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Industrial application of fish cartilaginous tissues

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ABSTRACT

Cartilage is primarily composed of proteoglycans and collagen. Bioactive compounds derived from animal cartilage, such as chondroitin sulfate and type II collagen, have multiple bioactivities and are incorporated in popular health products. The aging population and increases in degenerative and chronic diseases will stimulate the rapid growth of market demand for cartilage products. Commercial production of bioactive compounds primarily involves the cartilages of mammals and poultry. However, these traditional sources are associated zoonosis concerns; thus, cartilage products from the by-products of fish processing has gained increasing attention because of their high level of safety and other activities. In this review, we summarize the current state of research into fish-derived cartilage products and their application, and discuss future trends and tasks to encourage further expansion and exploitation. At present, shark cartilage is the primary source of marine cartilage. However, the number of shark catches is decreasing worldwide, owing to overfishing. This review considers the potential alternative fish cartilage sources for industrialization. Three keys, the sustainable production of fish, new fish-processing model, and market demand, have been discussed for the future realization of efficient fish cartilage use. The industrialization of fish-derived cartilage products is beneficial for achieving sustainable development of local economies and society.

1. Introduction

Bone and cartilage comprise the endoskeleton of vertebrates. During embryonic development in most vertebrates, hyaline cartilage develops first, and this is subsequently replaced by endochondral bone (Seidel et al., 2017). However, cartilaginous fish, such as sharks, rays, and their relatives, retain the cartilaginous skeleton into adulthood and consequently lack bony tissue at any stage of their life cycle (cf. Hall, 1982; Seidel et al., 2017).

Cartilage is a gel-like tissue produced by chondrocytes that originate from mesenchymal precursor cells (Seidel et al., 2017; Witten et al., 2010). The extracellular matrix (ECM), which is abundant in proteoglycans and collagen, comprise 90% of the dry weight of cartilage (Knudson and Knudson, 2001; cf. Peng et al., 2021; Witten et al., 2010). Proteoglycans are complex macromolecules comprised of a core protein and glycosaminoglycan (GAG) side chain(s) (Merly and Smith, 2013). The major GAGs in cartilage are chondroitin sulfate (CS), keratan sulfate (KS), dermatan sulfate (DS), and heparan sulfate (HS) (cf. Vázquez et al., 2013). The varieties of collagen are named types I–XXIX (Sotelo et al., 2016), and they play different roles in tissues. Hereafter, the different types of collagen are referred to as C–I, C-II, and so on, using Roman numerals to show collagen type. In cartilaginous tissues, C-II is the primary type (Witten et al., 2010). Depending on the location and degree of maturation, other collagen types, such as C–I, C-X, and C-XI, are also present (Cumming et al., 2019; Dai et al., 2018; Knudson and Knudson, 2001; Witten et al., 2010).

Since GAGs and collagen regulate cell development and activities in the cartilage (Knudson and Knudson, 2001), these compounds have found roles in functional foods, pharmaceuticals, and cosmetics (cf. Silvipriya et al., 2015; cf. Volpi, 2019), and research into the bioactivity of animal cartilaginous tissues is receiving increased attention. For example, CS and C-II extracted from animal cartilage are prescribed to improve osteoarthritis (OA) symptoms (Dai et al., 2018; cf. Volpi, 2019); oral administration reduces the associated inflammation reactions (Cantley et al., 2013; Dai et al., 2018). CS is recommended as a symptomatic slow-acting drug (SYSADOA) in Europe, and in dietary supplements in the United States, for the treatment of knee, hand and hip OA (Restaino et al., 2019). OA is a global health issue with high incidence and economic impact. Due to an aging population and rising obesity, OA is predicted to rise dramatically in the future (cf. Peng et al., 2021, cf.

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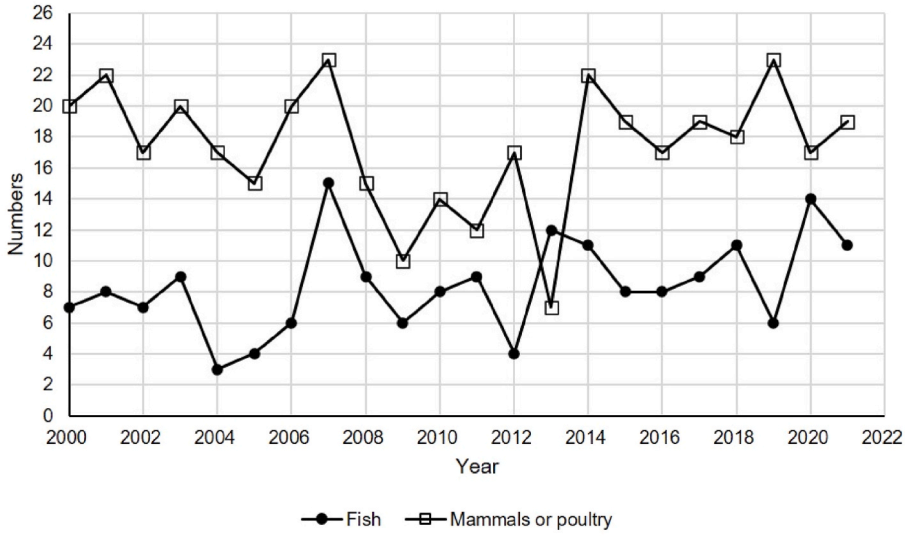

Fig. 1. Publication output of the studies of bioactivity compounds from fish, mammals and poultry cartilage. Scopus search (https://www.scopus.com/) was used to collect data. The data of fish was from the key words (TITLE-ABS-KEY (cartilage OR cartilaginous) AND TITLE-ABS-KEY (bioactivity OR collagen OR chondroitin AND sulfate) AND TITLE-ABS-KEY (fish OR shark OR skate OR sturgeon OR salmon)). The data of mammals and poultry was from the key words (TITLE-ABS-KEY (cartilage OR cartilaginous) AND TITLE-ABS-KEY (bioactivity OR collagen OR chondroitin AND sulfate) AND TITLE-ABS-KEY (fish OR shark OR skate OR sturgeon OR salmon)).

Volpi, 2019). Since the regeneration of cartilage is extremely limited, demand for CS and C-II products that relieve OA symptoms is increasing.

Various animals provide a resource for cartilage, including terrestrial mammals (bovines and porcines), poultry (chicken), fish (cartilaginous and bony fish), marine invertebrates (squid), and amphibians (giant salamander) (Miyazaki et al., 2015; cf. Silvipriya et al., 2015; cf. Volpi, 2019; Zhu et al., 2018), though commercial production of bioactive compounds mainly involves mammals and poultry. Although cartilage products from fish processing by-products have gained growing attention because of their high safety and activities, the use of fish-derived cartilage resources has fallen behind that from terrestrial vertebrates in scientific research and market share (FAO, 2020; Stevens et al., 2018).

In this review, progress in research and application of fish cartilaginous tissues are addressed, with comparisons to those from terrestrial vertebrates where appropriate. This is the first review to summarize the research progress on the application of cartilage products from fishery processing waste and discuss sustainability of the fish-production industry.

2. Potential bioactive compounds in fish cartilage

The primary commercial sources of cartilage are terrestrial vertebrates such as bovines, porcines, and chickens, yet although fish cartilage shows great potential for industrial use, data on the bioactivity of compounds obtained from fish cartilage are lesser than those on domestic animals cartilage (Fig. 1). In this part, we discuss the structure, production method, and bioactivity of compounds obtained from fish cartilage and compare them with those obtained from the cartilage of terrestrial vertebrates.

