatin molecules from the remaining solution. The resulting molecules show a size range between 100–200 nm. The gelatin particles can further harden by the addition of crosslinking agents, aldehydes such as formaldehyde or glutaraldehyde. The crosslinking step can also prevent the degradation of the nanoparticles. The pH and stirring conditions affect the size range and yield of these particles (Kommareddy et al., 2005).

By a single step desolvation process, a heterogeneity in molecular weight of the parental gelatin polymer can produce nanoparticles with an un-uniform size range. To overcome this problem, and forming smaller, uniform gelatin nanoparticles, a second desolvation step was proposed by Coester et al. (2000). The high molecular weight (HMW) gelatin was precipitated in the first desolvation step to remove the low molecular weight which is separated and discarded. The HMW sediment obtained is resolved (re-dissolved in suitable aqueous medium) to carry out a second-stage desolvation process where gelatin nanoparticles obtained have high stability (predominantly by virtue of high molecular weight) and do not show any aggregation or flocculation (Brzoska et al., 2004).

**Role of biopeptides in the biotechnology industry**

Proteins are the building blocks of living things. Molecular composition of proteins comprises of biopeptide chains of varying length, mostly containing 2-20 amino acids (FitzGerald and Meisel, 2003) and molecular masses of less than 6000 Da (Sun et al., 2004) which can be obtained by enzymatic hydrolysis or solvent extraction. Each biopeptide chain so obtained from parental protein molecule can exhibit various biological activities such as immune modulatory, anticancer, antifungal, antimicrobial and potent antioxidants. However, upon storage and administration into the human body, these biopeptides are subjected to proteolysis, chemical modification, and denaturation which may affect its intended effect. In order to overcome this problem, these high molecular weight polypeptides can be cleaved into constituent peptide chains and can be incorporated, suspended and dispersed or encapsulated into different forms such as emulsions,

<table>
<thead>
<tr>
<th>Fish variety</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Baltic cod, salmon, herring, Atlantic salmon</td>
<td>Koodlijpka et al. (2008), Amnesen and Gildberg (2007)</td>
</tr>
<tr>
<td>Megrim, Hake, Dover sole</td>
<td>Sarafka et al. (2000)</td>
</tr>
<tr>
<td>Black tilapia</td>
<td>Jamilah and Harvinder (2002)</td>
</tr>
<tr>
<td>red tilapia</td>
<td>Iwaradi et al. (2009)</td>
</tr>
<tr>
<td>Bigeye snapper</td>
<td>Jongpreeonrat et al. (2006)</td>
</tr>
<tr>
<td>browntripe red snapper</td>
<td></td>
</tr>
<tr>
<td>Skate</td>
<td>Cho et al. (2000)</td>
</tr>
<tr>
<td>Blue shark</td>
<td>Yoshimura et al. (2000)</td>
</tr>
<tr>
<td>Dover sole</td>
<td>Gamarra et al. (2005)</td>
</tr>
<tr>
<td>Calfish</td>
<td>Yang et al. (2007), Liu et al. (2008)</td>
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<tr>
<td>Sn croaker</td>
<td>Chiew et al. (2007)</td>
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liposomes, nutraceuticals, and other edible biopolymers to gaining their optimum functionality, bioavailability, stability and targeted effectiveness (Amar-Yuli et al., 2010; Livney, 2010; Patel and Velikov, 2011; Elzoghby et al., 2012) for use in the pharmaceutical and food industry. Recent studies have established their role in treating many diseases as well as improving the quality and nutritious value of the food we consume. A large number of carriers have been designed for safe, controlled and targeted delivery of these biopeptides such as liposome, biosome, polymeric nanoparticles, solid lipid nanoparticles as well as gelatin nanoparticles to further enhance their utilization as well as stabilize these agents (Mishra et al., 2008).

Production of biopeptides

Proteins are the starting materials for biopeptide production and the common approaches used in the production of biopeptides are presented in the following sections.

