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Review

The Potential of Marine Collagen Peptides (MCPs) as Natural Cryoprotective Agents for Frozen Food Applications

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Abstract

Marine collagen peptides have emerged as promising natural cryoprotective agents due to their ability to regulate ice crystal growth and reduce freeze-induced quality deterioration in frozen foods. This review examines the current understanding of collagen structure, marine collagen sources, peptide production, antifreeze mechanisms, functional characterization, and food applications, with emphasis on their potential as alternatives to conventional cryoprotectants. The available literature on collagen extraction, enzymatic hydrolysis, peptide purification, and methods used to evaluate antifreeze functionality, including thermal hysteresis, ice recrystallization inhibition, rheological analysis, and water solubility, is comprehensively summarized. Current evidence suggests that low-molecular-weight marine collagen peptides, particularly those under ~3 kDa, exhibit promising cryoprotective properties associated with peptide molecular weight, amino acid composition, and amphiphilic structure. The review also identifies salted, dried cannonball jellyfish (*Stomolophus meleagris*) as an abundant yet underutilized marine collagen source with potential for producing functional antifreeze collagen peptides. Although significant progress has been made, additional research is needed to clarify structure–function relationships, standardize antifreeze evaluation methods, improve production scalability, and validate performance in complex food systems to facilitate commercial application of marine collagen-derived cryoprotective ingredients.

Keywords: marine collagen peptides; antifreeze proteins; ice recrystallization inhibition; cryoprotection; frozen foods; *Stomolophus meleagris*

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1. Introduction

Collagen, an abundant and essential structural protein in animals, and its derivatives, including gelatin and collagen peptides, have been widely utilized in food, cosmetic, medical, and pharmaceutical industries [1]. In foods, collagen-derived products are increasingly valued for their nutritional attributes and functional roles in complex formulations, including frozen and refrigerated foods. The current demand for collagen-based ingredients stems from their desirable functionalities, such as water solubility and emulsification properties. Collagen is often extracted from animal connective tissues, including skin and bones, where it exists in the extracellular matrix as a structural scaffold that supports cell function, growth, and metabolism.

Lower-molecular-weight collagen peptides are often associated with enhanced biological availability, although functional performance depends on amino acid composition and sequence. The primary amino acid sequence of collagen is typically glycine-proline-X or

glycine-X-hydroxyproline, where every third amino acid is glycine, and "X" can be one of the other 17 amino acids [2]. This triple-helix configuration allows collagen to endure stress and maintain structural integrity, while also serving as a precursor for functional peptides when subjected to controlled hydrolysis [2].

The molecular lattice arrangement, surface charge, and chemical properties of collagen impact its self-assembly and binding characteristics. When collagen is broken down via hydrolysis, lower-molecular-weight peptides are formed; these peptides often contain a repeating tripeptide sequence, typically in the form of Gly-X-X or GLY-Y-X, where X is any amino acid residue, and Y is Proline (Pro) or Hydroxyproline (Hyp) [2]. Certain collagen-derived peptides containing these motifs have been reported to exhibit antifreeze and cryoprotective properties by inhibiting ice recrystallization in food matrices [3,4]. The amphiphilic nature (molecules with hydrophilic and hydrophobic properties) of collagen peptides allows them to interact with water and ice surfaces, thereby limiting ice crystal formation and growth during freezing and freeze–thaw cycling. Hydrophilic residues participate in hydrogen bonding with water molecules, while hydrophobic domains influence collagen peptide organization and adsorption behavior at ice interfaces [5]. This balance between polar and nonpolar residues provides a mechanistic rationale for using collagen peptides as naturally derived antifreeze agents, offering a potential alternative to conventional synthetic cryoprotectants used in food, biomedical, and agricultural applications.

Marine collagen has attracted increasing interest in this context because of its distinctive amino acid composition, nutritional characteristics, and functional behavior at low temperatures. Among marine sources, cannonball jellyfish (*Stomolophus meleagris*), particularly in salted and dried form, represents an underexplored raw material for producing collagen-derived peptides. This review critically evaluates the evidence for marine collagen- and gelatin-derived peptides as cryoprotective ingredients in frozen foods, with emphasis on structure–function relationships, methods used to demonstrate antifreeze activity, performance in real food matrices, processing–activity relationships, and practical feasibility. Salted, dried cannonball jellyfish is considered as a case study of an abundant marine collagen resource rather than as the sole focus of the review.

Literature Search, Selection, and Evidence Hierarchy

This narrative review used a structured literature-search approach to improve transparency and reproducibility. Peer-reviewed literature was identified primarily through Web of Science, Scopus, PubMed, and Google Scholar using combinations of terms including, "marine collagen peptide", "collagen hydrolysate", "gelatin hydrolysate", "antifreeze peptide", "cryoprotective peptide", "ice recrystallization inhibition", "thermal hysteresis", "frozen food", "surimi", "seafood", "ice cream", "frozen dough", "jellyfish collagen", and relevant species names. Emphasis was placed on English-language peer-reviewed studies available through August 2026, while older foundational studies were retained when necessary to explain collagen structure, canonical antifreeze proteins (AFPs), and established assay principles. Studies were prioritized when they (i) characterized collagen-, gelatin-, or protein-hydrolysate-derived peptides; (ii) reported direct ice-related endpoints such as ice recrystallization inhibition (IRI), thermal hysteresis (TH), ice crystal size/morphology, or ice-binding behavior; or (iii) evaluated cryoprotective performance in a defined frozen food or biological matrix with a comparator. General AFP studies were included only when needed to establish mechanistic or methodological context.

For synthesis, evidence was interpreted hierarchically. Highest mechanistic weight was assigned to studies combining defined collagen/gelatin-derived peptides or well-characterized hydrolysate fractions with direct ice-related measurements (IRI, TH, ice-binding, or controlled ice crystal growth) and, ideally, a food-matrix outcome. Studies

reporting only quality endpoints such as water-holding capacity, protein stability, rheology, texture, or drip loss were classified as evidence of cryoprotection rather than direct antifreeze activity unless an ice-specific endpoint was also measured. Canonical AFP studies were treated as mechanistic or application context and were not used as direct evidence that collagen-derived peptides possess equivalent activity. Because the literature is heterogeneous in peptide source, purification, concentration, freezing protocol, and outcome metrics, the evidence was synthesized narratively rather than by meta-analysis.

Despite rapid advances in identifying collagen-derived AFPs, structure–function relationships remain only partially resolved, with most work correlating activity to broad features such as molecular weight, hydrophobicity, and amino acid composition rather than to specific sequence motifs and three-dimensional binding interfaces at ice surfaces [6–8]. In parallel, antifreeze activity in foods is typically assessed using disparate TH, splat, sandwich, and model freeze–thaw protocols, often in simple sucrose or buffer systems. This lack of standardized, food matrix-relevant IRI methodologies complicates comparison across studies and hinders rational formulation in complex products such as ice cream, surimi, and frozen seafood [9–14]. From a technological and regulatory standpoint, most collagen-derived AFPs are still produced at laboratory scale using multi-step enzymatic hydrolysis, fractionation, and chromatography, raising questions about cost, process, scalability, and consistency of peptide profiles. Additionally, regulatory frameworks for approving novel AFPs as ingredients, including safety, allergenicity, and labeling requirements, remain underdeveloped relative to established phosphates and sugars [14–16]. Addressing these gaps is essential to transform marine collagen resources such as salted, dried jellyfish into safe, economically viable, and mechanistically understood cryoprotective ingredients for frozen food applications.

2. Collagen Structure, Sources, and Functional Properties

2.1. Molecular Structure and Composition of Collagen

Collagen is a major structural protein in mammals, but its whole-body abundance is species-, sex-, and tissue-dependent and should not be generalized as a fixed 30% of total protein. Recent whole-animal measurements in mice reported collagen contents of approximately 12% of total protein in females and 17% in males, whereas collagen represented much larger fractions in collagen-rich tissues such as skin, bone, and tendon [17]. Collagen is found primarily in connective tissues, including skin, bone, cartilage, and tendons, where it provides mechanical and structural strength and stability [18]. Collagen's triple helix comprises three coiled chains, known as tropocollagen molecules, which form the basic building blocks of collagen's fibrillous structure [19,20]. These fibers consist of three polypeptide chains coiled around one another. Each polypeptide chain is assembled from amino acids, most commonly following Gly-Pro-X and Gly-X-Hyp motifs, and is stabilized by covalent and noncovalent interactions. The repetition and regularity of amino acid sequences contribute to helical stability, strength, and elasticity of collagen molecules [20].

2.2. Mammalian Sources and Collagen Types

The growing demand for collagen-derived products in the food, nutraceutical, and cosmetic industries has driven the search for new collagen sources and the development of innovative processing technologies. Traditionally, mammalian collagen is derived from connective tissues, such as bovine and porcine skin and bone. However, the raw material, extraction method, and processing significantly affect the molecular weight distribution, functionality, and flavor profile of the resulting collagen peptides. Mammalian collagen hydrolysates have extensive applications across the food and nutraceutical industries, but they also present challenges. For example, limited availability and land requirements

pose sustainability concerns for mammalian collagen sources [21]. Additionally, disease transmission, such as spongiform encephalopathy, is a concern in bovine collagen sources, and certain religious prohibitions prevent some populations from consuming mammalian collagen [22]. Due to these ethical, health, and sustainability concerns associated with mammalian collagen sources, marine collagen has gained popularity as an alternative [23].

Mammalian tissues are a rich source of various collagen types, with 28 identified to date [24,25]. Among these, types I, II, and III are the most abundant, and account for around 90% of the total collagen found in the human body [26,27]. Type I collagen is the most prominent within the entire collagen family and the most relevant in the human body [25]. It can be found in tissues and organs throughout the human body, primarily including bone, skin, tendons, ligaments, respiratory organs, and teeth. Type II collagen is mostly found in cartilage, and Type III collagen is found in a variety of tissues, including skin, muscle, and vascular walls [27,28]. The rest of the collagen types are found in lower concentrations and are classified based on their unique structures and functions defined by their extracellular matrix [28,29].

2.3. Marine Sources and Collagen Types

Marine collagen shares structural components with mammalian collagen but also offers unique advantages. For example, marine collagen has a lower denaturation temperature and molecular weight, a lower risk of disease transmission, and simpler extraction methods compared to certain animal sources, such as cowhide or bones [27]. Aquatic organisms, including fish, shellfish, jellyfish, sea cucumbers, marine sponges, and mollusks, have been utilized as sources of collagen to produce high-quality collagen peptides with low molecular weights and excellent functional properties. Specifically, studies have explored marine collagen sources, including cod, Alaska pollock, tilapia, tuna, and shellfish [30–33]. Multiple extraction methods exist to yield collagen from these species.

