

## Unlocking the production, bioactivity properties, and potential applications of hydrolyzed collagen from different sources: a review

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### Abstract

Collagen plays a crucial role in maintaining the structure of different organs and tissues. In contrast, hydrolyzed collagen is a group of peptides with a low molecular weight obtained enzymatically. This review covers different methods applied for collagen extraction, the bioactivities possessed by collagen, and the potential application of hydrolyzed collagen in many sectors. Acid, alkali, and enzyme extractions are the standard methods used for collagen extraction. Still, the enzyme extraction method was the most efficient and preferred due to less waste and shortened processing time. Although advanced collagen extraction techniques, such as deep eutectic solvent extraction, supercritical fluid extraction, and microwave-assisted extraction, offer potential benefits, they currently face hurdles related to optimization, product stability, initial capital investment, and operational complexity. The hydrolyzed collagen provides good functional properties because of its bioactivities, such as antifreeze, antioxidant, antihypertensive, and anticancer properties. The applications of hydrolyzed collagen in the functional food, biomedical, and cosmetics industries are also discussed extensively. As far as the research is concerned, there still needs to be a review of various hydrolyzed collagen sources for their bioactive properties and potential applications. Therefore, the present review examines the production, bioactive properties, and potential application of hydrolyzed collagen from different sources.

*Keywords:* collagen; extraction method; hydrolyzed collagen; bioactive properties; collagen application

### Introduction

Collagen, the most prolific animal protein, plays a crucial role in the structural maintenance of different tissues and organs (Shaik *et al.*, 2023). In contrast, hydrolyzed collagen comprises low-molecular weight peptides (Seong *et al.*, 2024). Collagen is an essential structural protein

in extracellular matrix, and the term originates from the Greek word 'kola,' meaning 'glue making' (Senadheera *et al.*, 2020). It is a white and opaque protein absent from branched and fibrous chains. It is present in the skin, cartilage, bones, teeth, tendons, ligaments, and blood vessels of animals. Collagen is distinguished by repeated proline-rich tripeptides, such as glycine, proline, and

hydroxyproline or hydroxylysine, which are crucial in flexibility characteristics (Hamdan and Sarbon, 2019). Different collagen extraction methods are reported because this is a critical step in enhancing this protein's yield, purity, and structural integrity while saving time and lowering process costs.

Collagen extraction is done in three ways: acid extraction, alkali extraction, and enzyme extraction (Xu *et al.*, 2022). In acid extraction, several acids are utilized, including organic acids, such as acetic acid, chloroacetic acid, citric acid, and lactic acid, and inorganic acids, such as hydrochloric acid (Hadfi and Sarbon, 2019). Meanwhile, alkali extraction has a high hydrolysis capacity for collagen fibers (Hamdan and Sarbon, 2019). For enzyme extraction, pepsin, papain, and trypsin are common enzymes used to extract collagen protein, where large quantities of hydrolyzed collagen proteins are generated by hydrolysis (Schmidt *et al.*, 2016). In addition, sodium carbonate and magnesium oxide are generally utilized to recover collagen protein from leather waste (Yang and Shu, 2014).

Hydrolyzed collagen, or collagen hydrolysate or collagen peptides, is a form of collagen broken down into more readily soluble amino acids (Gee *et al.*, 2019). Hydrolyzed collagen can increase the food's functional activity, including water-holding capacity and solubility (Villamil *et al.*, 2017). For instance, hydrolyzed collagen is utilized in processed foods, such as sausages, to substitute for swine fat. As a result, the finished product exhibited a higher water-holding capacity, better after-cooking stability, and enhanced texture-like hardness and chewiness (Sousa *et al.*, 2017). Collagen hydrolysate exhibits exceptional techno-functional properties, including viscosity, emulsifying and foaming abilities, solubility, and water and oil absorption capacities, making it highly valuable for applications in the food and pharmaceutical industries (Tawalbeh *et al.*, 2025). Additionally, hydrolyzed collagen demonstrates excellent functional properties attributed to its bioactive characteristics.

Bioactive is an alternate term for biologically active substance (Ishak and Sarbon, 2018). A bioactive substance is most accurately described as one that seems to impact, trigger a reaction, or prompt response in live cells (Akbarian *et al.*, 2022). Studies have found that the bioactivity of hydrolyzed collagen possesses antifreeze, anticancer, antioxidant, and antihypertensive properties, antimicrobial, self-assembly, and cell-penetrating activities (Xu *et al.*, 2023). It is helpful in various biomedical applications, such as tissue regeneration, tissue engineering, wound healing, and drug delivery, including the design of nanostructures (Furtado *et al.*, 2022). In addition, antioxidants have been proposed as a possible option for treating and preventing illnesses caused

by active oxygen species, particularly cancer disorders (Hecht *et al.*, 2024).

Hydrolyzed collagen is applied in various sectors, including food, medicines, biomaterials, and cosmetics (Villamil *et al.*, 2017). Hydrolyzed collagen is used for food applications to treat mineral shortages (Hajj *et al.*, 2024). Marine collagen in the pharmaceutical industry is utilized to prevent bleeding because it has sponge-like structure, is very absorbent, and can store several times its weight in fluid (Salvatore *et al.*, 2020). Meanwhile, as hydrolyzed collagen has low molecular weight and amino acid composition in the biomaterial sector, creating a hydrolyzed collagen biomaterial could be helpful in treating bone and joint diseases. In addition, hydrolyzed collagen is employed as a moisturizer in the cosmetic sector because its moisture absorption and retention capability is superior to that of the control group (glycerol) (Li *et al.*, 2020).

Over the years, intensive research on the uses of collagen has been motivated by the great potential of collagen application. Collagen's significant sources of industrial exploitation are bovine and porcine, which have raised concerns recently (Senadheera *et al.*, 2020). Different sources of collagen are contested due to religious restrictions regarding the avoidance of porcine and bovine goods as well as the recent massive epidemic of bovine spongiform encephalopathy (BSE) (Liu *et al.*, 2022). In this regard, possible *halal* collagen sources, such as marine and poultry, are investigated to produce *halal* collagen products. Still a review is needed to discuss various hydrolyzed collagen sources on bioactive properties and potential applications. Therefore, the review aims to unlock hydrolyzed collagen's production, bioactive properties, and potential applications from different sources. To provide a comprehensive overview of this review, Figure 1 summarizes the interconnected aspects of hydrolyzed collagen, such as its sources, extraction methods, physicochemical and bioactive properties, and diverse applications across various industries.

## Collagen

Collagen is a structural protein found in connective tissues, including the skin, cartilage, tendons, and bones, accounting for 25–30% of all proteins in the body (Miranda *et al.*, 2021). Its molecular structure consists of three polypeptide chains twisted together to form a triple helix of around 300-nm length with a molecular weight of 105 kDa. These molecules can either be homomeric (with identical  $\alpha$  chains) or heteromeric (with genetically distinct  $\alpha$  chains). Each strand is first generated with left-handed symmetry before being confirmed as a right-handed triple helix (Senadheera *et al.*, 2020).