2.1. *Chondroitin sulfate*

2.1.1. *Chondroitin sulfate characteristics*

In both terrestrial vertebrates and fish, CS is the major GAG in the extracellular matrix of the cartilage, and it is covalently linked to a protein core to form a proteoglycan (cf. Restaino et al., 2019). CS is a polysaccharide chain having a repetitive disaccharide unit composed of -4GlcAβ1-3GalNAcβ1- (GlcA: glucuronic acid, GalNAc: N-acetyl-galactosamine) (Restaino et al., 2017). It is characterized by differences in sulfation, including non-sulfated disaccharides (CS–O), mono-sulfated (CS-A: di-4S CS and CS-C: di-6S CS), and di-sulfated units (CS-D: di-2,6S CS and CS-E: di-4,6S CS) (cf. Restaino et al., 2019). CS formulation varies with tissue, organ, and species (Volpi, 2007). Data on CS formulation and molecular weight differ somewhat depending on research conditions, and are impacted by processing and purification methods (cf. Martel-Pelletier et al., 2015; Volpi, 2007). During manufacturing, desulfation and depolymerization may occur (cf. Martel-Pelletier et al., 2015). Table 1 summarizes the formulations of CS derived from animal cartilage. CS from cartilaginous fish is characterized by a high percentage of di-sulfated disaccharides and CS-C, whereas that from terrestrial vertebrates contains either no or trace amounts of di-sulfated disaccharides and a higher percentage of CS-A (Arima et al., 2013; Restaino et al., 2017; Maccari et al., 2015). The formulation of CS from bony fish cartilage is more complex. In salmon, the CS formulation is similar to that of cartilaginous fish, whereas in sturgeon the formulation shows the characteristics of CS from terrestrial vertebrates (Im et al., 2010; Maccari et al., 2015; Restaino et al., 2017). CSs from different tissues also have different formulations in fish. For example, CS from bony fish bone contains a high percentage of CS-A and di-sulfated disaccharides (Arima et al., 2013; Restaino et al., 2017). Notably, CS from monkfish bone contains high CS-E, which is a typical characteristic of squid CS (Maccari et al., 2015). Some reports give conflicting data; for

Table 1
The CS formulations of cartilage of different origins.

	Mammal			Poultry	Cartilaginous fish			Bony fish		
	Bovine	Pig	Whale	Chicken	Shark	Skate	Chimaera	Salmon	Sturgeon	Bone[a]
CS-C/CS-A	0.5–1	<0.5	0.5–1	<0.5	>1	>1	>1	>1	<0.5	0.5–1
Di-sulfated (%)	<0.5	<0.5	<0.5	<0.5	>10	1–10	>10	0.5–1	<0.5	1–10
References	[1, 2]	[1]	[1]	[1]	[1–4]	[5, 6]	[7]	[3]	[8, 9]	[3]

References: [1]Restaino et al. (2019); [2]Volpi (2007); [3]Maccari et al. (2015); [4]Vázquez et al. (2018); [5]Li et al. (2019); [6]Hashiguchi et al. (2011); [7]Vázquez et al. (2019); [8]Im et al. (2010); [9]Yamagata et al. (1987).

[a] The data are obtained from bones of bony fishes (salmon, tuna, monkfish, codfish).

Table 2
The bioactivities of fish-derived CS.

Sources	Activities	References
Shark	anti-inflammation, cartilage regeneration, neurite outgrowth promoting activity, cell activation, anticoagulant	[1–8]
Skate	neuritogenic activity, anti-obesity, anti-inflammation	[9–11]
Salmon	anti-obesity[a], anti-oxidant, metal chelation	[12–14]
Sturgeon	wound healing, antithrombotic activity	[15–16]
Shark CS oligosaccharide	neuroprotective properties	[17]
Skate CS oligosaccharide	anti-obesity[a]	[10]

References: [1]Surapaneni et al. (2014); [2]Volpi (2002); [3]Imada et al. (2010); [4]Nishimoto et al. (2005); [5]Nadanaka et al. (1998); [6]Volpi et al. (1993); [7]Nair et al. (2015); [8]Krichen et al. (2018); [9]Hashiguchi et al. (2011); [10]Li et al. (2019); [11]Song et al. (2017); [12]Han et al. (2000); [13] Ajisaka et al. (2016); [14]Uchisawa et al. (2001); [15]Im et al. (2010); [16]Gui et al. (2015); [17]Zhang et al. (2015).

[a] Activities reported in fish CSs only.

example, the ratio of CS-C/CS-A in skate cartilage CS is given as either “more than 2” or as “near 1”, which may be related to the purity of the sample and detection methods (Li et al., 2019; Volpi, 2007). In summary, CS formulations from different fish species and tissues differ, a factor that requires adequate study in the future since CS formulations influence CS bioactivity.

2.1.2. *Chondroitin sulfate extraction and purification*

CS is extracted and purified from animal cartilaginous tissues; microbial production alternatives, as in the case of hyaluronic acid, have proved difficult (Schiraldi et al., 2010, 2012). In general, CS extraction and purification include three steps: hydrolysis of cartilage, selective precipitation of CS, and purification of CS. First, fish cartilage is hydrolyzed by chemical methods or proteases (Murado et al., 2010; cf. Vázquez et al., 2013). Considering environmental protection, enzymatic hydrolysis using proteases, such as papain, subtilisin, and peptidase, is recommended (Hashiguchi et al., 2011; Novoa-Carballal et al., 2017; cf. Vázquez et al., 2013). After protein fraction breakdown and cartilage structure degradation, the CS fraction is selectively precipitated by alcoholic treatment, usually using ethanol (Murado et al., 2010; cf. Vázquez et al., 2013). The redissolved CS sediment is purified by column chromatography or ultrafiltration membranes. Hashiguchi et al. (2011) used gel filtration and anion-exchange chromatography to purify ray CS, while rabbitfish (*Chimaera monstrosa*) CS was purified in the last stage using membrane technologies (Vázquez et al., 2019). Ultrafiltration membranes are more suitable for large scale production because they are cheaper, simpler, easier to scale-up, and provide satisfactory efficiency (Vázquez et al., 2019).

2.1.3. *Chondroitin sulfate bioactivity*

In this part, we summarize the bioactivity of CSs obtained from cartilage of mammals and chicken, and then consider fish CSs (Table 2).

CS derived from the cartilage of terrestrial vertebrates is mainly used for the treatment of OA (Schiraldi et al., 2010). OA is the most common form of arthritis, whose characteristics are degeneration and inflammation of the cartilage in joints. The beneficial effects of CS in the treatment of OA symptoms include reduction of apoptosis of chondrocytes, increased synthesis of articular cartilage proteoglycans, and reduction in inflammation reactions (Cantley et al., 2013). As already stated, CS is recommended as a SYSADOA in Europe, and in the United States as a dietary supplement for the treatment of knee, hand, and hip OA (cf. Restaino et al., 2019). As a food supplement, the daily CS recommended dosage is between 800 and 1200 mg (cf. Restaino et al., 2019).

CS from terrestrial vertebrates also has the following effects: neuroprotective (cf. Egea et al., 2010), antioxidative (cf. Campo et al., 2006; Canas et al., 2007), inflammation modulation (Melgar-Lesmes et al., 2016), wound healing (Zou et al., 2009), antithrombotic (Bjornsson et al., 1982), metal chelation (Ajisaka et al., 2016), and intestinal microbiota modulation (Shang et al., 2016). For example, bovine or porcine CS have found therapeutic use in diseases of the central nervous system (CNS) (Canas et al., 2007; cf. Egea et al., 2010). Canas et al. (2007) reported CS-A of bovine or porcine cartilage protects neuroblastoma SH-SY5Y cells from oxidative stress by inducing heme oxygenase-1, the dominant antioxidant gene in cells. Moreover, CS shows great promise in applications involving medical biomaterials since it combines with other polymers to form cellular scaffolds for cartilage and bone regeneration. A scaffold made of poly(ethylene glycol) containing bovine trachea CS enhances chondrogenic gene expression and cartilage-specific matrix production compared to a scaffold without CS (Varghese et al., 2008). CS-based nanocarriers are used for drug/gene delivery with the advantages of directional transmission (cf. Zhao et al., 2015).