In marine organisms, many collagen types overlap with those found in mammalian sources. A key difference among marine organisms is the strong dominance of Type I collagen over other collagen types. Type I collagen can be isolated and characterized from most fish species, including cod, tilapia, tuna, eel, salmon, carp, sea bass, and parrotfish; it is commonly extracted from skins, scales, bones, fins, and swim bladders [27]. Type I collagen can also be extracted from the bodies of several invertebrate species, including squid mantle, jellyfish, and sea urchin tissues, with reports of classification overlap with other collagen types. Most marine collagens used in food, cosmetic, and biomedical products are labeled as Type I [34].

Type II collagen is mainly extracted from cartilage from species such as the silvertip shark [27,35]. Direct isolation of Type III collagen from marine vertebrates is rare, but several jellyfish species have been reported to contain collagen domains associated with mammalian types I, II, and III [34]. The classification of jellyfish collagen is often debated due to low sequence conservation and reports of mixed I/II/III preparation types and is sometimes referred to as 'type 0' [36,37]. In general, collagen isolated from marine invertebrates is classified primarily by homology and function rather than by structure. Classification of marine collagen, outlined by type, organism, and species, can be referenced in Table 1.

Table 1. Outline of marine species, group, source and isolated collagen type(s).

Marine Species	Group	Tissue/Source	Isolated Collagen Type(s)	References
Atlantic cod (*Gadus morhua*)	Teleost fish	Swim bladder, skin	Type I	[32,38]
Barramundi/Asian sea bass (*Lates calcarifer*)	Teleost fish	Skin, scales	Type I	[39]

Table 1. *Cont.*

Marine Species	Group	Tissue/Source	Isolated Collagen Type(s)	References
Nile tilapia (*Oreochromis niloticus*)	Teleost fish	Skin, scales	Type I	[39–42]
Grass carp (*Ctenopharyngodon idella*)	Teleost fish	Skin, scales, swim bladder	Type I	[4,43,44]
Giant croaker (*Nibea japonica*)	Teleost fish	Skin, swim bladder	Type I	[45,46]
Bester sturgeon (hybrid *Huso huso* × *Acipenser ruthenus*)	Teleost fish	Skin, scales, swim bladder	Type I	[46]
Lizardfish (*Saurida tumbil*)	Teleost fish	Skin, bone, scales	Type I	[47]
Yellowfin tuna (*Thunnus albacares*)	Teleost fish	Skin, scales	Type I	[30,48]
Greenland halibut (*Reinhardtius hippoglossoides*)	Teleost fish	Skin	Type I	[49]
Brownbanded bamboo shark (*Chiloscyllium punctatum*)	Cartilaginous fish	Cartilage	Type I and II	[35,50]
Blacktip shark (*Carcharhinus limbatus*)	Cartilaginous fish	Cartilage	Type II	[35,51]
Silvertip shark (*Carcharhinus albimarginatus*)	Cartilaginous fish	Cartilage	Type II	[35]
Blue shark (*Prionace glauca*)	Cartilaginous fish	Skin, cartilage	Type I (skin) and Type II (cartilage)	[50]
Sea cucumber (*Holothuria parva*)	Echinoderm	Skin	Type I-like	[52]
Sea cucumber (*Holothuria cinerascens*)	Echinoderm	Body wall	Type I-like	[53]
Sea urchin (*Anthocidaris crassispina*)	Echinoderm	Test/skin	Type I-like	[54]
Sea urchin (*Paracentrotus lividus*)	Echinoderm	Peristomal membrane	Type I-like	[54]
Starfish (*Asterias pectinifera*)	Echinoderm	Whole body wall	Type I	[55]
Sponge (*Axinella cannabina*)	Sponge	Whole tissue	Type I	[56]
Sponge (*Suberites carnosus*)	Sponge	Whole tissue	Type I	[56]
Sponge (*Chondrosia reniformis*)	Sponge	Whole tissue	Type I and IV	[56]
Jellyfish (*Acromitus hardenbergi*)	Cnidarian	Bell and oral arms	Types I, II, and III	[57]
Jellyfish (*Chrysaora* sp., ribbon jellyfish)	Cnidarian	Umbrella	Type II	[58]
Jellyfish (*Catostylus mosaicus*)	Cnidarian	Umbrella and arms	Type I	[59]
Jellyfish (*Rhizostoma pulmo*)	Cnidarian	Umbrella and oral arms	Type I and Type II-like	[60]
Jellyfish (*Rhopilema esculentum*)	Cnidarian	Mesoglea (bell, arms)	Type I	[61]
Jellyfish (*Stomolophus meleagris*, cannonball)	Cnidarian	Whole	Mixed Types I, II, IV, and V	[62]
Squid (*Loligo vulgaris*)	Mollusk	Mantle	Type I and V	[63]
Antarctic squid (*Kondakovia longimana*)	Mollusk	Skin	Type I	[45]
Sub-Antarctic squid (*Illex argentinus*)	Mollusk	Skin	Type I	[45]
Blue mussel (*Mytilus edulis*)	Mollusk	Byssus (byssal threads)	Type IV-like	[64]
Crab (*Scylla serrata*)	Crustacean	Abdominal/leg muscles	Type V	[65]

3. Hydrolyzed Collagen

The main amino acids found in collagen, Gly, Pro, and Hyp, make up tropocollagen and collagen fibers. The size and shape of collagen fibers differ by source, type, and extraction conditions. For instance, marine collagens often exhibit lower Hyp content, resulting in weaker thermal stability and a lower denaturation temperature (~25–30 °C) compared to mammalian sources (~40 °C) [27]. Thermal denaturation causes the triple helix to unravel, forming three randomly coiled chains. Further hydrolysis, often with the use of organic acids and proteolytic enzymes, yields hydrolyzed collagen, also known as collagen peptides. They are typically more bioavailable and easier to absorb in the gastrointestinal (GI) tract due to their smaller molecular weight before digestion, which also allows for faster circulation through the bloodstream [22,66,67]. This gives collagen hydrolysates an advantage over native collagen, as their high digestibility rate contributes to increased absorption, distribution, and utilization in the human body. Generally, a lower-molecular-weight peptide exhibits better bioactivity [68]. While collagen peptides exhibit multiple bioactivities including antioxidant, angiotensin converting enzyme (ACE)-inhibitory, and

anti-inflammatory activities, these properties do not automatically confer antifreeze or IRI activity [5,7,32,40,43,64]. Collagen peptides with different molecular weight sizes can show different properties; for example, peptides in the 1–3 kDa range show superior IRI activity; however, these peptides may not show high ACE-inhibitory and antioxidant activity. Generally, the amino acid composition and molecular weight of collagen peptides affect their bioactivities [9,69,70]. Therefore, a collagen hydrolysate formulated to maximize one bioactivity (e.g., antioxidant potential) may not be ideal for cryoprotection, requiring separate optimization strategies tailored to the intended functional application.

The hydrolysis step of the collagen peptide production can differ depending on the collagen source and type. However, the general process remains the same: preparation, extraction, and recovery. Raw collagen sources are prepared for extraction by washing, trimming, crushing, blending, chopping, and other similar processes. The primary objective is to remove as much non-collagenous material as possible prior to the extraction process. Following extraction, collagen may be thermally denatured to disrupt the triple helix and produce gelatin. Acid or alkali treatments are commonly used during pre-treatment or extraction, but they do not necessarily convert collagen to gelatin unless conditions are sufficient to denature its triple-helical structure. Gelatin's molecular weight ranges from 20 kDa to 220 kDa, which is lower than that of native collagen (300 kDa to 330 kDa) [28]. The molecular weight of collagen-derived gelatin influences the gelling temperature and strength of the resultant gelatin gels, which vary depending on the source [71].

Gelatin can be further hydrolyzed with proteolytic enzymes, increased temperatures, and longer hydrolysis times. Partial hydrolysis of collagen or gelatin produces peptide mixtures spanning a broad molecular-weight range, often including fractions from approximately 1 to 100 kDa depending on processing conditions [72]. Complete hydrolysis, in contrast, would ultimately yield free amino acids rather than peptides. Compared to gelatins and native collagen, collagen peptides exhibit higher water solubility and bioavailability [28]. Longer, more extensive hydrolysis conditions result in lower-molecular-weight peptides and, in some cases, the complete breakdown of peptide chains into individual amino acids. The degree of hydrolysis is often used to measure the extent of the hydrolysis process, which can also alter the functionality and structure of collagen peptides.

Bioactivity and Reported Functionality

Low-molecular-weight collagen peptides are highly water-soluble and readily dispersible, properties that are useful for incorporating them into food formulations [73]. Although collagen peptides have also been studied for nutritional and physiological effects, those applications are outside the primary scope of this review. Here, the relevant emphasis is on how peptide size, composition, sequence, solubility, and molecular interactions influence IRI, TH, protein stabilization, and quality retention in frozen foods.

4. Freezing and Cryoprotection in Food Systems

4.1. Mechanisms of Ice Crystal Formation and Frozen Food Quality Loss

Freezing and frozen storage are widely used to preserve food quality and extend shelf life. The frozen food market is projected to reach $404 billion by 2027 [74]. Maintaining a proper cold-chain distribution and frozen conditions is crucial to many frozen foods, including frozen dairy desserts, meat, seafood, fruits, and vegetables. Low temperatures inhibit the growth and activity of microorganisms and spoilage-related enzymes [75]. Commercial frozen storage is typically maintained at or below −18 °C [76]. During freezing, a portion of water crystallizes as ice while dissolved solutes are excluded from the growing ice lattice and become increasingly concentrated in the remaining unfrozen phase, a process known as freeze concentration [77]. The rate at which food is frozen affects the

number and size of ice crystals formed, which in turn influence the quality and sensory properties of frozen foods [78].