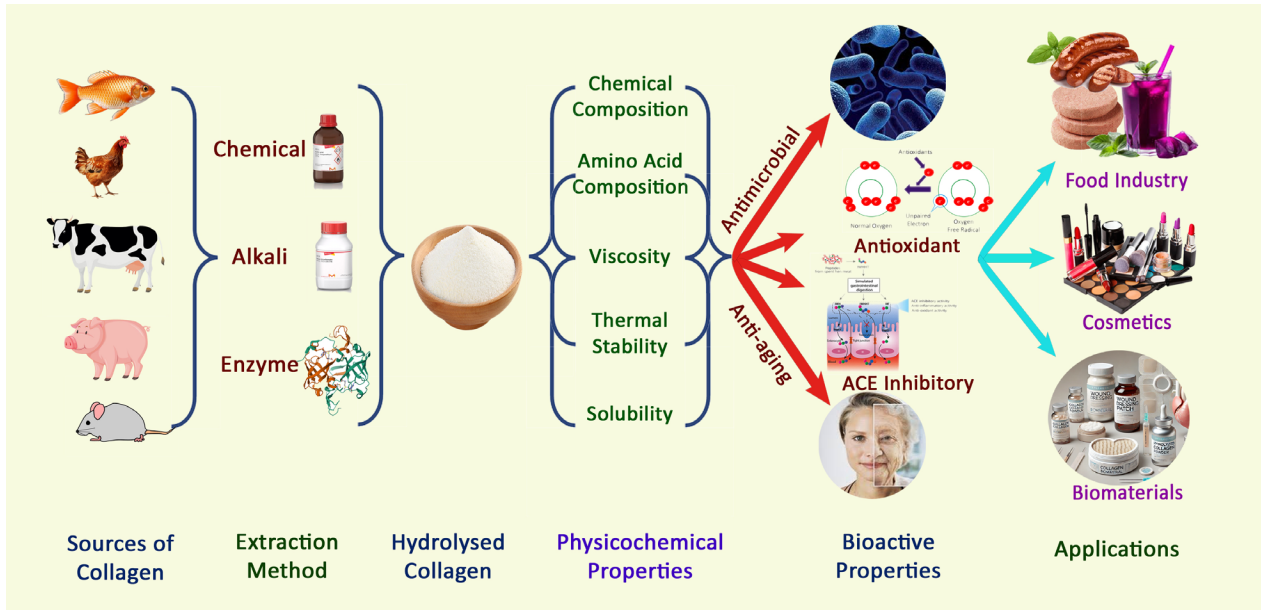


Figure 1. Overview of hydrolyzed collagen production, properties, and applications.

Each chain in the right-handed helical form comprises a glycine-X-Y repeating sequence, where X and Y are frequently referred to as proline or hydroxyproline (Islam and Mis Solval, 2025). All glycine residues are found in the core of this motif, whereas other amino acids (X and Y) are positioned on the surface (Senadheera *et al.*, 2020). Interchain N-H (Gly) O=C(x) hydrogen bonds and electrostatic interaction reinforce this stiff rod-like structure. A triple helix is the most distinguishing aspect of the collagen structure. On the other hand, the triple helix might vary depending on the type of collagen in the system (Fidler *et al.*, 2018). Additionally, this sequential homogeneity is uncommon in other proteins. Because of collagen’s uniformity, several investigations have examined its potential as a future biomaterial with many uses.

**Basic structure of collagen**

Collagen is a unique and essential structural protein composed of three intertwined  $\alpha$ -helical chains that form a triple helix (Figure 2). Each chain contains repeating sequences of three amino acids: glycine, proline, and hydroxyproline, which provide stability and strength (Shaik *et al.*, 2024b). Each collagen molecule has a molecular weight of approximately 360 kDa, a length of 28 nm, and comprises about 1,000 amino acids per chain (Schmidt *et al.*, 2016). To date, researchers have been identified 28 types of collagens (Type I– XXVIII), categorized based on their discovery (Hajj *et al.*, 2024).

Tropocollagen, a three-stranded polypeptide molecule, is the primary component of collagen. Owing to its wide

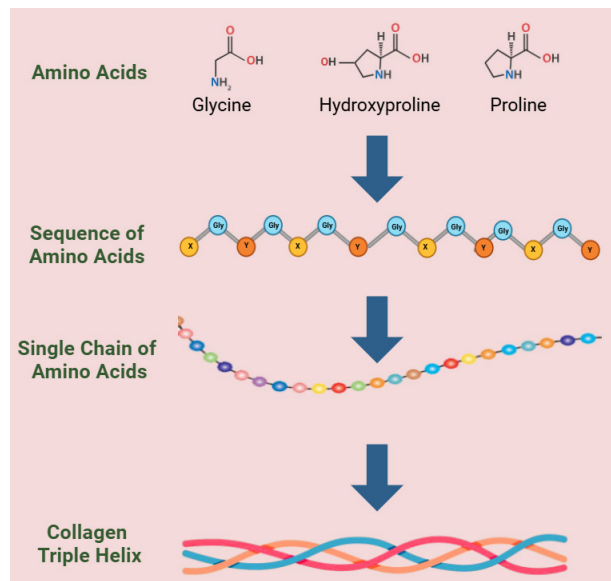


Figure 2. Structure of collagen.

structural variety, the collagen family is divided into several divisions (Wang *et al.*, 2014). Different helix lengths, including non-helical components, helix interruptions, alterations in the basic polypeptide chain assembly, and variations in helical domain terminations, contribute directly to many collagens. The basic collagen types include fibrillar collagen, short-chain collagen, beaded filament collagen, fibril associated collagens with interrupted triple helices (FACIT), FACIT-like collagen, basement membrane collagens, transmembrane collagens, and unclassified collagens (Gordon and Hahn, 2010).

The helix length and fraction of non-helical components vary with collagen type.

Numerous studies have revealed that type I collagen is the most frequent form found in the fibrillar group (Amirrah *et al.*, 2022). The fibril-producing collagen, known as fibrillar collagen, is produced by the fibrillogenic process as soluble precursor molecules (procollagen). Each polypeptide chain comprising N- and C-propeptides at the end of the triple helix contributes to the process of synthesis (Senadheera *et al.*, 2020). The fibrils that develop have a noticeable banding caused by the collagen aggregating pattern. The stability of fibrillar collagen is based on nonreducible covalent cross-links within the triple helix (Senadheera *et al.*, 2020).