Table 2 summarizes the reported biological activities of fish CSs. First, fish-derived CSs are useful for OA treatment due to their anti-inflammatory and cartilage regenerative activities. For example, oral administration of CS from shark fins had an anti-inflammatory effect and reduced OA symptoms (Volpi, 2002). Shark CS-C increased C-II mRNA expression in a three-dimensional culture of porcine chondrocytes (Nishimoto et al., 2005). Second, fish-derived CSs find application in the nervous system; for example, shark and ray cartilage CS have neurite outgrowth-promoting activity through the HGF signaling pathway (Hashiguchi et al., 2011; Nadanaka et al., 1998). Third, fish-derived CSs are potential health supplements. Skate (*Raja kenojei*) CS shows anti-inflammation activity through attenuation of lipopolysaccharide-induced liver damage in mice. Shark (*Mustelus mustelus*) CS has anticoagulant activity and can be used in the prevention and treatment of thromboembolic diseases (Krichen et al., 2018). Sturgeon (*Acipenser baerii* × *Acipenser schrenckii*) CS also acted as a strong antithrombotic through its anticoagulant, anti-platelet, and thrombolysis activities (Gui et al., 2015). Sturgeon (*A. sinensis*) CS has wound healing activity; it increases fibroblast adhesion and induces their proliferation and migration through its action on MAPK signaling pathways (Im et al., 2010). Fourth, fish CS is suitable for medical biomaterials. For example, shark CS nanoparticles incorporated into chitosan-poly (hydroxybutyrate-co-valerate) hydrogels significantly enhanced the viability and chondrogenic differentiation of mesenchymal stem cells, which offer great potential for nucleus pulposus tissue engineering (Nair et al., 2015).

Interestingly, anti-obesity action of CS has been reported using CSs from skate (*R. pulchra*) and salmon (Han et al., 2000; Li et al., 2019). Since bovine CS modulates obesity-related inflammation (Melgar-Lesmes et al., 2016; Stabler et al., 2017), CS can be used in functional foods for chronic health problems.

CS in its original form (polysaccharide) has low absorptivity in the digestive tract, which probably detracts from its potential in health foods or medications (Wang et al., 2016; Yamada et al., 2013). Therefore, CS oligosaccharides with low molecular weight have been developed. Skate (*R. pulchra*) CS oligosaccharides (2–14 sugar units), prepared by hydrolysis of CS polysaccharides with a subcritical water microreaction system by high temperature (180–190 °C) and high pressure (25 MPa) (Yamada et al., 2013) have anti-obesity action *in vitro* and *in vivo* (Li et al., 2019). Low molecular weight shark CS (2–5 kDa) possessed neuroprotective properties against toxic effects induced by amyloid-beta peptides both *in vitro* and *in vivo* that are beneficial for preventing Alzheimer’s disease (Zhang et al., 2015).

Based on their sulfation patterns, CSs from different origins provide specific biological functions (cf. Volpi, 2019). Since CSs from fish cartilage show different sulfation patterns compared to those from terrestrial vertebrates, there is a possibility that some of the reported biological activities of fish CSs are specific. As shown in Table 2, some of

Table 3
Imino acid and denaturation temperature of fish-derived pepsin soluble type II collagen.

Sources	Imino acid/ 1000 residues	Denaturation temperature (°C)	References
Silvertip shark *Carcharhinus albimarginatus* skeletal	156	31.25	[1]
Whale shark *Rhincodon typus* cartilage	155	34.02	[2]
Skate *Raja kenojei* fin cartilage	188	–	[3]
Amur sturgeon *Acipenser schrenckii* cartilage	226	35.71	[4]
Amur sturgeon *Acipenser schrenckii* notochord	232	33.5	[5]
Bester sturgeon *Huso huso* × *Acipenser ruthenus* notochord	212	36.3	[6]
Chick sternal cartilage	232	43.8	[7]
Bovine articular cartilage	206	38	[8]
Squid cartilage	171	31.9	[9]

-: no data.
References:[1]Jeevithan et al. (2014); [2]Jeevithan et al. (2015); [3]Mizuta et al., 2003; [4]Liang et al. (2014); [5]Zhang et al. (2019); [6]Zhang et al. (2014); [7]Cao and Xu (2008); [8]Herbage et al., 1977; [9]Dai et al. (2018).

the activities were reported only in fish CS. However, in the future, more detailed comparative studies are needed to elucidate the bioactivities specific to CS from different fish species.

2.2. *Type II collagen*

2.2.1. *Type II collagen characteristics*

Collagen accounts for approximately 30% of total protein in animals and is present in at least 29 different types, each with a distinctive amino acid sequence and molecular structure (Sotelo et al., 2016). C–I accounts for 80%–85% of the collagen in the body (cf. Subhan et al., 2015). To date, most research on fish collagen has been based on C–I extracted from the skin. However, C-II is the most abundant type of collagen in the ECM of vertebrate cartilage (Merly and Smith, 2013) and is the most important component in the development and maturation process of chondrocytes in articular cartilage (Dai et al., 2018). Thus, as described later, C-II or C-II-derived biomaterials are suited to mimicking the microenvironment around chondrocytes in the human body and for use in the treatment of and research into arthritis. C-II is also one of the most common types of collagen found in cosmetics and supplements (cf. Felician et al., 2018). Therefore, fish-derived C-II should also be a focus of research.

Fish C-II molecules consist of a homotrimer of α chains, which contrasts with the existence of two types of α chain in mammalian C–I and three types of α chain in fish C–I (Zhang et al., 2014, 2016, 2019). In common with mammalian C-II, a characteristic feature of fish C-II is that it binds more glycosides than C–I. Zhang et al. (2019) showed that notochord C-II from Amur sturgeon contained 31.8 μg/mg galactose and 33.7 μg/mg glucose, whereas skin C–I contains only 2.08 μg/mg galactose and 2.64 μg/mg glucose. The molecular denaturation temperature and the ability to assemble into fibrils also differ between C–I and C-II, both of which are also common in fish and other vertebrates. C-II has superior thermal stability to C–I in sturgeons (Zhang et al., 2014, 2019). C-II has a longer lag time for assembly, slower fibril formation speed, and lower fibril formation rate than C–I in humans, calf, and sturgeon (Birk and Silver, 1984; Fertala et al., 1994; Meng et al., 2020; Zhang et al., 2019). Fibrils formed from C-II are thinner and shorter (Zhang et al., 2019).

The principal differences between fish and other animal collagens include denaturation temperature and glycosylation (Bu et al., 2017; cf. Subhan et al., 2015). Generally, C-II from land animal species that maintain a high body temperature had higher amounts of imino acids and superior thermal stability than those from fish, whose habitat temperature, and therefore body temperature, was lower (Cao et al., 2013). For example, C-II from chicken sternal cartilage has a higher imino acid content (about 232 residues per 1000 total residues) than C-II from shark cartilage (156 residues per 1000 total residues) and shows a higher denaturation temperature (Cao and Xu, 2008; Jeevithan et al., 2014) (Table 3). A higher imino acid content usually confers a more stable helical structure. However, C-IIs from sturgeons have similar imino acid content to those from land animals yet show lower denaturation temperature (Table 3). The relationship between the denaturation temperature and structural characterization of fish C-II needs further study.

Glycosylation patterns of collagen are crucial factors for immunologic tolerance (Bu et al., 2017). Fish C-IIs express 5%–30% glycosylation, and this percentage may vary from species to species (Bu et al., 2017). As already stated, Zhang et al. (2019) reported the glycosylation patterns of C–I and C-II in Amur sturgeon (*A. schrenckii*); however, the glycosylation patterns of fish C-II and the function of specific carbohydrate chains linked to collagens are not well understood (Merly and Smith, 2013).

2.2.2. *Type II collagen extraction and purification*

Before C-II from fish and terrestrial vertebrate cartilage can be extracted, it is necessary to remove non-collagenous substances. Usually, NaOH and/or EDTA is used as a pretreatment of cartilage to decrosslink non-collagenous proteins and minerals, respectively, from the cartilage (Dai et al., 2018; Jeevithan et al., 2015; Mizuta et al., 2003). Then, C-II is extracted by hydrolysis of cartilage with proteases and purified by salting out or gel-filtration column chromatography (Jeevithan et al., 2018; Meng et al., 2020; Zhang et al., 2014). For extraction, pepsin in an acidic solution is commonly used. Native C-II molecules from cartilage are composed of three identical α-chains with telopeptides that construct inter-molecular crosslinks. The telopeptides also possess immunogenicity (Cao et al., 2013). Pepsin cuts telopeptides making C-II molecules more soluble and reducing their immunogenicity (Cao et al., 2013). Fish C-II extraction and purification should always be conducted at low temperature to reduce denaturation.