Temperature deviations throughout the freezing process or during frozen storage are the leading cause of quality degradation in frozen foods [79]. Temperature fluctuations during storage can promote partial thawing and ice recrystallization and can induce transitions from a glassy to a rubbery state in the freeze-concentrated phase [80]. Ice recrystallization results from the instability of initially formed ice crystals, involving alterations in their shape, size, quantity, and spatial orientation. There are three types of recrystallizations: accretion, iso-mass, and migratory [81]. Accretion causes closely oriented ice crystals to merge into a singular, larger crystal. Iso-mass changes the overall structure of an ice crystal, without affecting its mass. This is common in ice crystals with an irregular shape or a high surface-to-volume ratio. Among the main recrystallization mechanisms, migratory recrystallization (Ostwald ripening) is most detrimental in foods due to the continuous growth of large crystals at the expense of small ones, leading to coarse ice structures, cellular rupture, and major textural damage [80,82–84]. Ice recrystallization generally accelerates as storage temperature increases and molecular mobility in the freeze-concentrated phase rises. The temperature at which recrystallization becomes pronounced is matrix-dependent and is influenced by composition, unfrozen-water content, and the glass-transition behavior of the maximally freeze-concentrated phase; therefore, approximately −20 °C should not be treated as a universal threshold [80,85,86].

The food industry uses quick freezing to produce frozen foods with small ice crystals; however, these crystals may be disrupted during storage and transportation, resulting in larger ice crystals. Ice formation, particularly in the form of large, irregular crystals, can significantly affect food quality [42,87]. The freezing rate affects the number and size of ice crystals that form. For example, slow freezing generally produces fewer, larger ice crystals that can disrupt cellular and muscle structure and adversely affect texture [88]. Any associated increase in lipid oxidation is indirect and matrix-dependent, arising from structural damage, redistribution of solutes, exposure of pro-oxidant components, and changes in oxygen accessibility rather than from freezing rate alone. The addition of additives, including sugars, polysaccharides, active proteins, and hydrolysates; to modify ice crystals and restrict water movement to frozen food matrices is often necessary to prevent quality degradation associated with ice recrystallization [82,89,90].

4.2. Existing Ice-Regulating Additives and Associated Limitations

Exploring ice-regulating strategies and additives is crucial to minimizing ice-recrystallization-related damage and maintaining acceptable frozen food quality. The term cryoprotectant is used differently in food science and cryobiology. Cell- and tissue-preservation systems commonly employ small-molecule cryoprotectants such as dimethyl sulfoxide (DMSO), ethylene glycol (EG), propylene glycol, and glycerol, but DMSO and EG are not conventional food ingredients and should not be grouped with food-grade cryoprotective formulations. In frozen foods, commonly used cryoprotective ingredients include sugars, sugar alcohols, phosphates, proteins, hydrocolloids, and related formulation aids that modify water mobility, ice formation, protein stability, or the freeze-concentrated phase [81,85,91–96]. Commercial seafood and surimi systems, for example, frequently use sucrose-sorbitol and phosphate combinations. Although such ingredients are effective, high use levels can alter sweetness, ionic strength, formulation composition, and sensory characteristics. These limitations motivate interest in alternative ice-regulating compounds that can function at lower concentrations or provide complementary mechanisms such as IRI.

5. AFPs

The potential application of AFPs in foods has received growing attention because these proteins can modify ice crystal growth and recrystallization at relatively low concentrations [13]. AFPs can effectively mitigate freeze-induced damage by inhibiting ice crystal growth and recrystallization. Naturally found in the blood and tissues of cold-adapted organisms, AFPs have demonstrated a broad range of applications in food systems, cellular cryopreservation, and plant frost protection [16]. These proteins do not prevent freezing but rather inhibit ice crystal growth and recrystallization. Through hydrophilic interaction with ice and specific binding to basal and prism surfaces, AFPs block the addition of water molecules to the ice lattice, thereby restricting further crystal growth [3,97,98]. This adsorption-inhibition mechanism produces non-colligative TH, defined as a gap between the equilibrium melting temperature and the lower temperature at which rapid ice growth resumes in the presence of an ice-binding protein [3]. Depending on the organism and AFP type, the adsorption-inhibition mechanism generates TH values ranging from 1 to 2 °C in fish, and up to >6 °C as reported for insects [86,97]. The efficiency and mode of binding strongly depend on the AFP's structural class, amino acid composition, and the surface hydrophobic-hydrophilic patterning, all of which influence their interaction with the ice lattice.

5.1. Sources, Classifications, and Ice-Binding Mechanisms of AFPs

AFPs can be broadly classified by their biological source, with major groups derived from fish, plants, insects, and microorganisms, all of which exhibit similar ice-binding properties. Fish-derived AFPs are the most extensively researched and are categorized into four groups (Types I, II, III, and IV) based on their primary sequences, three-dimensional conformations, and ice-binding mechanisms [99]. These classifications and their associated structural features are presented in Table 2. Visual 3D examples of these classifications (Types I–III), in the form of cartoon crystal structures from fish-derived AFPs, are shown in Figure 1. AFP production and isolation from insects have been reported, with the most well-known examples found in beetles (*coleoptera*), which exhibit exceptionally high TH activity [100]. Plant-derived AFPs have been identified in species adapted to seasonal freezing, including winter rye (*Secale cereale*), wheat (*Triticum aestivum*), carrots (*Daucus carota*), and perennial ryegrass (*Lolium perenne*), among others [16]. Psychrophilic bacteria such as *Marinomonas primoryensis* and *Colwellia* spp. also exhibit antifreeze behavior by binding to ice surface through extracellular adhesion proteins [101]. Among all natural sources, marine organisms represent the most promising candidates for food applications due to their high protein content, scalable biomass availability, and the strong ice-binding efficiency observed in many fish-derived AFPs.

Table 2. Classifications, examples, molecular weight, and structural and binding characteristics of fish-derived AFPs.

AFP Type	Molecular Weight (kDa)	Structural Components	Source Species Examples	Ice-Binding Characteristics	References
Type I	3.3–4.5	Predominately single long a-helix chain, alanine-rich	Winter flounder (*Pleuronectes americanus*), shorthorn sculpin (*Myoxocephalus Scorpius*)	Aligned threonine residues form ice-binding site, binds to prism and pyramidal planes, moderate TH activity	[102]
Type II	11–24	Globular, stabilized by disulfide bonds, cystine-rich, 11–24 kDa	Sea Raven (*Hemitripterus americanus*), Smelt (*Osmerus mordax*), Herring (*Clupea harengus*)	Ca^{2+} dependent binding, large number of non-glycoproteins, high TH activity	[102]

Table 2. *Cont.*

AFP Type	Molecular Weight (kDa)	Structural Components	Source Species Examples	Ice-Binding Characteristics	References
Type III	6.5–14	Globular, compact secondary structures	Ocean Pout (*Zoarces americanus*)	Affinity towards quaternary and sulfopropyl amines *, binds multiple planes (prism + pyramidal), reduced TH activity	[16,103]
Type IV	~12	Glutamine and glutamate enriched, helical bundle, structurally unique	Longhorn sculpin (*Myoxocephalus octodecemspinous*)	Hydrophilic/hydrophobic anti-parallel organization, very low TH activity	[104]
AFGP *	2.6–33.7	Glycoprotein, helical shape	Notothenioid fish	Flexible glycoprotein that binds through hydrophobic interactions of the disaccharide on Thr, strong recrystallization properties	[102,105]

* AFGP = Antifreeze glycoprotein; sulfopropyl amine = highly soluble compounds containing a 3-sulfopropyl group attached to an amine or ammonium nitrogen.

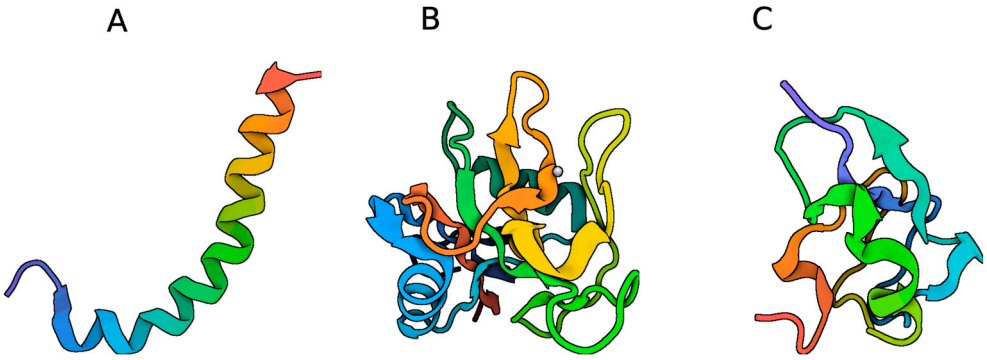


Figure 1. Cartoon sequence structures of (**A**) recombinant Type I Sculpin antifreeze protein, PDB: 1Y04; (**B**) Herring Type II antifreeze protein PDB: 2PY2; (**C**) Type III antifreeze protein from Antarctic Eel Pout PDB: 1UCS [106,107]. (Created with Biorender.com).

The ice-binding mechanisms of AFPs vary significantly by source and structure, with some binding scenarios being loosely defined. It has been suggested that AFP freeze inhibition involves the adsorption to specific ice crystal planes, accompanied by the ordering of water molecules around the protein surface. This interaction, supported by hydrogen bonding and hydrophobic hydration, inhibits ice crystal growth. Upon binding, AFPs curve the ice surface, thereby effectively lowering the freezing point via the Kelvin effect [108]. A curved ice surface is thermodynamically less favorable for rapid ice crystal growth compared to a flat ice surface. Additionally, protein hydration plays a critical role in the structure, function, and dynamic interactions of AFPs with water. The hydration shell, the layer of water surrounding the protein, is influenced by protein folding, molecular size, and binding interfaces and contributes to AFP's ability to interact with ice [109,110]. Furthermore, the number of ice-binding surfaces (IBS) per molecule significantly influences the antifreeze capabilities of the proteins [111].

IBS often display organized patches of hydrophobic and polar residues rather than being uniformly nonpolar. Threonine is a polar, uncharged residue whose hydroxyl group can participate in interactions with ordered water and ice, whereas residues such as Val and Ala are nonpolar; Pro is generally classified as nonpolar but can also influence backbone

conformation. The affinity and geometric organization of these residues at the ice-binding interface can strongly influence AFP activity [82]. Recent studies suggest that many IBSs combine hydrophobic surface character with strategically positioned polar groups. The relative contributions of hydrophobic effects, hydrogen bonding, hydration-shell organization, and surface complementarity remain active areas of investigation [82,89,110]. Figure 2 illustrates freeze-induced damage caused by uncontrolled ice crystallization in a non-AFP sample compared to freezing in a sample with AFP.