### Sources of collagen

Collagen contributes about 30% of the body protein because most animals' primary structural proteins are in the bones and skin (Pal and Suresh, 2016). Therefore, collagen could be acquired from natural and synthetic sources (Rezvani Ghomi *et al.*, 2021). The by-products obtained from slaughterhouses, such as bones, tendons, hides, cartilages, and recombinant collagen, are most often used as raw materials for synthesis of collagen. In industrial-scale processing, bovines and pigs are significant sources of collagen (Amyoony *et al.*, 2023). According to Introspective Market Research (2023), the global production of collagen in 2023 was reported to be 1,060 metric tons and is expected to reach 1,820 metric tons by 2032. Similarly, Grand View Research (2023) estimated the global collagen market at USD9.76 billion. It is projected to expand at a compound annual growth rate (CAGR) of 9.6% between 2024 and 2030. Moreover, with the increasing demand, it is essential to find more sustainable and cost-effective sources of collagen (Gaikwad and Kim, 2024). However, development of prion diseases, such as BSE has posed numerous challenges in using bovine collagen. On the other hand, swine flu has restricted the utilization of pig collagen (Senadheera *et al.*, 2020).

#### Marine species

Marine collagen is a practical alternative for bovine and porcine collagens because of the widespread occurrence of foot-and-mouth disease (FMD), avian influenza-like diseases, and BSE as well as various religious and societal restrictions (Dave *et al.*, 2019). In addition, marine collagen is preferred above other alternative sources in many industrial applications because of its unique biomaterial characteristic with exceptional biocompatibility and biodegradability (Shahidi *et al.*, 2019). Specifically, marine by-products have been employed in several bioprocessing methods to extract collagen and other collagen-related polymers (Dave and Routray, 2018).

Compared to collagen extraction from land-origin species, collagen extraction from marine is far less complex and time-consuming as well as affordable (Rajabimashhadi *et al.*, 2023). According to the available data, collagen recovered from marine vertebrates is primarily type I collagen (Table 1). Type I collagen is obtained from most species' skins, tendons, bones, and muscles. Meanwhile, type II collagen is obtained from fish cartilages (Benjakul *et al.*, 2012). According to Table 1, collagens are mainly isolated from skin tissues of most species because of increased concentration of collagen. Numerous marine species have been studied for collagen extraction, such as sea bass (Kim *et al.*, 2012), lizard fish, yellowback seabream (Minh-Thuy *et al.*, 2014), big-eye tuna (*Thunnus obesus*) (Ahmed *et al.*, 2019), fringe scale sardinella (Hamdan and Sarbon, 2019), clown featherback (Petcharat *et al.*, 2020), cuttlefish (*Sepia pharaonic*) (Hazeena *et al.*, 2022), barracuda (*Sphyraena sp.*) (Matarsim *et al.*, 2023), parrotfish (*Scarus sordidus*) (Jaziri *et al.*, 2023), blue shark (*Prionace glauca*) (Pan *et al.*, 2023). Research on using collagen from *Ameiurus nebulosus* skins in mayonnaise (Wang *et al.*, 2013) and the characterization of marine eel-fish collagen (Veeruraj *et al.*, 2015) are reported. Collagen from the skin of catfish (*Heteropneustus teropfossilis*), mullet fish (*Mugil cephalus*), and Indian salmon (*Eleutheronema tetradactylum*) are researched as well for its potential use as an emulsifier and foam stabilization agent in food processing (Kumar *et al.*, 2019).

#### Land mammals

Land mammals, such as bovine, porcine, goats, elephants, and monkeys, are good sources of collagen. Generally, type I collagen is extracted from the bones and skins of bovine and porcine (Hamdan and Sarbon, 2019). Bovine and porcine are essential sources of collagen for the industrial sector (Hadfi and Sarbon, 2019). Bovine collagen is the most common source of synthetic collagen used in beauty, pharmaceutical, and non-biomedical products (Rizk and Mostafa, 2016). Apart from the possibility of BSE, roughly 3% of the population is intolerant to bovine collagen. This intolerance is primarily due to allergic or hypersensitive reactions and digestive discomfort, resulting in its limited usage (Silvipriya *et al.*, 2015). Porcine collagen is extracted from pig skin and bones (Silvipriya *et al.*, 2015). Pig rind is well known for its application in manufacturing culinary items, such as edible films and sausage casings.

Furthermore, porcine collagen is frequently used in dermal replacement treatment, particularly for reconstructive surgery implants (Salvatore *et al.*, 2023). Pig skin-extracted type-I collagen has properties of human collagen and has various applications in the medical and culinary sectors (Maione-Silva *et al.*, 2019). However, collagen derived from porcine and bovine is associated with allergic reactions

Table 1. Collagen type and total collagen content based on different sources.

| Sources of collagen | Species  | Body part             | Total collagen content (%) | Type of collagen | References                |                      |
|---------------------|--|-----------------------|----------------------------|------------------|---------------------------|----------------------|
| Marine              | Blue Shark<br>( <i>Prionace glauca</i> )         | Cartilage             | –                          | Type II          | Pan et al., 2023          |                      |
|                     | Cuttlefish<br>( <i>Sepia pharaonis</i> )         | Skin                  | 8.79                       | Type I           | Hazeena et al., 2022      |                      |
|                     | Barracuda<br>( <i>Sphyraena sp.</i> )            | Skin                  | 10.06                      | Type I           | Matarsim et al., 2023     |                      |
|                     | Rainbow trout<br>( <i>Oncorhynchus mykiss</i> )  | Skin                  | 23.84                      | Type I           | Heidari and Rezaei, 2022  |                      |
|                     | Sponge<br>( <i>Aplysina fulva</i> )              | Body                  | 43.9                       | Type I           | Batista et al., 2022      |                      |
|                     | Lizardfish<br>( <i>Synodus macrops</i> )         | Scales                | 4.7                        | Type I           | Chen et al., 2021         |                      |
|                     | Clown featherback<br>( <i>Chitala ornata</i> )   | Skin                  | 80.81                      | Type I           | Petcharat et al., 2020    |                      |
|                     | Shortfin scad                                    | Bones and skin        | 3.45                       | Type I           | Sulaiman and Sarbon, 2020 |                      |
|                     | Jellyfish<br>( <i>Rhopilema esculentum</i> )     | Filament              | 4.31                       | Type I           | Ferrario et al., 2020     |                      |
|                     |  | Mesoglea              | 0.40                       |                  | Cheng et al., 2017        |                      |
|                     | Sea cucumber<br>( <i>Holothuria cinerans</i> )   | Body wall             | 72.20                      | Type I           | Li et al., 2020           |                      |
|                     | Golden pompano<br>( <i>Trachinotus blochii</i> ) | Skin                  | 21.81                      | Type I           | Cao et al., 2019          |                      |
|                     |  | Bone                  | 1.25                       |                  |                           |                      |
|                     | Starfish ( <i>Acanthaster planci</i> )           | Body wall             | 2.26                       | Type I           | Wijanarko et al., 2017    |                      |
|                     | Squid<br>( <i>Kondakovia longimana</i> )         | Muscle                | 1.05                       | Type I           | Coelho et al., 2017       |                      |
|                     |  | Skin                  | 2.12                       |                  |                           |                      |
| Land mammals        | Goat   | Shoulder              | 2.49                       | Type I           | LaRoche et al., 2022      |                      |
|                     |  | Breast                | 3.06                       |                  |                           |                      |
|                     |  | Leg                   | 1.78                       |                  |                           |                      |
|                     | Bull   | Hides                 |                            | 109.95*          | Type I                    | Noorzai et al., 2020 |
|                     |  |                       |                            | 110.92           |                           |                      |
|                     | Cow  |                       | 105.83                     |                  |                           |                      |
|                     | Lamb   | Slaughter by-products | 12.50                      | Type I           | Vidal et al., 2020        |                      |
|                     | Sheep  |                       | 18.00                      |                  |                           |                      |
| Poultry             | Spent-hens                                       | Bones                 | 9.63                       | Type II          | Cao et al., 2023a         |                      |
|                     | Broiler chicken                                  | Stomach               | 9.8                        | Type I           | Prokopová et al., 2022    |                      |
|                     |  | Sternal cartilage     | 20.59                      | Type II          |                           |                      |
|                     | Chicken  | Feet                  | 32.16                      | Type I           | Dhakal et al., 2018       |                      |
|                     |  | Feet                  | 42.67                      | Type I           | Mokhtar et al., 2017      |                      |
|                     |  | Lungs                 | 31.25                      | Type II          | Zou et al., 2020          |                      |
|                     | Quail  | Feet                  | Not evaluated              | Type I           | Yousefi et al., 2017      |                      |
|                     | Duck   | Feet                  | Not evaluated              | Type I and II    | Kim et al., 2016          |                      |