One of the characteristic features of the collagen obtained from both cartilaginous fish and bony fish is that it contains not only C-II but also other types of collagen, such as C–I (Seidel et al., 2017). Contamination by other types of collagen arises from the characteristic morphological features of fish cartilaginous tissues. The primary cartilage in cartilaginous fish is hyaline-like cartilage that persists throughout life and is never converted to or replaced by bone. Instead, mineralized tiles (tesserae) cover the outer cartilage surface to provide stiffness (Seidel et al., 2017). The calcified surface and the cartilage core contain different collagens, C–I and C-II, respectively (Seidel et al., 2017). Bony fish cartilaginous tissues include not only hyaline cartilage but also tissues intermediate between “bone and cartilage” or “connective tissue and cartilage” (Witten et al., 2010). These bone-like cartilages and cartilage-like connective tissues cause difficulties in teleost cartilage identification. Also, because fish grow throughout life, many cartilaginous tissues may not be fully differentiated (Witten et al., 2010). Thus, there is always a possibility of getting complex mixtures of cartilaginous tissues from bony fish, consisting of different types of collagen.

Here, we mention a characteristic teleost cartilage-related tissue, the notochord. The notochord is a rod-like tissue that develops anteroposteriorly and functions as an axial skeleton in the embryonic stages until other tissues, such as vertebral bones or cartilage, replaces it. The notochord, known as vesicular cartilage, has the same matrix components as hyaline cartilage (derived from chondrocytes) but is produced by notochord cells (Witten et al., 2010). The notochord is a unique tissue that expresses only C-II, making it a good source of C-II with minimal C–I contamination (Zhang et al., 2019). In sturgeon species, because of incompletely developed vertebral cartilage, the notochord exists throughout life.

Table 4
The bioactivities of fish-derived type II collagen.

Sources	Activities	References
Shark	anti-inflammation, osteogenesis-activating activity, anti-oxidant	[1–6]

References:[1]Bu et al. (2017); [2]Chen et al. (2012); [3]Jeevithan et al. (2016b); [4]Jeevithan et al. (2018); [5]Jeevithan et al. (2016a); [6]Jeevithan et al., 2015.

2.2.3. *Type II collagen bioactivity*

Since research on and applications of fish C-II are limited, we first summarize information on C-II from animals of terrestrial origin, and then move on to fish C-II.

Collagen is widely used in foods, cosmetics, pharmaceuticals, and biomaterials (cf. Hashim et al., 2015; cf. Silvipriya et al., 2015). Currently, health supplements are the most frequent applications of C-II from terrestrial vertebrates. As a collagen supplement, C-II helps in reducing the destruction of body C-II, modulating the inflammatory response, and increasing joint flexibility (cf. Hashim et al., 2015). One of the most significant functions of C-II in supplements is for the treatment of arthritis, including OA and rheumatoid arthritis (RA). RA is a systemic autoimmune disease that involves hyperplasia of synovial tissues and structural damage to cartilage, bone, and ligaments (Cho et al., 2007). Many reports have shown the effectiveness of oral administration of C-IIs against RA symptoms. For example, oral administration of bovine C-II suppresses pro-inflammatory cytokine expression of T cells in arthritis-induced mice (Ju et al., 2008). In a double-blind, placebo--controlled trial, oral treatment with chicken C-II showed positive effects in the relief of RA symptoms (Barnett et al., 1998). A 24-week, double-blind, double-dummy, randomized, methotrexate -controlled study also confirmed the efficacy and safety of C-II in the treatment of RA (Wei et al., 2009). In a randomized, double-blind trial, patients taking chicken C-II for three months showed a decrease in joint swelling and tenderness (Trentham et al., 1993). Additionally, squid C-II induced cartilage repair in an osteoarthritis-induced cartilage lesion model *in vitro* and *in vivo* (Dai et al., 2018). The long-term observations in large numbers of patients are expected to confirm the efficacy on RA of C-II and determine the optimal doses of orally administered C-II.

The function of C-II as a biomaterial is a current research focus in cartilage tissue engineering. C-II is the natural scaffold of chondrocytes, regulating chondrogenic differentiation of multipotent stem cells and supporting cartilage repair *in vitro* (Calderon et al., 2010; Raabe et al., 2010). A porous scaffold made of porcine C-II can increase rates of biosynthesis of cartilage matrix by chondrocytes (Lee et al., 2003). Porcine C-II sponge supports chondrocytes to maintain their phenotype and promote re-differentiation (Francioli et al., 2010). In hydrogel based on C-II and activated CS, chondrocytes survived well and showed round or oval morphology, and the surrounding matrix was remolded by the embedded chondrocytes (Gao et al., 2018). This hydrogel was injectable and was useful for delivering chondrocytes for cartilage regeneration (Gao et al., 2018). However, provision of a sufficient and stable supply of medical-grade C-II in the market, and clinical trials of C-II-derived scaffolds for cartilage regenerative medicine, remain future challenges.

Research on the bioactivity of fish C-II is in its infancy (Table 4). The information that does exists is limited almost entirely to shark C-II, and is mainly divided into three categories. First, in line with terrestrial sources, C-II from blue shark (*Prionace glauca*) and whale shark (*Rhincodon typus*) cartilage have anti-inflammatory activity (Bu et al., 2017; Jeevithan et al., 2016b). Chen et al. (2012) showed that oral administration of C-II from blue shark suppresses RA in experimentally-induced RA model rats. Second, whale shark C-II stimulates osteogenesis and suppresses osteoclastogenesis when used as cellular scaffolds (Jeevithan et al., 2018), and blue shark C-II scaffold promotes osteoblast cell formation (Jeevithan et al., 2016a). Third, whale shark C-II has antioxidant activity (Jeevithan et al., 2015). As described in Section 3, sharks are threatened by overfishing, therefore, research on the bioactivities of C-II derived from fish species other than sharks is crucial in the future.

2.3. *Other products*

2.3.1. *Proteoglycans*

Proteoglycans (PG) are biopolymers consisting of a core protein covalently attached to GAG chains (Ono et al., 2018). PGs can be extracted from cartilage using acetic acid (Kobayashi et al., 2017). The predominant PG in cartilage is aggrecan, which binds CS as GAG chains (Knudson and Knudson, 2001). In mammals, the core protein of aggrecan has multiple functional domains, including three globular domains, G1, G2, and G3 (cf. Kiani et al., 2002). A region for attachment of GAG chains (the attachment domain) separates the G2 and G3 domains (cf. Kiani et al., 2002; Knudson and Knudson, 2001). The CS attachment domain in mammalian aggrecan is the repeating amino acid sequence containing serine–glycine (Kakizaki et al., 2011; cf. Kiani et al., 2002). However, unlike mammalian aggrecans, the apparent repetitive sequence is not seen in zebrafish, pufferfish, and salmon aggrecans (Kakizaki et al., 2011). This may indicate the specificity of fish PGs.

We found that research on the bioactivity of cartilage PG was limited to salmon nasal cartilage PG. PG from salmon (*Oncorhynchus keta*) nasal cartilage is aggrecan with a 143-kDa core protein and GAG chains (Ono et al., 2018). The disaccharide units in the GAG chains contain 60% CS-C (Ono et al., 2018). Salmon PG has an immunomodulatory effect. Oral administration of PG attenuates allergic responses in a respiratory inflammation mouse model, regulates collagen-induced arthritis, and reduces obesity-induced inflammation (Hirose et al., 2017; Ono et al., 2018). Considering the low intestinal absorptivity of PG, the immunomodulatory effect of PG possibly comes from its effect on the intestinal microbiota, since daily oral administration of salmon PG enhances probiotics and short-chain fatty acid-producing bacteria (Asano et al., 2013; Ono et al., 2018). Salmon PG also has anti-angiogenesis activity arising through the inhibition of endothelial cell adhesion and production of matrix metalloproteinases (Kobayashi et al., 2017). The anti-angiogenesis activity of PGs may explain why cartilage lacks blood vessels (Kobayashi et al., 2017). We believe that PG from salmon nasal cartilage will show other forms of biological activity, and that it has great potential for medical application since PG is involved in the regulation of cellular activities (Hirose et al., 2017; Kobayashi et al., 2017). The results of studies into the biological activity of PGs from other fish species will open a new window for the future industrial application of PGs.