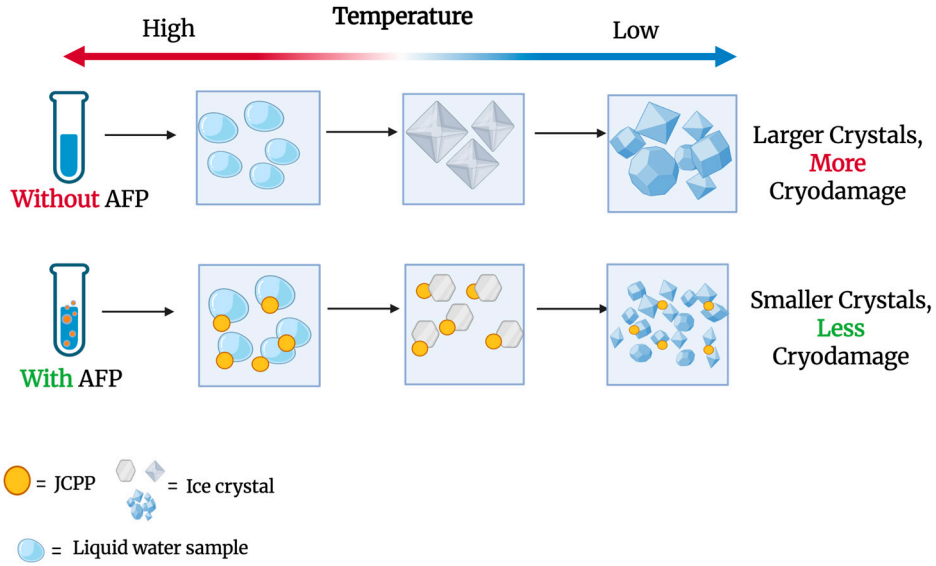


Figure 2. Illustration of freezing-induced ice crystallization and resultant damage with and without AFP. JCPP = jellyfish collagen peptide powders.

5.2. TH and IRI

The antifreeze capability of AFPs can be measured via TH and IRI. TH is the difference between the freezing point (T_f) and the melting point of a solution (T_m) [110,111], whereas IRI refers to the ability of AFPs to prevent small ice crystals from forming larger ones during freezing, storage, and thawing cycles [112,113].

Although TH and IRI are closely related functions of AFPs, they indicate distinct mechanisms. For example, hyperactive insect AFPs can exhibit TH values as high as 5 to 6 °C, whereas most marine AFPs show modest activity (0.3–1.5 °C). Despite lower TH, marine AFPs often exhibit strong IRI, reducing crystal growth by up to 90% [112]. This functional profile is particularly advantageous for certain food applications, since a high TH can disrupt controlled freeze–thaw behavior and potentially compromise food safety. As previously described, ice recrystallization refers to the enlargement of ice crystals during frozen storage, a process that IRI-active compounds aim to inhibit.

Low-molecular-weight peptides, including collagen-derived peptides and certain plant AFPs, often show weak or undetectable TH yet retain noticeable IRI activity. This is partly due to the difficulty of detecting TH in small peptides, even during interactions with ice interfaces. Since ice recrystallization during freeze–thaw cycles generates large ice crystals that can damage cells, alter texture, and reduce product quality [113], the IRI activity of AFPs is crucial to their potential as cryoprotective food ingredients. For instance, in frozen chicken breast stored at −18 °C for 75 days, the addition of 0.25% AFP significantly reduced ice crystal equivalent diameter and area relative to the control ($p < 0.05$), yielding much finer crystals and a tighter muscle microstructure [114].

6. Marine Collagen Peptides with Antifreeze-like Activity

6.1. Evidence of Ice-Inhibiting Activity in Marine Collagen Peptides

Direct evidence for marine collagen-derived peptides is smaller than the broader AFP literature but is increasingly substantive. For example, tilapia skin collagen hydrolysate has been evaluated both for direct ice-related behavior and for preservation of frozen scallop muscle; fractionation identified the tetrapeptide NHGK (454.26 Da), which showed substantially greater TH activity than the unfractionated hydrolysate, while the hydrolysate improved protein and water-retention indices during frozen storage [115]. Cod collagen peptide fractions have been studied at the molecular and food-matrix levels, with evidence linking primary structure and peptide–ice interactions to preservation of myofibrillar proteins during freeze–thaw exposure [5]. Membrane-separated silver carp hydrolysate fractions have also been examined using experiments and molecular-dynamics simulation, providing evidence that peptide composition and intermolecular interactions influence ice crystal growth [44]. Tilapia-skin antifreeze peptide preparations have additionally improved survival during cryopreservation of *Lacticaseibacillus rhamnosus*, although this is a biological cryopreservation model rather than a frozen food matrix and should therefore be interpreted separately [33]. Fish-gelatin hydrolysates provide complementary evidence that low-molecular-weight peptide fractions can inhibit ice crystal growth, but gelatin-hydrolysate results should not automatically be generalized to all marine collagen sources [69]. Collectively, these studies suggest ice-regulating potential while also showing that activity depends on source, hydrolysis, fractionation, sequence, concentration, and assay conditions.

6.2. Mechanistic Insights: Amino Acid Composition and Ice Interaction

Mechanistic evidence for collagen-derived peptides should be separated from that for canonical AFPs. For collagen-derived systems, direct evidence includes the squid-skin sequence DVRGAEGSAGL, the tilapia-skin tetrapeptide NHGK, and fractionated fish-gelatin peptides; these studies show that ice-related activity depends on peptide sequence, charge distribution, molecular size, and the assay used [3,69,115]. Gly and Pro are generally classified as nonpolar by side-chain polarity, whereas residues such as Glu, Asp, Arg, Lys, Ser, Thr, and Hyp contribute charged or polar side-chain functionality. Nevertheless, peptide hydration cannot be inferred from side-chain classification alone because backbone carbonyl and amide groups also participate in hydrogen bonding. Thus, statements that collagen peptides interact with ice should be reserved for preparations evaluated by TH, IRI, ice-growth, or ice-affinity measurements. Canonical fish AFPs, including winter flounder and ocean pout proteins illustrated in Figure 3, provide mechanistic context for organized ice-binding surfaces, but their residue patterning and binding behavior should not be extrapolated directly to collagen hydrolysates [82,99,101].

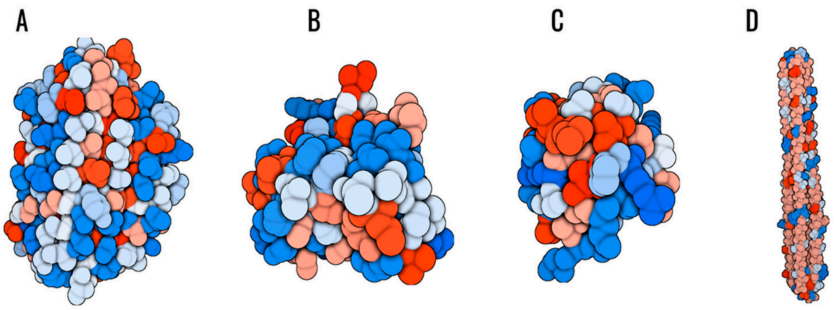


Figure 3. Representative structures of ice-binding proteins: (**A**) Antarctic sea-ice bacterium Colwellia sp., PDB: 3WP9; (**B**) Herring Type II antifreeze protein, PDB: 2PY2; (**C**) North Atlantic ocean pout Type III antifreeze protein, PDB: 1KDF; and (**D**) winter flounder Type I antifreeze protein, PDB: 4KE2. These canonical AFP/ice-binding protein structures are shown for mechanistic context and are not collagen-derived peptides. Source: Białkowska et al. [101].

6.3. Structure–Function Correlations in Marine Collagen-Based AFPs

Structural features of marine collagen-derived AFPs appear to correlate with antifreeze function, IRI and TH. Across numerous studies, collagen-derived AFPs with the highest recorded activity fall within a narrow molecular weight range of approximately 0.5–3 kDa [3,69,116–120]. Fish collagen peptides in this range size exhibited the highest inhibition of ice crystal growth in ice cream products, while peptides smaller or larger in size were less effective [69]. Peptides of this size are long enough to facilitate a patterned binding interface, but short and flexible enough to conform to the geometry of ice lattice planes. Marine collagen hydrolysates (fish, jellyfish, squid) with molecular sizes between 0.5 and 5 kDa can potentially be used for their antifreeze functionality in food systems [3].

Marine collagen peptides are rich in Gly, Pro, and Hyp, residues that strongly influence backbone conformation and hydration behavior. Gly and Pro should not be classified as hydrophilic on the basis of side-chain polarity; Gly is commonly treated as nonpolar; meanwhile, Pro is nonpolar and conformationally restrictive, and Hyp contains a hydroxyl group that can contribute polar interactions. Charged and polar residues such as Glu, Asp, Arg, Lys, Ser, Thr, and Hyp can contribute side-chain interactions, while peptide-backbone carbonyl and amide groups also participate in hydration. Consequently, experimentally demonstrated ice interactions should be distinguished from general water affinity. For example, squid-skin collagen hydrolysate produced with acid protease yielded the active sequence DVRGAEGSAGL [3], whereas tilapia-skin hydrolysate generated with Alcalase yielded NHGK [115]; the different sequences, enzymes, fractionation schemes, and assay endpoints preclude assigning activity to bulk hydrophilicity alone.

6.4. Beyond Molecular Weight: Sequence, Charge, Hydrophobicity, Conformation, and Hydrolysis

Molecular weight alone does not explain antifreeze performance. Study-level comparisons indicate that enzyme specificity and fractionation can generate preparations with markedly different sequence, charge, and conformational properties. Acid-protease hydrolysis of squid-skin collagen produced a hydrolysate containing a substantial low-molecular-weight fraction and yielded DVRGAEGSAGL as the principal sequence in the most active chromatographic fraction [3]. By contrast, Alcalase hydrolysis of tilapia-skin collagen generated a <2.5 kDa hydrolysate with TH of 0.50 °C, while the purified 454.26 Da peptide NHGK reached 2.63 °C [115]. Fish gelatin hydrolyzed with Alcalase showed maximum ice-growth inhibition after 25 min at pH 9.0 and 45 °C; subsequent fractionation concentrated activity in a cationic fraction containing peptides of 1850.82 and 2036.88 Da, indicating that charge and sequence can be as important as size [69]. Porcine skin collagen provides a further contrast: alkaline-protease hydrolysis at pH 8.0, 55 °C, and 15% degree of hydrolysis followed by chromatography yielded GLLGPLGPRGLL (1162.8 Da), with strong TH and ice-cream recrystallization effects [121]. The studies support preparation-specific optima that depend on cleavage specificity, degree of hydrolysis, sequence, charge, hydrophobic/polar patterning, conformational flexibility, concentration, and the measured endpoint. Mixtures and purified peptides should therefore not be treated as functionally equivalent, and apparent inconsistencies across studies should be interpreted in light of their different assays and matrices.