Solvent extraction

Solvent extraction is the main technique for biopeptide production, and by using buffer saline solutions. The solvent mixture best used to purify biopeptides from fermented protein hydrolysates consists of three elements including trifluoroacetic acid (TFA), water, and acetonitrile (Wang et al., 2012). After protein hydrolysis, the solution is centrifuged followed by filtration of the supernatant. While solvent extraction is a relatively simpler process for biopeptide production, it may leave some residual solvent even after filtration, which can further be purified by liquid chromatography.

Enzymatic hydrolysis

Among the main techniques to produce biopeptides is enzymatic hydrolysis of the protein molecules. Enzymatic hydrolysis is the most favored protein degradation system due to lack of any toxic chemicals or residual solvents which render it safe for human consumption. Pepsin and trypsin have played a major role in production of many known biopeptides, for example angiotensin-converting enzyme (ACE)-inhibitory peptides and calcium-binding phosphopeptides (CPPs), are derived from the parental protein molecule by the action of trypsin (FitzGerald and Meigel, 2003; FitzGerald et al., 2004; Gobbetti, Minervini and Rizzello, 2004). Several digestive enzymes and different enzyme combinations of proteinases—including alcalase, chymotrypsin, pancreatin, pepsin and thermolysin as well as enzymes from bacterial and fungal sources—have also been utilized to generate bioactive peptides from various proteins (Kilara and Panyam, 2003; Korhonen and Pihlanto, 2003). In the case of antioxidant peptides, they have been generated using a mixture of alcalase and flavorzyme or pepsin and pancreatin (Aluko and Monu, 2003; Saiga, Tanabe and Nishimura, 2003; Gibbs et al., 2004; Sakanaka et al., 2004; Parrado et al., 2006; Blanca et al., 2007).

<table>
<thead>
<tr>
<th>Source</th>
<th>Activity</th>
<th>Enzyme used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower seeds</td>
<td>Peptides fractions</td>
<td>Alcalase, Flavoglyme</td>
<td>Villarroya et al. (1999)</td>
</tr>
<tr>
<td>Beans</td>
<td>Antioxidant and metal chelating peptides</td>
<td>Pepsin and pancreatin</td>
<td>Carrasco-Castilla et al. (2012)</td>
</tr>
<tr>
<td>Chickpea</td>
<td>Iron chelating</td>
<td>Pepsin and pancreatin</td>
<td>Fuentes et al. (2012)</td>
</tr>
<tr>
<td>Anchovy</td>
<td>Antimicrobial activity</td>
<td>Pepsin</td>
<td>Xu et al. (2009)</td>
</tr>
<tr>
<td>Sesame seeds</td>
<td>Zinc chelating activity</td>
<td>Papain, Alcalase</td>
<td>Wang et al. (2012)</td>
</tr>
<tr>
<td>Tofu</td>
<td>Antioxidant activity</td>
<td>Alcalase</td>
<td>Gilgin et al. (2015)</td>
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</tbody>
</table>


**Microbial fermentation**

The dairy industry utilizes starter cultures for their fermented goods which are highly proteolytic, such starter and non-starter bacteria can, therefore, be used to generate bioactive peptides. Lactic acid bacteria (LAB), e.g. *Lactococcus lactis*, *Lactobacillus helveticus* and *Lb. delbrueckii* spp., *Bulgarcus* make for a well-known proteolytic system and has found wide application inabiopeptide generation. This proteolytic system comprises of a cell wall-bound protease and a number of distinct intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases and dipeptidases (Christensen et al., 1999).

**Types of biopeptides and their properties**

Proteins have always been a major part of the human diet, however recently their applications have been widely increased. Proteins upon digestion in the gastrointestinal tract and enzymatic hydrolysis produce physiologically active biopeptides which show better uptake in the bloodstream and faster impact on the body metabolism and may ultimately influence health (Kitts and Weiler, 2003). As such upon oral administration of theses bioactive peptides, which are specific protein fragments, may affect the major body systems namely, the cardiovascular, digestive, immune and nervous systems depending on their amino acid sequence. Epidemiological studies have linked antihypertensive, antioxidant, antithrombobiopoteptides to the cardiovascular system; opioidbiopeptides to the nervous system; antiappetising and antimicrobial to the gastrointestinal system; and Cytolmodulatory and immunomodulatory to the nervous system.