2.3.2. *Cartilage hydrolysate and peptides*

Tissues or proteins are commonly hydrolyzed by enzymes, such as papain, pepsin, properase E, flavourzyme, and trypsin, to obtain tissue hydrolysates or peptides (cf. Felician et al., 2018). Bioactive peptides usually have 3–40 amino acid residues, and their activities are based on amino acid composition and molecular weight (cf. Ngo et al., 2012). Over the years, many researchers have tried to obtain tissue hydrolysates and peptides from fish-processing waste and have studied their biological activities. These activities include antioxidant, anti-hypertensive, anti-inflammation, anti-proliferative, anti-coagulant, calcium-binding, anti-obesity, anti-diabetic, anti-cancer, and so on (cf. Halim et al., 2016; cf. Ngo et al., 2012). Although the number of reports on the bioactivity of fish cartilage-derived hydrolysates or peptides are much fewer than that of skin-derived hydrolysates or peptides, several researchers have demonstrated antioxidant activity of fish cartilage-derived hydrolysates or peptides (Li et al., 2017; Pan et al., 2016; Tao et al., 2018). For example, three peptides isolated from shark (*M. griseus*) cartilage hydrolysate scavenge radicals, inhibit lipid peroxidation, and protect H_2O_2-stressed HepG2 cells (Tao et al., 2018). Antioxidant peptides from skate (*R. porosa* and *R. pulchra*) cartilage have also been reported (Li et al., 2021b; Pan et al., 2016). Apart from this, the alcalase-hydrolyzed cartilage of shark (*P. glauca*) exerts

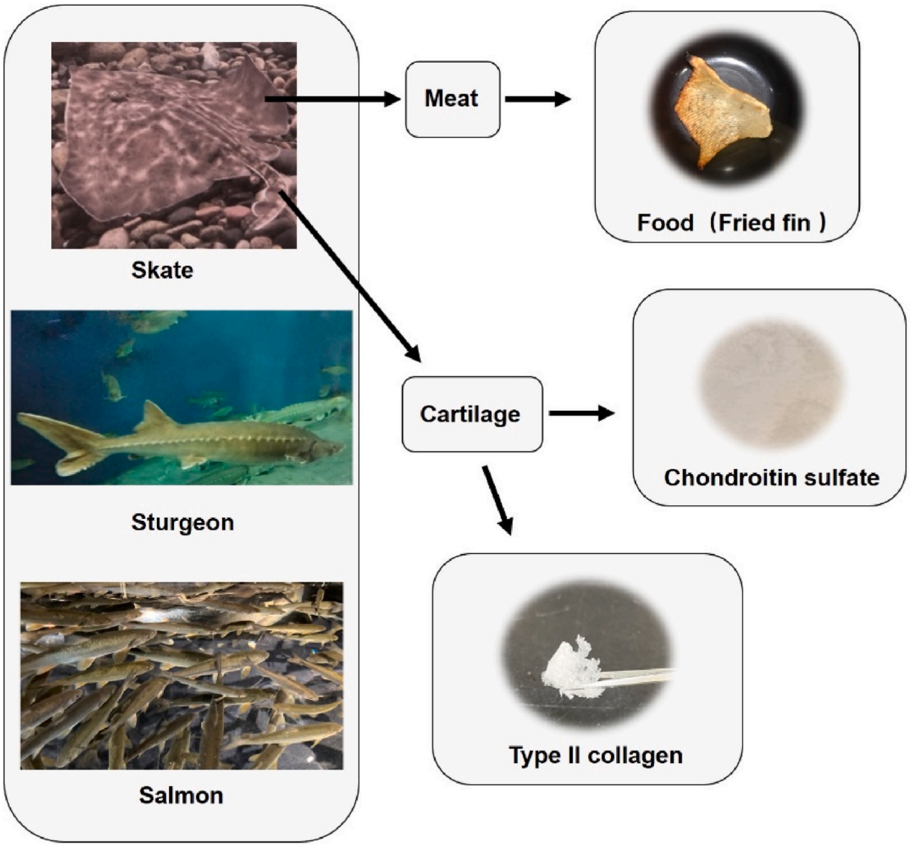

Fig. 2. Images of fish and the representative cartilaginous products produced from them.

anti-hyperuricemic activity after oral administration to rats (Murota et al., 2014). In addition, a double-blind, placebo-controlled clinical study reported oral supplementation with hydrolyzed fish cartilage improves the morphological and structural characteristics of the skin (Maia Campos et al., 2021). These results suggest that fish cartilage hydrolysates or peptides have potential as functional foods or in other fields.

2.3.3. *Other derivatives*

In addition to the products that we have discussed, fish cartilage can be used in the production of cartilage powder, gelatin, various GAGs, and others (Jeevithan et al., 2014; Knudson and Knudson, 2001). Their biological activities deserve further study. However, the possible industrialization of these products needs to be considered from the viewpoints of extraction and purification complexity, cost, and stability, sufficiency, and sustainability of the supply of raw materials (cf. Olsen et al., 2014).

3. Potential fish cartilage sources for industrialization

3.1. *Cartilaginous fish*

Cartilage is the primary skeletal tissue in elasmobranchs (cartilaginous fish), including sharks, skates, rays, and relatives. Therefore, cartilaginous fish have been the primary candidate as the source of fish cartilage.

Until now, the cartilage of sharks has been one of the major sources of commercial CS. However, due to overfishing, commercial mass killing of sharks is not supported by international treaties (Arima et al., 2013; Sotelo et al., 2016). Some researchers have found that by-catch during fishing operations includes a certain quantity of low-value sharks (e.g., lantern shark and catshark) worthy of exploitation (Novoa-Carballal et al., 2017; Sotelo et al., 2016). These species generally become “discards” that are not retained on board during fishing operations because their relatively low commercial value makes it unprofitable to bring them to shore (Blanco et al., 2015; Sotelo et al., 2016). However, fishery discards are currently controversial, and the European Commission has published rules that prohibit discards (Sotelo et al., 2016; Vázquez et al., 2018). Therefore, in Europe, the wise use of by-catch sharks is an alternative for dealing with shark discards.

Another example is the dominant by-catch species in Chinese fisheries, the blue shark (*P. glauca*). The amount of cartilage remaining after consumption of the fresh or frozen meat appears to be increasing and forms a resource that should be utilized (Bu et al., 2017).

Regardless, shark catches are decreasing worldwide and have become controversial; thus, alternatives to shark cartilage have been widely sought. Other major cartilaginous fish, skates and rays, are an alternative resource because these species are the target of commercial fisheries. Skate or ray fin has become a popular food in many countries. For example, Hongtak, made from the pectoral fins of skate (*R. kenojei*), is an indigenous fermented product in Korea (Mizuta et al., 2003). Skate (Kasube) fin is a familiar food in Hokkaido, Japan, and there are various recipes in restaurants, including boiled, deep-fried, or sashimi. In Spain, 2581 tons of skate and rays were landed at the Port of Vigo in the year 2008 (Murado et al., 2010). Skate are also commercially harvested on both coasts of North America. In Alaska, skate (family Rajidae) are not overfished, and the development of skate fisheries is encouraged (Farrugia et al., 2015). Seafood exporters are interested in buying skate from Alaska to sell in Asia (Farrugia et al., 2015).