6.5. Distinguishing Antifreeze Activity, IRI, and Broader Cryoprotection

In this review, "antifreeze activity" is used as an umbrella description only when a peptide preparation directly modifies ice behavior. IRI is a specific phenotype: suppression of the growth of larger ice crystals at the expense of smaller crystals during annealing or temperature fluctuation. Meanwhile, TH is a distinct, non-colligative property defined by the temperature gap between equilibrium melting and the lower temperature at which rapid

ice growth resumes. Neither IRI nor TH should be inferred solely from improved frozen food quality. "Cryoprotection" is therefore used more broadly for preservation during freezing or frozen storage, including reduced protein denaturation or oxidation, improved water-holding capacity, lower drip loss, maintenance of enzyme activity, and improved texture. Such outcomes may arise from direct ice modulation but can also reflect viscosity, hydration, antioxidant effects, or peptide–protein interactions. Importantly, IRI itself may not fully explain cryoprotective performance, even for established antifreeze proteins [122]. The strongest evidence for collagen-derived peptides therefore combines molecular characterization, a direct ice-specific endpoint, and a food-matrix outcome [5,9,44,69,123].

7. Functional Characterization and Antifreeze Assessment of Marine Collagen Peptides

7.1. Water Solubility and pH-Dependent Behavior

Water solubility is an important formulation property for marine collagen peptides because it affects dispersion, concentration control, and accessibility of peptides within aqueous food matrices [124]. However, solubility alone does not establish antifreeze activity and should not be interpreted as evidence of IRI, TH, or direct ice binding. These functions require dedicated ice-based assays. Soluble peptides may have greater opportunity to interact with the aqueous and ice phases, but any resulting effect on ice recrystallization must be demonstrated experimentally.

Increasing temperature increases the thermal energy of peptide chains, enhancing mobility and reducing viscosity by destabilizing hydrogen bonds between the chains and water [123]. This same principle applies to systems containing native collagen, where the triple helix is still intact. A drop in complex viscosity with increasing temperature reflects the disruption of the hydrogen-bond network and the unwinding of the triple helices. Even though the helix is already denatured in peptide solutions, this H-bond weakening leads to more mobile chains [123]. For practical applications, a higher temperature causes peptides to dissolve faster and contribute to less viscous solutions at a given concentration due to increased speed of diffusion and weakened peptide–peptide H-bonding. Dry marine collagen peptide powders, such as jellyfish collagen peptides, are thermally stable up to relatively high temperatures. A study by Cruz et al. [124] confirmed the endothermic peaks associated with denaturation of jellyfish collagen peptides were observed around 103–119 °C, indicating the peptide chains themselves do not degrade at typical food processing temperatures.

Marine collagen peptides are inherently highly soluble in water, but solubility behavior changes with concentration and pH adjustment. This behavior is well documented in studies using collagen and gelatin systems [2,40,125–127]. MCP solutions behave analogously, meaning that below a formulation-specific threshold, increasing the concentration contributes to higher viscosity with complete dissolution. Above that threshold, especially near the protein's isoelectric point (pI) or in the presence of salt, a phase separation is observed, turbidity increases, and a gel-like behavior can contribute to an overall apparent loss of solubility [126,128]. Hydrolysates with smaller peptide sizes and high degrees of hydrolysis generally exhibit higher water solubility due to shorter chains, more exposed polar groups, and less tendency to self-associate [129,130].

Numerous methods exist for evaluating the water solubility of collagen peptides. For example, a study using lumpfish and starfish collagen tested water solubility using isoelectric precipitation (IP) by dissolving powdered samples across a pH range to generate a solubility curve and identify zeta potential values and pI [123]. Like most protein peptides, collagen peptides have a pH-dependent solubility. Under acidic conditions, collagen peptides demonstrate high water solubility, compared to lower solubility at neutral or

alkaline pH conditions [131]. It is hypothesized that the water solubility of marine collagen peptides will depend on solution pH, solid concentration, and solution's temperature, with lowest water solubility occurring near the pI.

Acid pre-treatment and thermal conditions can alter collagen solubilization by disrupting intermolecular interactions and, under sufficiently severe conditions, promoting limited bond cleavage [132]. Acid pre-treatment should therefore be considered separately from enzymatic hydrolysis. Its necessity and optimal conditions are source- and process-dependent rather than universal. For marine peptide production, acid type, concentration, pH, exposure time, temperature, and subsequent enzyme treatment should be optimized together to control recovery, molecular-weight distribution, mineral removal, and sensory quality. Direct IRI or TH measurements are still required to determine whether these processing choices improve ice-related functionality.

7.2. Rheological and Thermophysical Properties

Rheological behavior provides insight into the molecular stability of a collagen peptide solution. Meanwhile, amino acid composition affects inter- and intramolecular interactions. Low quantities of Pro and Hyp in fish skin collagen were seen to affect the rheological behavior of the collagen solution, making it less stable against external forces [22]. Additionally, the viscoelastic modulus of a collagen peptide solution is temperature dependent. As temperature increases, enhanced molecular mobility reduces viscoelastic stability [133]. Recent studies have reported that extended hydrolysis affects the viscoelastic properties of the resultant collagen peptide solutions [73,129,134,135]. Steady shear, dynamic frequency sweep, and temperature sweep tests are common methods of analyzing viscoelastic properties of a collagen peptide solution in relation to temperature [2,133]. Rheological testing provides insight into the behavior of marine collagen hydrolysates in solution with applied strain, which is helpful for determining potential applications in sub-zero conditions. Rather than directly measure recrystallization, rheological properties provide indirect evidence of structural stability post-freeze–thaw.

7.3. IRI

The IRI of AFP-based solutions can be measured via splat assay, sandwich assay, cold-stage microscopy, and direct image analysis to measure ice crystal size.

Splat and Sandwich Assays

The splat assay and sandwich assay are microscopy-based methods commonly used to evaluate IRI, but they differ in sample preparation and thermal history. In a conventional splat assay, a small droplet of the test solution is rapidly frozen on a precooled surface, forming a thin polycrystalline ice wafer. The sample is subsequently annealed at a defined sub-zero temperature, allowing smaller crystals to disappear while larger crystals grow. IRI activity is determined by comparing ice crystal size or growth in peptide-containing samples with an appropriate control using metrics such as mean grain size or mean largest grain size [11,12].

In the sandwich assay, a small volume of sample is confined between two coverslips, frozen, and maintained on a temperature-controlled cryostage. The same microscopic field can then be monitored during annealing, allowing changes in ice crystal size and morphology to be followed over time. Therefore, the coverslip configuration illustrated in Figure 4 is more appropriately described as a sandwich assay rather than a classical splat assay [9,10].

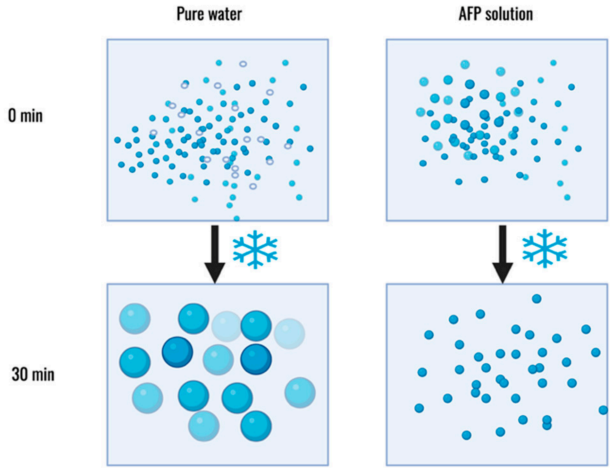


Figure 4. Visual representation of sandwich assay to study the expected behavior of microscopic ice crystal formation in pure water versus AFP-containing solution.

For both methods, appropriate composition-matched controls are essential. Peptide-containing samples and controls should have equivalent solvent, sucrose or salt concentration, buffer composition, sample thickness, freezing history, annealing temperature, and duration [12]. Comparing a peptide–sucrose solution with pure water, for example, may confound peptide effects with the influence of sucrose on freezing behavior and recrystallization. Results from splat and sandwich assays should also not be considered directly interchangeable because cooling rate, sample geometry, peptide concentration, annealing conditions, and image-analysis metrics can influence the measured response. Thus, IRI results should be interpreted within the specific experimental conditions used and should not, by themselves, be considered evidence of thermal hysteresis or broader cryoprotection in food systems.

A study by Kaganovich et al. [136] measured the rate of ice recrystallization by freezing a mixture of peptides in a sucrose solution, then warming to an annealing temperature just under melting to monitor crystal size over time, against pure water. The sample containing AFP peptides demonstrated a considerable inhibition of ice crystal growth and alteration of the ice crystal shape due to direct binding of the peptide to prism planes.

7.4. TH Behavior

TH is the non-colligative gap between the equilibrium melting temperature and the nonequilibrium ice-growth/freezing temperature produced by an ice-binding molecule [110,112]. For canonical AFPs, TH is most reliably measured by observing a single ice crystal in a nanoliter osmometer or temperature-controlled microscopy system and identifying the melting point and the temperature at which rapid crystal growth occurs. Bulk DSC freezing exotherms can be strongly affected by stochastic supercooling and nucleation and therefore should not automatically be interpreted as TH. Appropriate controls should match peptide concentration, buffer/salt/sugar composition, cooling and warming rates, sample volume, and thermal history. Replicate measurements are essential because nucleation temperature is inherently variable.