\*The collagen content is given as a percentage of either a wet or dry basis.

and various diseases, including BSE, FMD, scrapie in ovine and caprine, and other zoonotic infections.

### Poultry

Compared to the collagen derived from bovine and porcine sources, poultry collagen enjoys broader consumer

acceptance across various belief systems (Jayaprakash et al., 2024). Collagen can be extracted from different poultry species, such as chickens, ducks, turkeys, and geese. It is claimed that collagen can be derived from poultry's by-products as well, such as bones, skin, and cartilage. Furthermore, for Muslims, chicken is not

subjected to any religious restrictions, except that it must be slaughtered in a *halal* manner (Goyal *et al.*, 2013). However, due to the emergence of avian influenza, use of poultry as a collagen source has been restricted (Maione-Silva *et al.*, 2019).

For example, chicken sternal cartilage is used to extract type II collagen (Akram and Zhang, 2020). In contrast, spent-hen's feet (Cao *et al.*, 2023a), broiler chicken's stomach (Prokopová *et al.*, 2022), chicken's sternal cartilage (Akram and Zhang, 2020), quail's feet (Yousefi *et al.*, 2017), duck's feet (Kim *et al.*, 2016), and chicken's skin and bones (Munasinghe *et al.*, 2015) are used to extract type I collagen. Chicken foot collagen is used to produce jelly drinks, which is a well-received product by consumers (Almeida *et al.*, 2012). Compared to calfskin and pig skin collagen, type I collagen produced from quail feet appears to have more significant structural and thermal stability. As a result, it could be used as an alternative to mammalian collagen in biomaterials, functional foods, cosmetics, and medicines (Yousefi *et al.*, 2017). However, mammalian collagen is favored over avian collagen for industrial applications. Expensive and complex extraction procedure correlates with the restricted use of avian collagen (Senadheera *et al.*, 2020).

## Collagen extraction

### Pretreatment

Collagen preparation methods involve a series of crucial steps, such as pretreatment, extraction, separation, purification, and characterization. The cross-linked collagen in animal connective tissue degrades slowly, even in hot water, due to its inherent properties. Hence, a mild chemical treatment is indispensable to disrupt these cross-links before extraction (Schmidt *et al.*, 2016). This step, using low-concentrated acids and bases, is vital, as it partially hydrolyzes collagen, preserving collagen chains while breaking cross-links (Prestes, 2015). The removal of covalent intra- and intermolecular cross-links is a key aspect of collagen extraction (Schmidt *et al.*, 2016). The extraction of collagen involves pretreatment of raw material, with the preparation method varying according to the type of raw material.

In collagen extraction techniques, acidic and alkaline pretreatments are commonly utilized (Soroushanna *et al.*, 2019). Since collagen has a cross-linked structure, pretreatment is typically performed before extraction. This allows the collagen to be extracted with fewer cross-linking points (Schmidt *et al.*, 2016). In contrast, the primary solution in alkaline pretreatment eliminates non-collagenous proteins, lipids, colors, calcium, and other inorganic substances. Removing non-collagenous components from alkaline solutions depends on

various factors, such as temperature (Silva *et al.*, 2014). According to Schmidt *et al.* (2016), NaOH at a concentration of 0.05–0.10 M is acceptable for pretreatment. Moreover, temperatures ranging from 4°C to 20°C and the same concentration range inhibit acid-soluble collagen and cause structural alterations. While at 15°C and 20°C, 0.5 M NaOH produces structural changes, whereas 0.2 M and 0.5 M NaOH induce loss of acid-soluble collagen. Additionally, the alkaline method treats thick and hard raw materials for intense penetration to dissolve inter- and intramolecular cross-links of collagens (Schmidt *et al.*, 2016).

Demineralization is eliminating mineral salts from basic materials, such as calcium. This technique is frequently required for raw materials with high mineral content, such as the bones, cartilages, and scales, to improve the efficacy and purity of extracted collagen (Jafari *et al.*, 2020). In addition, the demineralized raw material has a porous structure with a larger surface area, making the collagen extraction process easier (Silva *et al.*, 2014). Researchers investigated ethylene-diamine tetraacetic acid (EDTA), hydrochloric acid (HCl), formic acid (CH<sub>2</sub>O<sub>2</sub>), and a combination of EDTA and HCl as demineralization agents (Pang *et al.*, 2021). HCl is the most effective demineralization agent, as it rapidly removes a significant portion of mineral content while preserving collagen structure. EDTA is identical to HCl in mineral removal and preservation of collagen integrity. However, treating with EDTA is a lengthy process (Park *et al.*, 2017). Based on surface data, CH<sub>2</sub>O<sub>2</sub> worked best but failed to demineralize the inside portion of a sample (Hamed *et al.*, 2012).