Since only the fins of skate and rays are used as food, 50%–80% of their carcasses end up as waste following industrial processing (Farrugia et al., 2015; Li et al., 2019; Murado et al., 2010). In particular, the head and axial skeleton of skate, which are rich in cartilage, are mostly discarded. In terms of the availability of raw materials, skate and ray cartilage could be an alternative to that from shark.

Another potential source of cartilage would be the ancient cartilaginous chimera fish. Rabbit fish (*C. monstrosa*) appears as by-catch in deep-water North Atlantic trawlers, and its cartilage has been studied with a view to isolating CS (Vázquez et al., 2019).

Cartilaginous fish have low rates of potential population increase with little capacity to recover from threats such as overfishing, pollution, and habitat destruction (Cavanagh and Gibson, 2007). The sustainability of the cartilaginous fish fisheries depends on future demand and management.

3.2. *Cultured bony fish*

Thanks to their abundance and extensive culture programs, bony fish could serve as an excellent new source for commercial CS and collagen (Maccari et al., 2015).

Sturgeons are the top-rated source of cartilage among the bony fish because of their cartilaginous skeleton and notochord. Sturgeons are primitive bony fish and have a characteristic skeleton whose major component is cartilage, similar to chondrichthyans (Zhang et al., 2016). The natural sturgeon population is severely threatened by overfishing, habitat degradation, and pollution (Bronzi et al., 2019). However, there is now a trend towards sturgeon aquaculture to substitute the production from fisheries, with the practice now widely adopted in China, Russia, the Middle East, the Far East, Europe, and Japan (Bronzi et al., 2019), with China accounting for approximately 80% of global production (Gui et al., 2015). Globally, sturgeon fisheries are valuable for production of the luxury food, caviar. However, since a long cultivation period is required to obtain caviar, the production cost of sturgeon is higher than that of other fish (Zhang et al., 2014). Utilization of by-products could support further growth of the sturgeon aquaculture industry. Recently, 3D sturgeon fish cartilage scaffold had been reported, which can support the adhesion, proliferation and chondrogenic differentiation of human adipose stem cells (Khajavi et al., 2021).

Aquaculture of Salmonids (particularly Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss*) is a major contributor to the world production of farmed finfish. Atlantic salmon is the most commonly farmed salmonid, and its production exceeds 2 million tons annually (FAO, Cultured Aquatic Species Information Programme). Salmon farming is significant in Chile, Norway, Scotland, Canada, and the Faroe Islands, and the primary consumption occurs in the United States and Europe (FAO, Cultured Aquatic Species Information Programme). Because salmon is a high-yielding species, the major by-product of the salmon processing industry is the nasal cartilage.

As research on the active compounds in fish cartilage continues, opportunities to develop new industries using cartilage from cultured bony fish will significantly increase.

3.3. *Other bony fish*

Collagen from the nasal cartilage of hoki (*Macruronus novaezelandiae*) has been studied for potential application in cartilage scaffolds (Cumming et al., 2019). Hoki in New Zealand is an essential commercial deep-water species making up the largest tonnage fishery (Cumming et al., 2019). Furthermore, New Zealand hoki is considered one of the best-managed trawl fisheries in the world, certified as sustainable by the Marine Stewardship Council (MSC). Therefore, nasal cartilage from hoki represents a sustainable by-product resource.

Bioactive compounds derived from fish cartilage have been investigated only in a limited number of species (such as shark, skate, sturgeon, and salmon). Fig. 2 showed these species and the representative cartilaginous products produced from them. More research is needed to find alternative fish cartilage sources and new bioactive substances that can be applied in the pharmaceutical and nutraceutical industries.

4. Advantages and disadvantages of fish cartilage as a resource

The most prominent benefit of the industrial use of fish cartilage is that it decreases the waste of the fish-processing industry. In the last couple of decades, the cumulative total production from capture fisheries and aquaculture has continuously increased (FAO, 2020). The trend towards more processing of fish products within the supply chain is creating increasing quantities of by-products (FAO, 2020). By-products may constitute as much as 50%–70% of fish weight after industrial processing (FAO, 2020; cf. Olsen et al., 2014). Cartilage has been regarded as a low-value fish processing by-product and discarded. Recently, researchers have focused on transforming this waste into isolated bioactive components to provide value-added products (cf. Ferraro et al., 2010; cf. Olsen et al., 2014). This strategy can mitigate environmental pollution from solid waste, improve the economic profit of fishery and aquaculture industries, and could result in the production of more food from limited resources (cf. Ferraro et al., 2010; cf. Olsen et al., 2014).

The second benefit is the broader acceptance of the use of fish cartilage in society. Fish cartilage has a much lower risk of contamination with zoonotic diseases (such as bovine spongiform encephalopathy and influenza) than the traditional sources of cartilage (bovine, porcine, and chicken cartilage) (cf. Volpi, 2019). Also, fish cartilage has fewer religious restrictions (FAO, 2020). For Buddhists and Muslims, the use of mammal products is restricted.

Third, the cartilage content in fish species is higher than in terrestrial species. Mammalian cartilage represents only 0.6% of the body weight, whereas that in cartilaginous fish represents 6%–8% (Blanco et al., 2015).

Taken together, cartilage from fish by-products is a valuable resource. Moreover, the ecological diversity of fish gives different characteristics and properties to the isolated bioactive compounds. For example, gelatins extracted from warm-water fish have a thermal stability similar to that of mammalian gelatins, whereas gelatins from cold-water fish are less thermally stable (cf. Ferraro et al., 2010). The bioactive compounds isolated from fish cartilage may also show diverse characteristics, suggesting more flexible usage of the compounds. Those whose properties are close to those for to mammalian equivalents could act as substitutes, whereas those with specific characteristics could form the basis of new products to fill gaps in the market.

Though the use of fish cartilage has many advantages, only a few high-value products have been established and sold in large quantities, indicating a considerable gap between research and the market. Like other fish by-products, there are two main disadvantages of fish cartilage: the supply of raw materials from specific species is insufficient and irregular; and the high price of fish products relative to those from mammals, probably due to the higher cost of production (cf. Olsen et al., 2014).

5. Future challenges, significance, and influences

5.1. *Future challenges*

As we mentioned earlier, the challenges for commercialization of fish by-products currently arise from the insufficient supply of high-quality by-products and their high cost (cf. Olsen et al., 2014). Changes and efficiency improvements in the entire supply chain are required, and here we review three key aspects, fish production, a new fish-processing model, and the market, which, if addressed, could ensure future realization of fish cartilage by-products.

5.1.1. *Fish production*

Consideration must be given to the characteristics of different fish species and consumers' consumption preferences. As a viable source of cartilage, the fish species chosen should have a large body size, be available or produced in large quantities, yield sufficient cartilage, and,