**Contemporary food industry**

Various functional ingredients are added to food products worldwide to improve upon the taste, color, odor or preservation properties with the incorporation of bioactive peptides such as antioxidants or probiotics as the latest inclination. These inclusions add to the normal properties of food products, thereby making it ideal for the current fast paced lifestyle. However, a critical parameter for successful addition of these bioactive compounds is their stability upon addition and administration into the human body (Dordevic et al., 2014). Namely, health-promoting bioactive compounds such as vitamins, probiotics, minerals, polyphenols, omega-3-fatty acids, and phytosterols are sensitive to oxygen, light, heat, and water. Such factors can potentially limit their addition to food products as well as their shelf life and bioavailability (Champagne, 2007) as they might produce byproducts upon degradation such as off-flavors, off-colors, or carcinogenic compounds. Furthermore, upon oral consumption, these compounds undergo rapid gastrointestinal digestion and enzymatic hydrolysis which can lead to a potential change in the chemical structure of the compound thereby changing its bioactivities. Ensuring the stability of bioactive compounds, therefore, becomes the most critical step in preparation and storage of these fortified foods. It is also mandatory to stabilize these compounds in the gastrointestinal tract and allow controlled targeted delivery.

An additional critical factor in the implementation of fortified food products is the limited uptake in the bloodstream and bioavailability of theses bioactive compounds. Solutions are required to overcome all these issues such that the qualitative and organoleptic properties are maintained throughout the food manufacturing as well as digestion process. For that reason, encapsulation process where bioactive compounds can be protected within a protective biopolymeric matrix is a powerful tool against all the aforementioned issues (Thies, 2005). Encapsulation can help eliminate the issues of biomolecule degradation as well as safe targeted delivery within the gastrointestinal tract. Additionally, the interest for encapsulated bioactive compounds relies on the possibilities to modify physical properties of food materials, e.g., rheological properties, and to overcome solubility incompatibilities between ingredients, e.g., bioactive compounds and the food matrices

Food manufacturers worldwide need to generate commercially viable encapsulation techniques for functional food ingredients with broad spectrum applications such as to reduce the cost in use as well as facilitate scale up. The maximum acceptable cost of an encapsulation process is quite low, £0.1/kg of a new product which causes a lag in the manufacturing of fortified foods at a low level (1–5%) (Anabio Laboratories, 2014). Nevertheless, the development of encapsulation technologies has created in a new dimension in food processing and preservation (Nutraceuticals world, 2010). According to the reports from the Global Industry Analysts, Inc. (GIA), the global market of encapsulated food ingredients is projected to reach 60 billion US dollars by 2020. A large part of encapsulated food ingredients is accounted by nanometric delivery systems, incorporated in the packaging coatings, health-promoting products, and beverages, and this market is foreseen to grow to more than 20 billion US dollars in the next decade. The rapid interest in
encapsulation technology is driven by the growing demands for safe and sustained nutrition and supported by the fast increase in the global sector of preserved and packaged food products.

Conclusion

Although the exploration of utilization of fish gelatin as an alternative to mammalian gelatin is currently limited, there is an increasing demand for fish gelatin which calls for further research to meet the needs of the consumers. Great advancement has been made in terms of fish gelatin extraction from various fish parts including the skin, bones and other waste produced by the fish industry. This review has demonstrated the versatility and utility of fish skin gelatin as nanoparticles which can find application in the food industry as nanocarrier systems for controlled delivery of various food supplements and additives. A diverse variety of biopeptides can be added to the gelatin nanoparticles while preserving their innate biological activity and ensuring a sustained release upon consumption and degradation. As discussed in this review, gelatin extracted from the fish skin has been established as a good biomaterial to encapsulate a variety of biologically active molecules. While work continues to improve nanoparticle release technology, more research is required to understand the sorption as well as biological release profile within the human matrix of a wider range of bioactive molecules from such gelatin nanocarriers.

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