Defatting is a method of removing fat from raw materials with a high fat content. This can be done by immersing raw material in alcohol solution. To acquire high-purity collagen, defatting must be carried out. Three techniques for defatting of raw material were tested using NaHCO<sub>3</sub>, lipolytic enzyme, and 10 different solvent systems (Mrázek *et al.*, 2018). Defatting with NaHCO<sub>3</sub> solution yields raw material's leftover fat content, making it ineffective for future usage. The yield of residual fat content using enzyme-based approach grew significantly, reaching a relatively high value compared to the raw material's initial fat content (Mrázek *et al.*, 2018). The amount of enzyme used and the processing duration had no discernible influence on process efficiency (Nilsuwan *et al.*, 2021). The most efficient technique for defatting of the material is using a combination of ethanol and petroleum ether (the mixture was swapped for three times). The residual fat content was roughly 5%. The final product reported below is particularly collagen-rich (Mrázek *et al.*, 2018). Additionally, different raw material–solvent ratios (usually 1:10) and treatment periods were used to remove fat content.

### Collagen extraction process

#### Chemical extraction

There are two types of chemical hydrolysis of collagen: acid hydrolysis and alkali hydrolysis. Acidic solutions are used commonly, where organic and inorganic acids dissolve connections between collagen molecules, facilitating the extraction of collagen fibril. In addition, collagen molecules acquire a positive charge in acidic conditions (Yang and Shu, 2014), thus aiding their solubility by causing repulsion between tropocollagen molecules (Pal and Suresh, 2016). To isolate collagen, organic acids, such as acetic acid, citric acid, lactic acid, and chloroacetic acid, as well as inorganic acids, such as hydrochloric acid, are employed (Hukmi and Sarbon, 2018). Organic acids are generally more effective than inorganic acids in breaking the cross-links of collagen molecules, thereby improving collagen extraction. However, acetic acid is widely used due to its ability to alter collagen electrostatic properties, thereby enhancing its solubility and extractability (Islam and Mis Solval, 2025).

In general, 0.5 M acetic acid is often used for acid hydrolysis, and the reaction mixture is continually agitated

for 24–72 h (Ran and Wang, 2014). Then, the mixture undergoes sequential filtering, precipitation with NaCl, and centrifugation to obtain crude collagen powder. The filtrate should subsequently dissolve in 0.5 M acetic acid, and after 2 days of dialysis in 0.1 M acetic acid, it is distilled for another 2 days (Devita *et al.*, 2021). Similar findings were reported where the yield of extracted collagen from shortfin scad bone using 0.7 M acetic acid was higher (1.31%) than 0.5 M acetic acid (1.01%). These findings could be attributed to a more vital electrostatic repulsive force between single nominal charged groups in collagen extracted with 0.7 M acetic acid, compared to 0.5 M acetic acid (Baderi and Sarbon, 2019). Some extraction parameters differ depending on the type of raw material, as summarized in Table 2.

Moreover, citric acid and lactic acid have shown promising collagen yields, particularly if used in combination with enzymatic treatments; 0.5 M lactic acid and citric acid are used to extract 90% and 60% of collagen, respectively, from the skin of Baltic cod treated at 4°C for 48–72 h (Skierka and Sadowska, 2007). However, the yield dropped significantly to 18% with 0.15 M HCl under similar conditions, resulting in the lower efficiency

**Table 2.** Studies related to different extraction methods with various parameters.

| Extraction methods                        | Source   | Body part   | Hydrolysis parameter                                  | Extraction solvent                       | Yield (%)                    | References                  |
|---|--|---|---|--|------------------------------|-----------------------------|
| Chemical method                           | Cobia ( <i>Rachycentron canadum</i> )                  | Skin  | Temperature: 4°C<br>Period: 24 h<br>Ratio (w/v): 1:10 | 0.5M lactic acid                         | 36.70                        | Sukeri <i>et al.</i> , 2021 |
|   |  |   |   | 1.0M lactic acid                         | 22.23                        |                             |
|   | Shortfin scad ( <i>Decapterus macrosoma</i> )          | Bone  | Temperature: 4°C<br>Period: 24 h<br>Ratio (w/v): 1:5  | 0.5M acetic acid                         | 1.01                         | Baderi and Sarbon, 2019     |
|   |  |   |   | 0.7M acetic acid                         | 1.31                         |                             |
|   | Fringescale sardinella ( <i>Sardinella fimbriata</i> ) | Waste material  | Temperature: 4°C<br>Period: 24 h<br>Ratio (w/v): 1:2  | 0.5M acetic acid                         | 7.48                         | Hamdan and Sarbon, 2019     |
| Medusa fish ( <i>Centrolophus niger</i> ) | Skin   | Temperature: 4°C<br>Period: 72 h<br>Ratio (w/v): 1:25 | 0.5M lactic acid                                      | 45.00                                    | Bhumbar <i>et al.</i> , 2019 |                             |
|   |  |   | 0.5M formic acid                                      | 32.00                                    |                              |                             |
|   |  |   | 0.5M tartaric acid                                    | 31.00                                    |                              |                             |
|   |  |   | 0.5M citric acid                                      | 31.00                                    |                              |                             |
|   |  |   | 0.5M acetic acid                                      | 25.00                                    |                              |                             |
| Enzymatic method                          | Shortfin scad ( <i>Decapterus macrosoma</i> )          | Waste material  | Temperature: 4°C<br>Period: 30 h                      | 0.5 M acetic acid with 1.5% (w/w) pepsin | 0.10                         | Sulaiman and Sarbon, 2020   |
|   | Sharp nose stingray ( <i>Dasyatis zugei</i> )          | Skin  | Temperature: 4°C<br>Period: 30 h<br>Ratio (w/v): 1:40 | 0.5 M acetic acid with 1.5% (w/w) pepsin | 34.84                        | Ong <i>et al.</i> , 2020    |
|   | Golden carp ( <i>Probarbus jullieni</i> )              | Skin  | Temperature: 4°C<br>Period: 48 h<br>Ratio (w/v): 1:15 | 0.5 M acetic acid with 1.0% (w/w) pepsin | 79.29                        | Ali <i>et al.</i> , 2018    |
|   | Miiuy croaker ( <i>Miichthys miiuy</i> )               | Scales  | Temperature: 4°C<br>Period: 48 h<br>Ratio (w/v): 1:15 | 0.5 M acetic acid with 1.0% (w/w) pepsin | 3.87                         | Li <i>et al.</i> , 2018     |

of HCl for collagen extraction. Similarly, collagen isolated from the skin of black ruff fish achieved a yield of 45% with 0.5 M lactic acid and 31% with 0.5 M citric acid, whereas treatment with HCl resulted in negligible extraction yield, attributed to its limited efficiency (Bhumbar *et al.*, 2019). A recent study conducted by Hasanuddin *et al.* (2024) reported that acid-soluble collagen extracted from the bones and fins of purple-spotted bigeye exhibited the highest yield (1.93%) with 0.5 M citric acid, followed by a yield of 1.43% with 0.5 M lactic acid. These findings light-tight the effectiveness of citric acid and lactic acid as viable alternatives for collagen extraction compared to HCl.