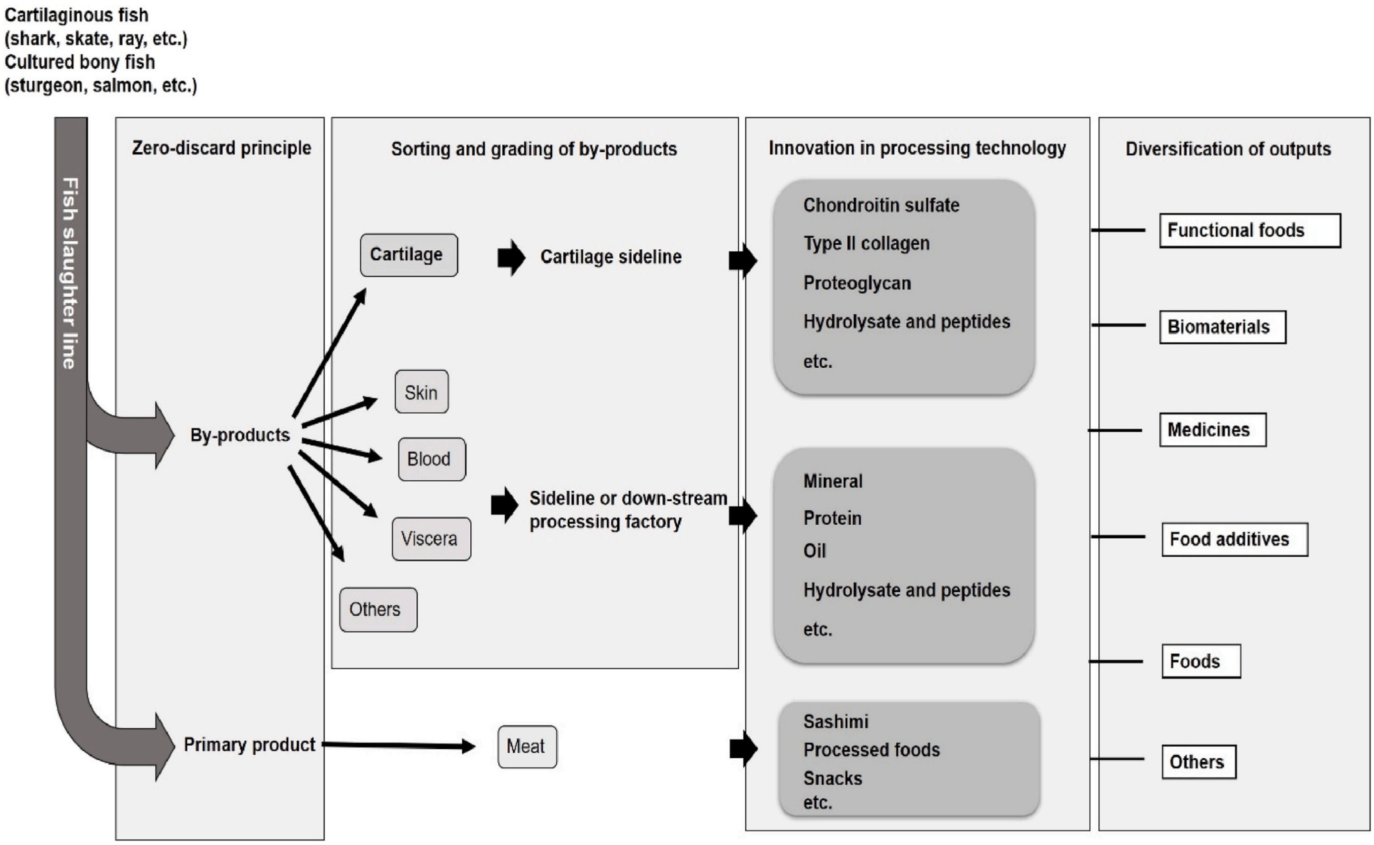

Fig. 3. A flow chart of an ideal zero-discard principle, sorting and grading of by-products, innovation in processing technology, and diversification of outputs of fish cartilage.

importantly, be processed in a factory. Factory processing will ensure integrated production of the mainstream fish products and the sideline processing of by-products including cartilage, as described in the next section. Sustainable production of the species must also be ensured, and it is probable that when this aspect is taken into account the importance of cartilage sourced from aquacultured fish species will increase. The design of factory processing should be incorporated as downstream industries of fish production.

The purchasing preferences of consumers affects the ability to exploit fish by-products. Traditionally, people have tended to buy whole fish for home cooking, which makes it difficult to collect fish waste products. However, consumption modes are changing; there is an increasing preference by consumers for ready-to-eat products (cf. Ferraro et al., 2010). Such a trend towards processed fish products within the supply chain creates increasing quantities of by-products (FAO, 2020), increasing the supply of resources suitable for cartilage, and raising opportunities to transform the value chain.

5.1.2. New fish processing model

As a first step, proper planning in the fish processing industry, to integrate the production of both primary products (usually fish meat) and secondary products (those from by-products), is essential, and this should cover the whole supply chain. The traditional fish processing factory has a primary product, and by-products (mixtures of non-edible parts) are discarded, however, the latter could be treated as in-house sideline products or as new products for down-stream processing factories. Here we discuss four key aspects necessary to implement this plan: zero-discard principle, sorting and grading of by-products, innovation in processing technology, and diversification of outputs. We proposed an ideal fish-cartilage processing flow (Fig. 3).

The principle of zero-discard, which considers processing of the mainstream products and sideline by-products as a whole to exploit the entire fish body, is essential. Since fish by-products, including cartilage, quickly deteriorate, the processing of by-products sequentially after their production is desirable. For example, streamlined modern facilities for seafood processing have been successfully built in Norway (cf. Olsen et al., 2014; Stevens et al., 2018). They can manage over 650,000 tons of seafood by-products each year (Stevens et al., 2018). The sideline products in the Norwegian salmon industry include silage (animal feeds), fish oil, and protein hydrolysates (cf. Rustad et al., 2011; Stevens et al., 2018). Efficiency is maximized by the transportation of by-products directly from the slaughter line to the oil factory within hours (cf. Rustad et al., 2011). The processing expertise, technology, and infrastructure of the poultry industry provide useful insights for the aquaculture industry (cf. Jayathilakan et al., 2012; Stevens et al., 2018).

The value of food-grade by-products, such as those used directly for human consumption or for the extraction of bioactive compounds, is high (cf. Olsen et al., 2014). Therefore, sorting and grading of by-products is required during processing since by-products that are low-grade can only be used as hydrolysates or animal feeds, products that are much less profitable. As already stated in this review, cartilage, which contains multiple bioactive compounds, is a highly valuable by-product, and its separation from other by-products is essential. After sorting and grading, different types of by-product are collected for potential conversion into multiple other products (Jayathilakan et al., 2012; Stevens et al., 2018). Unfortunately, most current by-product processing lines lack sorting and grading.

An appropriate by-product processing strategy is also crucial. Industrial production technologies should be low cost, high-efficiency, scalable, and environmentally friendly. For instance, large volumes of strong alkali or acid solution and chromatographic technologies are very challenging in use (cf. Vázquez et al., 2013). In contrast, the use of industrially available cheap enzymes, hydrothermal treatments (such as critical- or subcritical-water treatment), and membrane separation are more suitable technologies in large scale production.

The ideal model of fish processing is that a diverse range of products is produced from a single species, and sustainability is sought throughout the value chain. Stevens et al. (2018) reported that by maximizing by-product usage for human consumption the Scottish Atlantic salmon industry could increase food production from fish farming by over 60% in the UK. The resulting by-products could increase revenue by 803% and the industry bottom-line by over 5% without inputting more resources (Stevens et al., 2018). Other than as foods, salmon by-products could be processed into high-value consumables for human use, such as protein powder and hydrolysates, oil supplements, collagen and gelatin, polyunsaturated fatty acids, pharmaceutical products, and others (Stevens et al., 2018). CS and C-II could be obtained from salmon nasal cartilage, the major by-product of the salmon industry. In the case of fish cartilage by-products, the co-production of CS and peptides would be an excellent strategy to maximize output; Shen et al. (2019) liquefied chicken sternal cartilage using hydrothermal treatment, hydrolyzed this using trypsin and papain, and separated CS and peptides using a membrane ultrafiltration system.

5.1.3. *Market growth*

An aging population is becoming a global issue. With aging, increasing numbers of people will suffer degenerative diseases, such as RA, Parkinson's disease, and Alzheimer's disease. As already shown in this review, fish-cartilage products show high bioactivity for the treatment of degenerative diseases. For example, oral administration of fish-cartilage supplements (CS, C-II, or proteoglycans) improves arthritis symptoms (Trentham et al., 1993; Volpi, 2002). Fish-derived CS has the potential to be used as a treatment for diseases of the CNS due to its neuroprotective effects (Hashiguchi et al., 2011). Biomaterials for regenerative medicine also have enormous potential against degenerative diseases; for cartilage and bone regenerative medicine, fish C-II, CS, and their hybrid materials show great potential (Jeevithan et al., 2018; Varghese et al., 2008).