7.5. Methodological Limitations and Minimum Reporting Requirements

Cross-study comparisons of IRI and TH are difficult because assay conditions strongly influence the measured response. In IRI assays, peptide concentration, sucrose or salt concentration, initial freezing rate, annealing temperature, annealing duration, sample thickness, microscope field selection, and image-analysis metric should be matched between test and control samples. Crystal size may be reported as mean grain size, mean largest grain size, equivalent circular diameter, area, or growth rate; these metrics are not

interchangeable. Controls should include the same solvent and solute composition without peptide and, when feasible, a recognized positive control. In TH measurements, supercooling/nucleation must be distinguished from adsorption-mediated inhibition of ice growth; a bulk freezing onset alone is insufficient evidence of TH. TH values are also sensitive to ice crystal size, cooling protocol, concentration, and the criterion used to identify burst growth. Rheological improvements should likewise be interpreted as indirect evidence unless accompanied by microscopy, IRI, TH, or another direct ice-related endpoint. Standardized reporting of concentration on a mass or molar basis, peptide molecular-weight distribution, assay matrix, temperature history, number of replicates, and image-analysis method would substantially improve comparability. These limitations are especially important when extrapolating from model sucrose or buffer systems to multicomponent foods [9,69,121]. Figure 5 shows the mechanism of cooling and warming used to measure the temperatures at which crystals form, melt, and then rapidly reform. The difference between the melting and freezing onset temperatures determines the sample's thermal hysteresis.

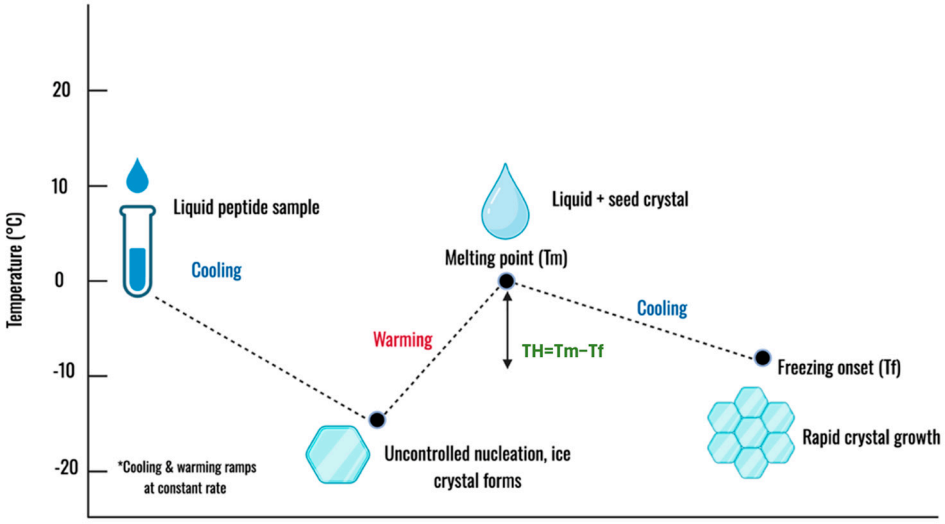


Figure 5. Thermal hysteresis schematic in a peptide sample showing cooling and warming to measure the temperature difference in crystal growth. * Cooling and warming rates are constant.

8. Production of Marine Peptides with Antifreeze Properties from Salted, Dried Jellyfish (SDJ)

8.1. Cannonball Jellyfish (CJ): A Sustainable Source of Marine Collagen

The CJ is one of the most abundant jellyfish species in the U.S. Southeast Atlantic and is harvested just off Georgia's coast [62,130]. The U.S. CJ biomass is expected to grow with rising populations, arguably due to climate change (warmer and more acidic oceans), overfishing of natural predators, and ocean sprawl linked to human activity [137,138]. Traditionally, CJ is harvested in the spring months and processed into SDJ. CJ has a protein-rich, largely collagenous makeup, with characteristics similar to those of fish-derived AFP raw materials [69]. The unique amino acid profile of marine collagen and the low molecular weight of its derivatives has been suggested to have potential cryoprotective effects [139,140]. For example, marine collagen peptides contain residues and peptide-backbone groups capable of extensive hydration; however, Gly should not be classified as a hydrophilic side-chain residue, and hydration alone does not demonstrate ice binding. Gly and Hyp are both prominent in CJ collagen [141]. Peptides in the 1–3 kDa range have frequently demonstrated strong IRI activity, although optimal size depends on sequence and matrix composition [8,44,69]. Several jellyfish-derived hydrolysates yield peptides within the 0.5–5 kDa range, which overlaps with molecular weight ranges reported to

exhibit IRI activity in other marine systems [62,142,143]. Therefore, the characteristics of CJ offer a promising source of marine collagen with potential antifreeze properties.

8.2. Acid Pre-Treatment Strategies

Seafood by-products used for obtaining marine collagen peptides undergo pre-treatment to improve the extraction and removal of non-collagenous components, such as minerals. Acid treatment prior to enzymatic hydrolysis facilitates the initial extraction and softening of tissue, thereby making native collagen more accessible for further denaturation through the addition of heat and enzymes, which yields low-molecular-weight peptides [119]. The choice of acids and enzymes affects the molecular weight, bioactivity, and sensory components of the resultant collagen peptide powders [19]. Acid treatments primarily employ low concentrations of organic acidic solutions to permeate the raw material, disrupting intermolecular salt bonds and Schiff bases, while causing tissue swelling and dissolution [143]. Two acids commonly used in pre-treatment strategies are citric and acetic. Concentration and time combinations vary according to raw material. Studies using different fish tissue types (scales, skin, swim bladder, and gills) utilized acetic acid as an extraction pre-treatment method [144–147]. Chiarelli and colleagues have previously utilized citric acid as a pre-treatment for producing collagen peptides from frozen fresh CJ [71]. Studies evaluating acid-solubilized collagen yield reported the impact of various acids, including acetic and citric, used individually [148]. Results suggest a correlation between acid type and the extent of protein denaturation; however, combinations of different acids have not been evaluated. It appears that the yields and outcome of collagen peptides depend on the initial protein content, enzyme and acid choice, concentration, and soak time. Furthermore, recent reports suggest that acetic acid maintains the integrity of the collagen molecule while facilitating the breakdown of intra- and intermolecular cross-links, whereas citric acid exhibits a hydrotropic swelling effect in marine collagen sources [38,119]. Acetic and citric acids differ in acid dissociation behavior, buffering capacity, chelation, and interactions with collagenous tissues; their effects therefore depend on solution pH, acid concentration, temperature, ionic strength, and treatment time rather than simply on the number of ionizable protons. Reported treatment times vary widely among raw materials and should be interpreted in the context of the actual process conditions [149]. Citric acid has also been described as a chelating agent, with the ability to remove non-collagenous minerals, including calcium and sodium [150]. Therefore, the synergetic potential between acetic and citric acid could lead to increased initial disruption of structural bonds, ultimately optimizing enzymatic extraction. Combined acid strategies may enhance tissue breakdown and disrupt the bonds that stabilize tightly coiled collagen fibrils, facilitating the production of low-molecular-weight peptides from SDJ.

8.3. Enzymatic Hydrolysis and Enzyme Selection

Controlled hydrolysis is a key step for converting marine collagen into lower-molecular-weight peptide mixtures, but hydrolysis by itself does not establish antifreeze activity. In general, collagen hydrolysis can be accomplished using chemical, physical, and/or enzymatic methods that affect the amino acid profile, peptide size, hydrophobic and hydrophilic residues of resultant peptides. A visual of the breakdown and denaturation of collagen's unique triple helix as a result of acid pre-treatment and enzymatic hydrolysis can be seen in Figure 6. Jellyfish collagen resembles human Type I collagen in structure, while also sharing similarities with types II, III, V, and IX mammalian collagen [151]. Its three-dimensional native structure is stabilized by hydrogen bond forces, along with other intramolecular noncovalent bonds, including Van der Waals forces, electrostatic attractions, and hydrophobic interactions. Pre-treatment, denaturation, and enzymatic hydrolysis can

be used to generate low-molecular-weight peptides; however, IRI, TH, or direct ice-binding activity must be demonstrated experimentally for the resulting fractions.

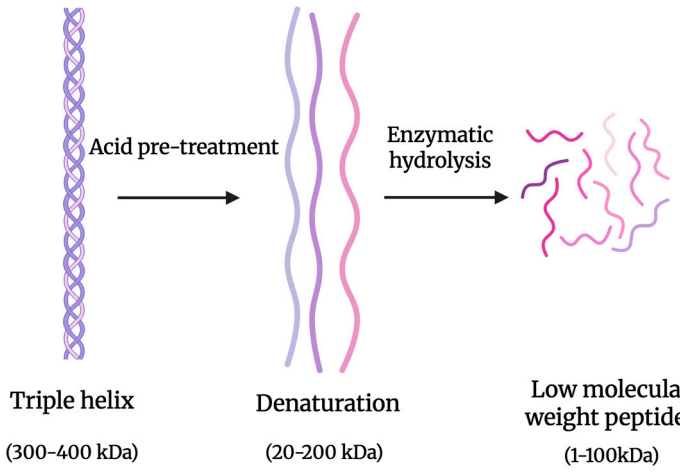


Figure 6. Schematic of the denaturation and breakdown of collagen triple helix into low-molecular-weight peptides through acid pre-treatment and enzymatic hydrolysis.

To successfully produce marine collagen peptides with antifreeze properties from jellyfish, an effective hydrolysis method must yield water-soluble, low-molecular-weight peptides. Collagen peptide powders can be produced using a variety of enzymes that affect the final collagen peptide size distribution, functionality, and sensory components. To choose the ideal enzyme, the optimal pH and temperature working ranges must be considered, along with the enzyme's mechanism for cleaving the peptide backbone [152]. Enzymes accelerate the biochemical reaction that breaks down proteins into smaller peptides and amino acids. This action is achieved by cleaving peptide bonds with water molecules, a process typically performed by hydrolytic enzymes. The majority of food-grade enzymes consist of proteases [144]. Proteolytic enzymes are broadly classified as endopeptidases, which cleave internal peptide bonds, and exopeptidases, which remove terminal amino acids. Enzyme selection influences peptide size, distribution, hydrophobicity, and functional performance. Among food-grade proteases, Alcalase ® is widely used due to its broad specificity and efficiency in generating low-molecular-weight peptides under mild processing conditions [72,73]. A table of the most common commercial enzymes used to produce marine-derived collagen peptides is presented in Table 3.

Table 3. Commonly used commercial enzymes for protein hydrolysis with their typical source, working pH, and temperature ranges, and simplified use.