Alkali hydrolysis uses robust alkali solutions to dissolve and break down collagen (Pal and Suresh, 2016). Commonly used alkalis include sodium hydroxide, potassium hydroxide, and extractants such as calcium hydroxide, calcium oxide, and sodium carbonate. Additionally, alkali has a high potential for hydrolysis. It may hydrolyze proteins by acting on collagen fibrils, although under severe extraction conditions, amino acids, such as cysteine, histidine, serine, and threonine, may be destroyed (Wu *et al.*, 2017). The problem with chemical collagen hydrolysis is that increasing acid or alkaline conditions to lower molecular weight often leads to a higher yield of free amino acids. Furthermore, alkali and alkaline processes are very corrosive to equipment, resulting in a high salt concentration in the final product upon neutralization (Hong *et al.*, 2019).

#### Enzyme method

Enzymes are a relatively ideal technique for extracting collagen proteins. This is because enzymes are more selective in their reactions and less damaging to amino acid residues. Various proteolytic enzymes are utilized in enzymatic extraction process, sourced from animals (pepsin and trypsin), plants (papain, bromelain, and ficin), or commercially available alternatives (Alcalase, proteinase K, collagenase, Nutrase, Flavoenzyme, and Protamex). Pepsin from animal sources is the most used enzyme (Pal and Suresh, 2017).

Pepsin is the most often employed enzyme for collagen (gelatin) extraction because it cleaves collagen telopeptides containing most intermolecular cross-links. This cleavage allows collagen to be digested more readily into low-molecular weight peptides (Hong *et al.*, 2019). Furthermore, because pepsin removes antigenic telopeptide region, pepsin digestion may diminish antigenicity. Finally, pepsin proved the most effective enzyme for preserving collagen's Gly-X-Y repeating structure (Yu *et al.*, 2018). Compared to heat extraction alone, pepsin enhanced gelatin production from bovine lung tissue by approximately nine times (Roy *et al.*, 2017).

#### Other methods

Heating is another method utilized for collagen extraction. By disrupting collagen's hydrophobic interactions and hydrogen bonds, heat increases the kinetic energy of molecules, causing vibrations that break the bonds holding collagen structure together (Hong *et al.*, 2019). Collagen has traditionally been extracted using heat. Common enzymes, such as trypsin, collagenase, proteinase K, and thermolysin, enhanced hydrolysis when bovine collagen was boiled for 5 min (Zhang *et al.*, 2013). However, heating is less effective for extracting collagen from strongly cross-linked animal tissues, such as wasted hen skin. Pyrrole and pyridinoline, covalently attached to collagen molecules, were discovered to be the cross-links (Hong *et al.*, 2019).

Another method used to extract collagen is ultrasonication. Developed as an alternative to traditional processes, ultrasonication aims to reduce processing time and increase extraction yield (Kim *et al.*, 2012; Shaik *et al.*, 2021a). Ultrasound operates at a high frequency (20 kHz), beyond human hearing capabilities (16 kHz), using sound wave energy for mass transfer in a wet process (Schmidt *et al.*, 2016; Shaik *et al.*, 2021b). A study considered the effect of the combinational approach of pepsin with ultrasound-assisted extraction for bovine tendon-derived collagen and discovered that the extraction efficiency was higher with improved collagen quality (Ran and Wang, 2014). Another study found that the combination might boost yield while decreasing the time required (Li *et al.*, 2018). As a recent approach, ultrasound-assisted extraction offers advantages over traditional extraction methods, including the absence of complex procedures, being environment-friendly, safe to use, quick processing time, and economically feasible.

#### Advanced methods

While traditional and conventional techniques for collagen extraction have been established for a long, recent research highlights the emergence of several advanced methods for obtaining collagen from diverse sources. These innovative approaches, including deep eutectic solvent (DES) extraction, microwave-assisted extraction (MAE), mechanical agitation, and supercritical fluid extraction (SFE), are garnering significant attention (Kiyak *et al.*, 2024).

Deep eutectic solvents (DESs) are particularly noteworthy as sustainable and environmentally benign alternatives to conventional organic solvents for collagen extraction from natural sources (Farooq *et al.*, 2024). DESs are complex ionic liquids formed by combining a hydrogen bond acceptor, such as quaternary ammonium salt, with a hydrogen bond donor, such as carboxylic acid or an amine (He *et al.*, 2024). These solvents have

demonstrated efficacy in extracting a broad spectrum of bioactive compounds from natural materials due to their low toxicity, biodegradability, and ability to solubilize various compounds. Notably, DESs can minimize the risk of collagen denaturation and enhance the quality of the extracted collagen. For instance, a study conducted by Bisht *et al.* (2021) reported obtaining higher yields of type I collagen from Atlantic codfish (*Gadus morhua*) using DESs with different combinations (urea–lactic acid and urea–propionic acid), compared to traditional acetic acid extraction. Silva *et al.* (2024) reported high-quality collagen extracted from codfish (*Gadus morhua*) skin using DESs with urea–propanoic acid mixture (in 1:2 ratio). Similarly, 90% effective collagen was extracted from cod skin by using a DESs composed of comprising choline chloride and oxalic acid (Bai *et al.*, 2017).

Supercritical fluid extraction (SFE) has gained widespread usage due to its advantages, such as increased extraction yields, enhanced selectivity, improved fractionation capabilities, and environment-friendly operation (Barzkar *et al.*, 2023). This method selectively separates bioactive components using a supercritical fluid as an extracting solvent. Carbon dioxide (CO<sub>2</sub>) is the widely used supercritical fluid in SFE due to its exceptional properties, such as low toxicity, chemical stability, high availability, cost-effectiveness, non-flammability, environment acceptability, and less operating conditions (typically near its critical point) (Gutierrez-Canul *et al.*, 2025). A recent study conducted by Sousa *et al.* (2020) demonstrated the efficacy of SFE using CO<sub>2</sub> to extract collagen from Atlantic cod (*Gadus morhua*) skins, achieving a high yield of 13.8%.

Microwave-assisted extraction (MAE) uses electromagnetic waves to disrupt efficiently the cellular structures of biological materials, thereby boosting the extraction of target compounds (Fauziyah *et al.*, 2023; Islam and Mis Solval, 2025). This technique allows microwave radiation to penetrate deeply into protein matrices, weakening cell walls, and facilitating the release of desired molecules (Zin *et al.*, 2025). The interaction of microwaves with polar substances generates heat through molecular friction, effectively using microwaves as an internal heat source (Feng *et al.*, 2022). Notably, MAE has been shown to enhance collagen yield from European plaice (*Pleuronectes platessa*) byproducts such as the skin, head, and backbone, particularly when combined with pre-treatments, such as enzyme hydrolysis and salt-washing (Kendler *et al.*, 2023). Furthermore, Jin *et al.* (2019) demonstrated that MAE hydrolysis of collagen from sea cucumber (*Acaudina molpadioides*) yielded extracts with significant bioactivities, including antioxidant properties.