Chronic diseases, such as obesity and hypertension, are also a growing threat to public health. Fish-cartilage products can be functional foods for chronic health problems. Shark and sturgeon CSs have antithrombotic activity suitable for the prevention and treatment of thromboembolic diseases (Gui et al., 2015; Krichen et al., 2018). Skate and salmon CSs have anti-obesity activity, and show potential for anti-obesity functional foods (Li et al., 2019; Han et al., 2000). Fish cartilage CS and C-II have antioxidant activity (Ajisaka et al., 2016; Jeevithan et al., 2015), suggesting their suitability as antioxidants in health supplements or cosmetics.

The aging population and increases in degenerative and chronic diseases will stimulate the rapid growth of market demand for cartilage products. For instance, potential growth in the CS market has been predicted at 10% annually (cf. Restaino et al., 2019). Moreover, fish-derived bioactive compounds are becoming increasingly popular due to their high safety and activity (cf. Schiraldi et al., 2010; cf. Subhan et al., 2015). It seems inevitable that market demand for fish cartilage products will increase dramatically in the future, therefore, future research should be directed towards the development of high-quality cartilage products from fish by-products.

5.2. *Significance and influences*

Usage of fish-derived cartilage has high significance for tackling world problems. The bioactivity of fish cartilage products contributes to treatment of human health problems, even in developed countries, as described above. Wise use of cartilage by-products is a "trash to treasure" transformation beneficial to the sustainable development of our society.

Fish cartilage products could contribute to improving the economic condition of poor households through livelihood diversification and income generation. If fish-cartilage processing forms an industrial chain from farming to market, it can influence related fishery or aquaculture industry. Extended downstream processing can develop the fish processing industry into the biotechnology industry. Such an industrial transformation is a way of increasing the income of fishery and aquaculture practitioners.

The transformation of the aquaculture and fishery industries is in line with government policy in many countries. In the Thirteenth Five-Year Plan for Economic and Social Development (2016–2020) in China, the Chinese government planned a substantial reduction in the amount of capture fisheries, a decrease in the growth rate of aquaculture production in the country, and a transformation and upgrade of the fisheries and aquaculture sector (FAO 2018). The plan aims to improve product quality and to optimize the industry structure, including the processing sector. In Europe, the downstream processing of fisheries by-catch is encouraged by governments (Sotelo et al., 2016; Vázquez et al., 2018). Industry transformation is dependent on innovations in fish utilization and processing. In many countries, innovations are being made in areas like processing and packaging technology, the efficient use of raw materials, and product diversification (FAO, 2018).

Moreover, new industries can activate regional areas economically. In Japan, young people are pouring into big cities like Tokyo, due to weaker regional economic conditions. Population loss, especially of young people, further weakens regional economies. This negative cycle will eventually lead to the decline of local towns, as witnessed in New Zealand and Germany (Lovell et al., 2018; Wirth et al., 2016). The traditional fisheries industries, such as fishing and aquaculture, have limited appeal to young people and are less profitable. Developing high value-added and high-technology new industries can create new jobs and high profits, attract talent inflow, and activate the local economy.

The United Nations launched the 2030 Agenda for Sustainable Development, and the 2020 edition of The State of World Fisheries and Aquaculture (FAO, 2020) is devoted to the topic of Sustainability in Action. Social sustainability in fisheries and aquaculture is a major focus of development of fisheries and aquaculture value chains. Therefore, the industrialization of fish-derived cartilage products will be a good and promising practice.

6. Conclusions

Animal cartilage products are popular in the market as health supports. The present two major products produced from animal cartilage in the market are the CS and C-II, which used for arthritis treatments. Both CS and C-II also have various bioactivities other than for anti-osteoarthritis; they are also potential biomaterials for cartilage regenerative medicine intending to heal osteoarthritis. Owing to their safety and bioactivity, fish-derived cartilage products have gained increased attention. However, research on the bioactive compounds in fish cartilaginous tissues is rather limited. The bioactivity of fish cartilaginous tissues needs further clarification to enable development of new products from fish cartilage by-products. Although sharks are the most studied species and the primary marine cartilage source, they are endangered. Thus, sustainable fishery, with precise resource management, is the prerequisite for the sustainable development of fish cartilage industry. Also, research to discover other sources of fish cartilage is essential. Cultured bony fish could serve as a new source, but we need to learn more about bony fish cartilaginous tissues, including its structure, composition, CS and C-II characteristics, and others.

For the realization of products from fish cartilage by-products, the entire supply chain must be transformed. It needs to be integrated from upgrading efforts of the fish farming forms operating at the industry's upstream node, and development of deep-processing of by-products at the industry's downstream node. Diversification of products will give a revolution of the aquaculture value chain.

Here, we propose an ideal skate-cartilage processing flow after years

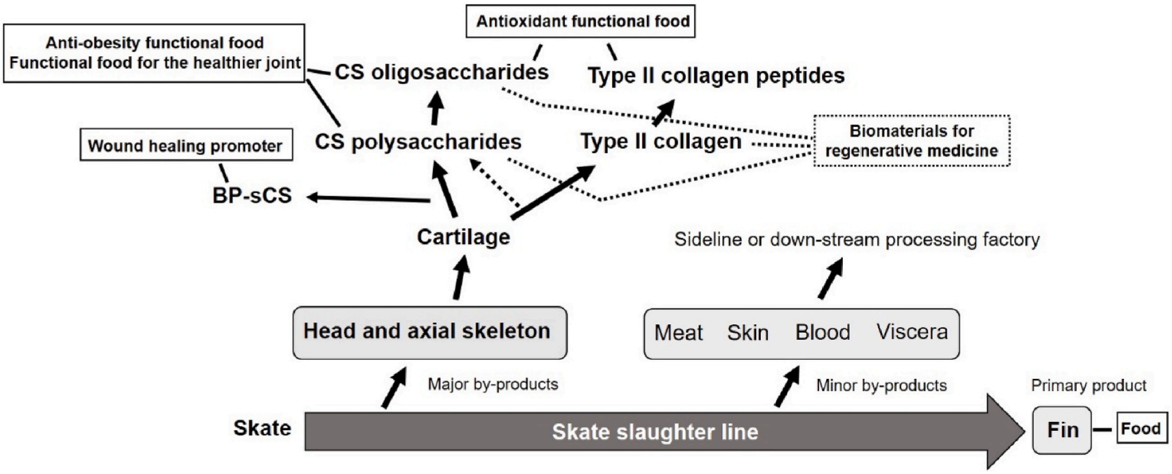

Fig. 4. Proposed skate-cartilage processing flow chart. Dotted lines and the dotted arrow indicate future tasks.

of research as a representative of the future model of fish cartilage industry (Fig. 4). In the skate-processing factory, fins are processed as the primary product (food), and other parts, including head, axial skeleton, viscera, skin, and blood, are obtained as by-products. The by-products are sorted and graded in the slaughter line. After the sorting, cartilage is transferred from the slaughter line to the sideline. At the same time, other by-products (skin, viscera, and blood, etc.) are also transferred to another sideline or down-stream processing factory. The cartilage sideline branches into two ways. On the one way, CS polysaccharides are extracted and purified. The by-product of this step is BP-sCS and used as a wound-healing promoter for the bioactive wound dressing (Li et al., 2021a). CS oligosaccharides are produced from CS polysaccharides by thermal hydrolysis. Both CS polysaccharides and oligosaccharides are used as functional foods for healthier joints and anti-obesity (Li et al., 2019). CS oligosaccharides can also be applied as antioxidant functional foods (Li et al., 2021b). On the other way, C-II is extracted and purified and then hydrolyzed to obtain collagen peptides. The by-products of this way contain CS polysaccharides and peptides of non-collagenous proteins, but further studies are needed to realize the utilization of these by-products. As a result, diversified products (fins, CS, BP-sCS, C-II, and C-II peptides) will make the high efficiency of the skate-processing industry. We believe that innovations in technologies and business models will stimulate growth of products derived from fish cartilaginous tissues that are applicable to human health. The industrialization of fish-derived cartilage products is beneficial for achieving sustainable development of local economies and society.

CRediT authorship contribution statement

Wen Li: Conceptualization, Writing – original draft preparation. **Kazuhiro Ura:** Supervision. **Yasuaki Takagi:** Supervision, Writing - review & editingWriting- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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