Enzyme (Common Name)	Type	Source	Optimal pH/Temp	Common Raw Materials	References
Alcalase	Endo (serine)	*Bacillus* species, *Aspergillus* species	pH 7.5–9.0/ 55–65 °C	Tilapia skin/scales, tuna skin, silver carp scales, jellyfish, fish bones, shrimp waste, skipjack viscera	[4,9,23,69,72,111,153–155]
Protamex ®	Endo (serine protease)	*Bacillus* protease complex	pH 6.0–7.5/ 50–60 °C	Striped tuna, black muscle, fish frames, kilka (whole fish), turbot by-products	[23,72,155–160]
Neutrase ®	Endo (metallo endoprotease)	*Bacillus amyloloquefaciens*	pH 7.0–8.0/ 45–55 °C	Yellowfin tuna viscera, fish skin, giant croaker swim bladders, skipjack scales	[23,90,153,155,159,161,162]
Papain	Endo (cysteine protease)	Papaya (*Carica papaya*)	pH 6.0–7.0/ 40–60 °C	Tilapia skin, grass carp scales, squid skin, fish skin gelatin, monkfish swim bladders	[23,25,127,137,159]

Table 3. *Cont.*

Enzyme (Common Name)	Type	Source	Optimal pH/Temp	Common Raw Materials	References
Bromelain	Endo (cysteine protease)	Pineapple (*Ananas comosus*)	pH 6.0–9.0/ 40–60 °C	Bigeye tuna skin, fish protein, tilapia skin gelatin	[155,163]
Pepsin	Endo (aspartic protease)	Porcine gastric mucosa	pH 1–6/ 37–45 °C	Fish skin, fish scales, swim bladders, tuna skin, jellyfish, bigeye tuna by-products	[38,46,154,164–169]
Trypsin	Endo (serine protease)	Porcine pancreas	pH 7.0–9.0/ 35–55 °C	Tilapia skin, grass carp scales, fish skin, skipjack scales, sturgeon by-products	[4,6,9,170–175]
Collagenase	Endo (metalloprotease)	*Clostridium histolyticum*	pH 6.0–8.0/ 20–40 °C	collagen-rich tissues, fish skin, swim bladders, cartilage	[19,73]
Flavourzyme ®	Endo + Exo mix	*Aspergillus oryzae*	pH 4.0–8.0/ 50–55 °C	Bighead carp, salmon scales, tilapia skin, fish protein hydrolysates	[127,176–178]
Alkaline Protease	Endo (serine protease)	*Bacillus* species	pH 8.0–10.5/ 40–60 °C	Tilapia skin, squid skin, fish gelatin, grass carp scales, silver carp skin	[6,9,70,137]
Acid Protease	Endo (aspartic protease)	Fungal sources (*Aspergillus* spp.)	pH 2.0–4.0/ 45–55 °C	Squid skin, fish collagen, specialized marine proteins	[4,137]

8.4. Post-Treatment Techniques for Purification

SDJ contains high amounts of minerals, which must be removed to produce a peptide suitable for industry standards. Jellyfish also shows a strong, fishy odor and taste. The primary objective of post-treatment strategies is to purify marine collagen peptides by removing non-collagenous material, pigments, and off-flavors. In a study with skipjack tuna, the effect and mechanism of hydrogen peroxide treatment on the quality and fishy taste of surimi gel were evaluated. Results indicated a reduction in fishiness as well as an improvement in texture and color of the final product [179]. Previous studies on jellyfish collagen peptide powders have evaluated activated-carbon adsorption and dialysis as post-treatment approaches to improve physicochemical and sensory-related properties [123,140]. By removing minerals, carbohydrates, and other non-collagen-containing materials, the concentration of collagen peptides is expected to increase.

The mechanism of each post-treatment process differs. Hydrogen peroxide works through chemical reactions, while activated carbon operates through a combination of physical and chemical mechanisms, and dialysis is a purely physical process. Each process is designed to produce a purer peptide powder from SDJ through demineralization, deodorization, and/or bleaching.

8.4.1. Hydrogen Peroxide Treatment

Food-grade hydrogen peroxide (H_2O_2) has been used to bleach and improve the visual quality of catfish skin gelatin processing, as well as deodorize seafood matrices in skipjack surimi gels [179,180]. The mechanism of deodorization and bleaching is attributed to oxidation. H_2O_2 can oxidize tertiary amines to amine oxides, which has been observed to remove fishy odors in industrially processed surimi gels [180]. According to Code of Federal Regulations (CFR 184.1366), hydrogen peroxide can be used as a food ingredient in processing proteins with the purpose of oxidizing, reducing, and bleaching if residual hydrogen peroxide is removed by appropriate physical and chemical means [181]. To adhere to these regulations, food-grade catalase enzyme can be added to quench residual H_2O_2 [182]. Under these conditions, H_2O_2 can be used as a post-treatment step in the hydrolyzed peptide solution.

8.4.2. Adsorption with Activated Carbon

Activated carbon has been utilized as an adsorption agent to effectively remove off-flavors from marine collagen products [124,182]. The mechanism of adsorption seen in activated carbon is attributed to its large surface area and ability to adsorb small, hydrophobic volatiles responsible for off-flavors. For example, activated carbon at a concentration of 0.5% effectively reduced fishy odors in tiger puffer skin gelatin [182]. Off-flavor removal in marine peptides by activated carbon is attributed to hydrogen bonding, van der Waals forces, and dispersion interactions. However, there is potential for peptide loss due to adsorption efficiency [124,182]. Although the adsorption properties of activated carbon are expected to clarify and deodorize marine collagen peptides, the influence on porosity, structure, and yield of the final product is unknown [41].

8.4.3. Dialysis

Dialysis separates collagen peptides with specific molecular weights in solution through diffusion across a semipermeable membrane with a defined molecular weight cutoff (MWCO). Diffusion is driven by a concentration gradient, allowing small molecules, including minerals, acids, and volatiles, present in the product to move from an area of high concentration to an area of low concentration until equilibrium is established [183]. Dialysis tubes are placed in a chamber of liquid. Smaller molecules pass through the membrane pores into the surrounding liquid, leaving larger molecules (peptides) inside the tube [184]. Dialysis is an effective method for removing minerals and impurities while retaining low-molecular-weight collagen peptides [185].

8.4.4. Ultrafiltration

Ultrafiltration is a pressure-driven membrane-separation process in which solutes are fractionated according to membrane MWCO, hydrodynamic size, and solute-membrane interactions. Unlike dialysis, which relies primarily on diffusion down a concentration gradient, ultrafiltration uses transmembrane pressure to generate permeate and retentate streams and is therefore more compatible with continuous or scaled processing. For collagen hydrolysates, selected membranes can enrich or remove peptide fractions according to size while also reducing salts and other low-molecular-weight components, although membrane fouling and incomplete size selectivity can affect yield and composition [72].

8.5. Processing–Activity Tradeoffs

Processing steps that improve purity or sensory quality can also change peptide recovery and composition, but direct processing–antifreeze comparisons remain sparse. Enzyme choice and hydrolysis time demonstrably affect cleavage patterns and the molecular-weight distribution of hydrolysates; in the squid-skin and fish-gelatin studies, activity differed among hydrolysis/fractionation conditions [3,69]. Activated carbon can improve odor-related quality, but published jellyfish work has not yet quantified IRI or TH before and after adsorption, so loss or retention of ice-active peptides remains an evidence gap [123,182]. Likewise, dialysis and ultrafiltration can alter molecular-weight distributions, yet direct antifreeze-activity yields for jellyfish peptides have not been reported. Therefore, proposed tradeoffs involving acid pre-treatment, deodorization, and membrane purification should be treated as hypotheses until mass yield, peptide recovery, sequence distribution, and direct IRI/TH activity are measured before and after each unit operation. Future process comparisons should report both mass yield and antifreeze-activity yield rather than assuming that greater purity confers greater activity.

9. Food Applications: Evidence for Collagen-Derived Peptides and Context from Canonical AFPs

9.1. Comparative Evidence Across Food Matrices

Table 4 shows canonical AFP food applications. Table 5 presents evidence for collagen- and gelatin-derived peptides as antifreeze or cryoprotective agents. The strongest aquatic/marine food-matrix evidence includes squid-skin collagen hydrolysate in shrimp [3], tilapia skin collagen hydrolysate in scallop muscle [115], fish-gelatin peptides in ice-cream/sucrose model systems [69], and Atlantic-cod skin collagen peptide in frozen dough [186]. Non-marine collagen studies are included to provide useful comparative evidence [70,121].

Table 4. Summary of AFP/AFPT food applications.

Food Matrix/Product	Canonical AFP Source, Type & Concentration	Main Functional Effects Reported	References
Ice cream & frozen desserts	Winter wheat-grass ice-structuring proteins; cold-acclimated oat AFP	Reduced ice recrystallization/smaller crystals in ice-cream systems; effects are AFP-specific and are contextual rather than collagen peptide evidence	[187,188]
Frozen meat	Recombinant ice-binding protein from Arctic yeast; recombinant Type I AFP analog from *Lactococcus lactis*	Reduced oxidative/quality deterioration or improved frozen-meat quality in the cited systems	[189,190]
Frozen dough & bread	Recombinant Type I AFP analog from *Lactococcus lactis*; barley AFP	Improved frozen-dough/bread quality during freezing or freeze–thaw exposure	[190,191]
Frozen fruits & vegetables	Type I AFP from *Drimys angustifolia* leaves; *Tenebrio molitor* AFP	Reduced freeze–thaw injury and/or ice-related structural damage	[192,193]

AFPT = Antifreeze peptide.

9.2. Canonical Marine AFPs as Contextual Evidence for Food Applications

Canonical marine AFPs provide useful proof of concept that direct ice regulation can improve quality in complex frozen foods, but they are structurally and functionally distinct from collagen-derived peptide hydrolysates. Their application history is therefore discussed here only as contextual evidence for the feasibility of ice-active proteins in food matrices, not as direct evidence for MCP efficacy. Studies using fish AFPs have demonstrated control of ice crystal growth and quality preservation in frozen products; however, these outcomes should not be extrapolated quantitatively to collagen peptides without peptide-specific testing.

For collagen-derived systems, direct food-matrix evidence remains limited but increasingly quantitative. Squid-skin collagen hydrolysate (1%, *w*/*w*) was evaluated through repeated −25 °C/4 °C freeze–thaw cycles in shrimp, whereas tilapia skin collagen hydrolysate was evaluated at 0.5–3 g/100 g in scallop adductor muscle stored at −18 °C for eight weeks after snap freezing [3,115]. Fish-gelatin fractions provide direct model-system evidence that molecular size and cationic character influence ice crystal growth inhibition [69]. More recent Atlantic-cod skin collagen peptide work extends marine collagen evidence to frozen dough, where 1.5% peptide preserved gluten structure and reduced hardness after four weeks of frozen storage [186]. Canonical fish AFP studies remain useful mechanistic comparators, but they are not counted as collagen peptide evidence.