Advanced collagen extraction methods are established for their potential to enhance yield kinetics, minimize

protein denaturation, preserve native bioactivity, increase product purity, and offer more environment-sustainable approaches. However, realizing the full potential of these methods necessitates comprehensive studies to address challenges related to process optimization and the stability of collagen during extraction from diverse biological sources. Furthermore, the considerable initial capital investment required for specialized equipment capable of maintaining specific operating parameters, coupled with increased operational complexity, warrants careful consideration by researchers.

## Hydrolysis of collagen

### Enzymatic hydrolysis

Using enzymes to extract collagen is a common practice and is recognized as one of the most practical biological approaches for commercial use (Schmidt *et al.*, 2016). The enzymatic extraction technique was developed to enhance collagen yield due to its high reaction selectivity and lesser negative impact on collagen's molecular structure (Yang and Shu, 2014). Furthermore, enzymatic hydrolysis is more efficient than chemical hydrolysis, as it has more advantageous features due to the milder process conditions (pH 6.0–8.0 and temperature 40–60°C) (Nasri, 2017). Despite its higher cost, enzymatic hydrolysis offers several advantages over chemical hydrolysis, including high specificity, moderate reaction conditions, controlled degree of hydrolysis, lowest salt content in final hydrolysate, reduced waste output, and a higher collagen yield (Senadheera *et al.*, 2020).

Over the past 60 years, numerous researchers have identified and documented enzymatic proteolysis from plant and animal sources. This approach is widely used to add value to underutilized species. Common commercial enzymes used for preparing collagen hydrolysates include Papain, alcalase,  $\alpha$ -chymotrypsin, Neutrase, pepsin, flavourzyme, trypsin, collagenase, and bromelain (Ong *et al.*, 2021). Pepsin from animal sources is the most commonly utilized enzyme (Pal and Suresh, 2017). Pepsin can cleave the non-helix peptide chain of collagen protein at the three-quarters position of N-terminus, leaving intact the helix peptide chains of collagen (Yang and Shu, 2014). However, compared to single-enzyme hydrolysis, multi-enzyme hydrolysis yielded more molecular weight in collagen peptides.

### Chemical hydrolysis

Protein hydrolysis in an acidic environment is a reliable method for obtaining peptides and amino acids. Organic acids, such as acetic acid, lactic acid, citric acid, formic acid, and inorganic acids, including hydrochloric acid (HCl), can be used for acid hydrolysis (Schmidt *et al.*, 2016). For example, HCl hydrolysis is often used to

determine the amino acid content of proteins. However, it has the disadvantage of complete or partial destruction of tryptophan, tyrosine, serine, and threonine in extreme circumstances, such as 6.0 M concentration at 110°C for 20 h (Hong *et al.*, 2019). Formic acid is preferred for collagen hydrolysis because it enters collagen molecules more quickly, is less corrosive to equipment than HCl or sulfuric acid, and cleaves Asp residues commonly found on cross-linked telopeptides. In addition, formic acid also kills viruses and germs in raw materials and may be separated for reuse. Finally, no inorganic salts are added to the hydrolysate (Hong *et al.*, 2018).

Alkaline hydrolysis is employed to break down various proteins to determine their amino acid contents, especially tryptophan, which is eliminated by acid hydrolysis (Senadheera *et al.*, 2020). Researchers have developed a rapid and sensitive method for quantifying collagen using alkaline hydrolysis, with a NaOH concentration of 7 mol/L used as a standard (Hong *et al.*, 2019). However, a significant issue arises due to the chemical hydrolysis of collagen, which lowers molecular weight by increasing acid or alkaline conditions, typically resulting in a larger yield of free amino acids. This problem underscores the need for further research and development in this area. Furthermore, the acid and alkali procedures are very corrosive to equipment and result in a high salt concentration in the final product upon neutralization (Hong *et al.*, 2019).

#### Subcritical water hydrolysis

Subcritical water hydrolysis, a method that operates within a temperature range of 100–374°C and a pressure of 0.1–22 MPa, is gaining popularity as an eco-friendly alternative to protein hydrolysis. This approach, which does not involve the use of chemicals or the generation of salt or hazardous waste, offers a rapid reaction time. The process is facilitated by the production of hydrogen ( $H_3O^+$ ) and hydroxide (OH) ions, which act as catalysts under subcritical conditions (100°C at 0.10 MPa to 374°C at 22 MPa) (Powell *et al.*, 2017).

Subcritical water hydrolysis is utilized in research to manufacture collagen hydrolysates from various sources. For example, Ahn *et al.* (2017) used an electric heater with 1.1 MPa pressure to heat a pressure chamber containing tuna skin to 190°C for 10 min. The resultant collagen hydrolysates had a molecular weight of 500 to 3000 Da. Jo *et al.* (2015) evaluated peptide yield from pig skin after sub- and supercritical water treatment. Subcritical water (300°C at 8 MPa) was marginally more successful than supercritical water (400°C at 280 MPa) in hydrolyzing porcine skin into low-molecular weight peptides (less than 1 kDa) within a short time (1 h). On the other hand, subcritical water hydrolysis entails the highest energy cost and requires the most advanced equipment, compared to enzymatic and chemical hydrolysis. Another disadvantage of subcritical water hydrolysis is the potential alteration in amino acid side chains following subcritical water treatment (Powell *et al.*, 2017).

### Physicochemical properties of hydrolyzed collagen

#### Chemical composition

Collagen is mainly composed of proteins but contains trace amounts of other elements, such as moisture, ash, and fat. Therefore, to determine the purity of collagen, it is necessary to first establish its composition. The chemical composition of collagen is influenced by its sources, extraction process, and solvent. Table 3 shows the chemical composition of various sources of hydrolyzed collagens.

Hydrolyzed collagen from snakehead (*Channa striata*) fish has the highest amount of moisture (74.33%) compared to other sources (Rosmawati *et al.* 2018) (Table 3). Lipid is one of the constituent of both skin and bone. The lipid content of fish bone is higher than that of the skin, which is also true for the leather jacket fish. Lipids must be removed during processing operations, particularly during bone extraction into gelatin. Lipids can lower

**Table 3.** Chemical composition of hydrolyzed collagen from various sources.

| Source  | Body part | Hydrolysis method            | Chemical composition (%) |         |       |       | References                     |
|---|-----------|------------------------------|--------------------------|---------|-------|-------|--------------------------------|
|   |           |                              | Moisture                 | Protein | Fat   | Ash   |                                |
| Snakehead fish ( <i>Channa striata</i> )        | Skin      | ND                           | 74.33                    | 18.49   | 2.99  | 0.20  | Rosmawati <i>et al.</i> , 2018 |
|   | Bone      |                              | 43.19                    | 15.9    | 4.19  | 32.05 |                                |
| Sockeye ( <i>Oncorhynchus nerka</i> ) salmon    | Head      | Thermal hydrolysis           | 3.89                     | 3.58    | 0.03  | 0.29  | Abuine <i>et al.</i> , 2019    |
|   | Head      | Enzymatic-thermal hydrolysis | 6.71                     | 6.21    | 0.07  | 0.43  |                                |
| Velvet elk ( <i>Cervus elaphus canadensis</i> ) | Antler    | Enzymatic hydrolysis         | 27.7                     | 51.37   | 2.82  | 26.09 | Kim <i>et al.</i> , 2016       |
| Chicken   | Feet      | Enzymatic hydrolysis         | 3.78                     | 91.38   | 17.35 | 4.84  | Kodous, 2020                   |

ND = no data presented by the researcher.

the grade of gelatin generated, which is usually accomplished by a degreasing process (Abuine *et al.* 2019). The snakehead fish bone's ash content is higher than that of the skin. A high ash level suggested a high mineral mass in the bone to represent mineral content, yet skin leather jacket fish ash was more significant than its bone (Rosmawati *et al.* 2018). This was based on the skin's thickness and biochemical composition.