Table 5. Study-level evidence for collagen/gelatin-derived peptides as antifreeze or cryoprotective agents.

Species/Tissue	Material & Processing	Peptide Size/Sequence	Dose & Matrix	Freezing/Storage Conditions	Comparator	Quantitative/Measured Outcome	Interpretive Limitation
Squid (*Dosidicus gigas*) skin [marine]	Collagen hydrolysate; acid protease, 6000 U/g, 40 °C, 3 h; Sephadex G-25 fractionation	82.3% <5 kDa; 22.69% at 1–2 kDa; active sequence DVRGAEGSAGL	1% (*w*/*w*) CH-SS; shrimp (*Penaeus penicillatus*) muscle	Freeze–thaw cycles: −25 °C for 24 h, then 4 °C thawing; 2, 6, 10, and 14 cycles	Distilled-water control and 1% TSPP	Reduced myofibrillar denaturation/structural change, retained water-binding capacity, reduced ice crystal mechanical injury and protein oxidation; exact numerical values vary by endpoint	Food outcomes were measured for the hydrolysate, not purified DVRGAEGSAGL; multiple cryoprotective mechanisms may contribute [3]
Tilapia (*Oreochromis niloticus*) skin [aquatic/marine]	Collagen hydrolysate; Alcalase, 4000 U/g, 60 °C, 3 h; fractionation	<2.5 kDa hydrolysate; NHGK, 454.26 Da	0.5, 1, 2, and 3 g/100 g TSCP; scallop adductor muscle; purified NHGK assessed separately for TH	Snap frozen at −75 °C for 2 h; stored at −18 °C for 8 weeks	Untreated control and commercial phosphate cryoprotectant	Hydrolysate TH = 0.50 °C; NHGK TH = 2.63 °C; 3 g/100 g TSCP produced cryoprotection comparable to commercial phosphate and preserved salt-soluble protein, sulfhydryl content, Ca^{2+}-ATPase activity, and water-holding capacity	Purified NHGK TH and hydrolysate food performance are different endpoints and should not be conflated [115]
Carp fish skin [aquatic/marine gelatin]	20% (*w*/*w*) fish gelatin; Alcalase 0.176 Anson units/g gelatin, pH 9.0, 45 °C, 25 min; size-exclusion and ion-exchange fractionation	Cationic fraction contained prominent peptides of 1850.82 and 2036.88 Da	Up to 4% hydrolysate in ice-cream mix; sucrose model systems also evaluated	Controlled freezing/annealing ice-growth assays; samples cooled to −40 °C, then temperature increased to −14 °C, then 7 cycles between −14 and −12 °C.	Peptide-free composition-matched matrices; fraction comparisons	25 min hydrolysate showed maximal ice-growth inhibition; cationic fraction was most active	Activity depends on assay matrix, charge fraction, and concentration; carp fish species was not reported in the study [69]
Porcine skin collagen [non-marine comparative evidence]	Alkaline-protease hydrolysis; pH 8.0, 55 °C, 5% enzyme, DH 15%; column chromatography	GLLGPLGPRGLL (AP-3), 1162.8 Da	0.2% (*w*/*v*) purified AP-3; ice cream	Frozen ice-cream/recrystallization testing. Ice cream was frozen to −50 °C, then, the temperature was increased to −16 °C and cycled between −16 and −14 °C (7 cycles).	Ice-cream control and natural-AFP comparison	AP-3 TH = 5.28 °C; improved recrystallization and melt resistance; Tg increased to −17.64 °C	Non-marine source; DSC-derived TH is method-dependent and should not be compared directly with microscopy-based TH values [121]

Table 5. *Cont.*

Species/Tissue	Material & Processing	Peptide Size/Sequence	Dose & Matrix	Freezing/Storage Conditions	Comparator	Quantitative/Measured Outcome	Interpretive Limitation
Porcine skin collagen [non-marine comparative evidence]	Collagen hydrolysate produced with pepsin and alkaline protease; ice-affinity adsorption purification	GLLGPLGPRGLL, 1163.8 Da	0.3% (*w*/*v*) ice-structuring collagen peptide; frozen dough/steamed bread	Frozen at −40 °C for 2 h, then 4 weeks frozen storage at −20 °C	Untreated frozen-dough control	Purified peptide TH = 2.76 °C under the reported adsorption/purification conditions; significantly improved baking and textural characteristics after 4 weeks	Non-marine source; hardness and gumminess of steamed bread was improved after 4 weeks (compared to control) [70]
Atlantic cod skin collagen peptide [marine]	Commercial fish-skin collagen peptide, purity ≥ 90%	Mean MW ≈ 1 kDa	0–2.0% (*w*/*w*, flour basis); frozen dough and steamed bread; 1.5% highlighted	Dough was frozen at −70 °C for 1 h, and stored at −18 °C for 4 weeks	0% peptide control	At 1.5%: dough springiness +5.17%, hardness −30.74%, α-helix +5.29%, absolute zeta potential +35.94%, surface hydrophobicity −1.48%; TH reported at 0.62–1.24 °C depending on holding temperature	Recent marine food-matrix evidence; TH was measured under a separate thermal protocol and should not be treated as a direct causal measure of all dough-quality effects [186]

MW = molecular weight; TH = thermal hysteresis; TSCP = tilapia skin collagen hydrolysate; TSPP = Tetrasodium pyrophosphate.

10. Safety, Sensory Quality, Scalability, Cost, and Sustainability

Industrial feasibility depends on mass yield, activity yield, effective dose, and the number of unit operations required. Published collagen peptide studies demonstrate that hydrolysis and fractionation can enrich ice-active fractions [3,69,115,193], but comparable process-yield, cost, and life-cycle data are generally unavailable. Membrane fractionation and food-grade enzymatic hydrolysis are plausibly more scalable than repeated chromatography or laboratory dialysis, yet this is a process-engineering inference rather than demonstrated economic superiority for marine collagen peptides. Likewise, use of marine by-products may support resource valorization, but sustainability cannot be assumed from feedstock origin alone because sample conditioning, process water, transportation, enzyme use, membrane operation, and drying can contribute substantial environmental burdens. Techno-economic analysis and life-cycle assessment using source- and process-specific mass and energy balances are therefore required before industrial or sustainability advantages can be established.

Translation of marine collagen peptides into commercial frozen foods also requires source- and process-specific safety and sensory evidence. Potential considerations include marine allergens, environmental contaminants, high mineral loads in salted raw materials, residual processing aids, microbiological control, oxidation during deodorization, and lot-to-lot peptide variability. Marine hydrolysates may also be limited by bitterness, fishy volatiles, color, or salt at functionally effective doses. These issues are recognized from marine-protein and hydrolysate processing literature, but direct safety, sensory, and consumer-acceptance datasets for jellyfish-derived ice-active peptides remain limited. Accordingly, the present evidence supports these factors as required validation endpoints rather than as demonstrated advantages or disadvantages of the proposed ingredient.

11. Future Perspectives and Research Needs

AFP research has advanced substantially from initial discovery in polar organisms to demonstrated efficacy across biological and food systems. Fundamental AFP mechanisms are now well established, including IRI, TH, and ice crystal morphology modification, with IRI emerging as the most critical determinant of cryoprotective performance in food systems. Despite structural differences, AFPs converge functionally through flat, amphipathic ice-binding surfaces that irreversibly adsorb to ice crystals and suppress recrystallization.

While proof of concept for AFP use in frozen foods is well documented, large-scale adoption has been limited by cost, limited raw materials, and consumer resistance to recombinant or genetically modified sources. In this context, marine collagen-derived antifreeze peptides represent a compelling and underexploited alternative. Enzymatic hydrolysis of collagen-rich marine by-products yields low-molecular-weight peptides (approximately 0.5–3 kDa) enriched in Gly-Pro-X motifs and amphiphilic residues that exhibit AFP-like behavior. Importantly, marine AFPTs often provide dual functionality, combining ice recrystallization inhibition with antioxidant activity, therefore addressing both mechanical ice damage and oxidative degradation in frozen foods.

Despite these promising characteristics, several critical gaps remain. Quantitative sequence-function relationships for AFPTs are poorly defined, limiting rational optimization. AFP and AFPT performance are highly matrix-dependent, yet systematic studies in complex food systems are scarce. Safety, allergenicity, and regulatory evaluation of marine invertebrate-derived peptides remain incomplete. Additionally, long-term cold-chain validation under realistic storage and temperature abuse conditions is lacking.

Future research should focus on scalable AFPT production from abundant, sustainable marine sources, including jellyfish. Mechanistic studies in real food matrices, comprehensive safety assessments, and full cold-chain performance are necessary. Addressing

these needs may enable partial replacement of high-dose conventional cryoprotectants with natural, clean-label marine peptides, thereby improving frozen food quality, sustainability, and consumer health outcomes.

Edible jellyfish represents an underexplored marine collagen source with potential antifreeze functionality. However, systematic evaluations of how enzymatic hydrolysis conditions influence peptide composition, physicochemical properties, and ice-modulating behavior are lacking. In particular, there is a need to link processing parameters to functional outcomes such as ice recrystallization inhibition and rheological behavior in aqueous food systems.

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Abbreviations

The following abbreviations are used in this manuscript:

MCPs	Marine Collagen Peptides
CJ	Cannonball jellyfish
AFPs	Antifreeze proteins
IRI	Ice recrystallization inhibition
GI	Gastrointestinal
ACE	Angiotensin converting enzyme
UV	Ultraviolet radiation
DMSO	Dimethyl sulfoxide
EG	Ethylene glycol
PDB	Protein data bank
PG	Propylene glycol
IBS	Ice-binding surfaces
JCPP	Jellyfish collagen peptide powders
TH	Thermal hysteresis
pI	Isoelectric point
IP	Isoelectric precipitation

DSC	Differential scanning calorimetry
SDJ	Salted, dried jellyfish
MWCO	Molecular weight cutoff
AFPT	Antifreeze peptide
TSCP	Tilapia skin collagen hydrolysate
TSPP	Tetrasodium pyrophosphate

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