#### Amino acid composition

Collagen, the most abundant protein in the human body, mainly comprises amino acids glycine (33%), proline (12%), and hydroxyproline (10%) in a triplex helix composed of three chains (primary structure) (Thirukumaran *et al.*, 2022). Each alpha chain comprises 1,014 amino acids and has a molecular weight of approximately 100 kDa (León-López *et al.*, 2019a). Collagens differ in their chain composition, controlled by the repeat and length of the Gly-X-Y amino acid sequence, with and without interruptions. The occupancy of X and Y positions by proline and its hydroxylated analog, hydroxyproline, plays a role in this variation (Sorushanova *et al.*, 2019). It is important to know the amino acid composition of collagen, as this represents structural characteristics and significantly influence collagen's physicochemical properties (Luo *et al.*, 2020). Table 4 shows the amino acid composition of hydrolyzed collagen from various sources.

#### Viscosity

Viscosity is the consistency of fluid flow and is determined in Pascal-second (Pa. s) using a viscometer (Wei *et al.*, 2019). Collagen's viscosity is determined by its denaturation temperature ( $T_d$ ), the temperature at which the triple helix structure dissolves into irregular coils in a solvent or solution (Zheng *et al.*, 2024). Owing to the damaged hydrogen link in collagen molecules, the viscosity of collagen reduced with increasing temperature, causing the triple helix structure to disintegrate and convert into random coils (Chen *et al.*, 2019). Table 5 summarizes the previous research data. Hydrolyzed collagen from sheepskin showed a viscosity of 4,576 centipoise (Cp) after 1 h of hydrolysis with trypsin, compared to 6,800 Cp at 0 min (León-López *et al.*, 2019b). The existence of a high amount of molecular weight and chains could explain increased viscosity.

#### Thermal stability

Protein stability refers to the capacity of a protein to maintain its primary structure despite extreme conditions (Pucci and Rومان, 2017). Furthermore, one of the essential variables in assessing the possible application of collagen is its temperature stability. The composition of amino acids directly influences collagen's physical and chemical characteristics, particularly heat stability. Hydroxyproline's hydroxyl groups form hydrogen bonds that strengthen triple helix structure's stability

(Zhong *et al.*, 2015). Pepsin- and acid-soluble collagen derived from the body wall of sea cucumbers (*Stichopus hermanni*) exhibited greater enthalpy values ( $0.28 \pm 0.01$  and  $0.62 \pm 0.07$  J/g, respectively), indicating enhanced stability of collagen molecules (Shaik *et al.*, 2024b). A higher enthalpy value of collagen suggests that more thermal energy is needed to break its intramolecular bonds.

Collagen, like any other protein, is frequently defined in terms of its denaturation temperature ( $T_d$ ) or maximum transition temperature ( $T_m$ ) (Zhong *et al.*, 2015), as summarized in Table 5. For example, the thermal stability of collagen hydrolysate from sheep hides decreased gradually from  $153.3^\circ\text{C}$  (0 min) to  $137.91^\circ\text{C}$  after 4 h of hydrolysis (León-López *et al.*, 2019b). Furthermore, the data indicates that intermolecular helix formation decreases dramatically with progress in hydrolysis. This decrease began at the bimolecular nucleation step, where glycine and proline residues assisted an intramolecular  $\beta$ -turn (Choi *et al.*, 2018).

#### Solubility

Solubility measures the dissolution of a sample in a solvent. The solubility of collagen is evaluated by dissolving collagen in acid solutions of varying pH or salt (NaCl) solutions of different concentrations (Gaurav *et al.*, 2017). Collagen solubility is the most significant component and a great indicator of their functioning. Understanding collagen solubility provides essential information on protein consumption and functionality, particularly in foams, emulsions, and gels (Li *et al.*, 2018). Collagen from different sources may have varying molecular properties, which contribute to a wide range of collagen solubility characteristics. In case the pH is above or below the isoelectric point (pI) value, the protein molecule's net positive or negative charge rises, and the solubility is enhanced by the repulsive force between chains (Li *et al.*, 2020). In other words, when the pH value deviates from the pI value, the solubility of collagen increases. Collagen solubility is proportional to pH and pI values. Table 5 summarizes data on the solubility of collagen.

## Bioactive Properties of Hydrolyzed Collagen

### Antimicrobial properties

The antimicrobial properties of hydrolyzed collagen are the activities against some of the most dangerous antibiotic-resistant bacteria by destroying target cells quickly (Aleman and Martinez-Alvarez, 2013). Bioactive peptides with antimicrobial activities have potential uses in natural product food safety and protection. The content of amino acids first shape the molecule's configuration, establishing hydrophobic and hydrophilic areas, domains, and charges. Recently, antimicrobial

Table 4. Amino acid composition based on different sources (residue/1,000 amino acid residue).

|                      | Sockeye ( <i>Oncorhynchus nerka</i> ) head |                              | Sheep hides              |                      | Chicken feet         |                      | Red seabream ( <i>Pagrus major</i> ) skin |                   | Flounder ( <i>Paralichthys olivaceus</i> ) |                   | Snake head ( <i>Channa striata</i> ) skin |              | Common carp ( <i>Cyprinus carpio</i> ) skin |              |
|----------------------|--|------------------------------|--------------------------|----------------------|----------------------|----------------------|---|-------------------|--|-------------------|---|--------------|---|--------------|
|                      | Thermal hydrolysis                         | Enzymatic-thermal hydrolysis | Enzymatic hydrolysis     | Enzymatic hydrolysis | Enzymatic hydrolysis | Enzymatic hydrolysis | Acidic extraction                         | Acidic extraction | Acidic extraction                          | Acidic extraction | Acid soluble                              | Acid soluble | Acid soluble                                | Acid soluble |
| Aspartic acid (Asp)  | 0  | 0.01                         | 28.03                    | 42.2                 | 28.4                 | 29.6                 | 54.0                                      | 53.1              |  |                   |   |              |   |              |
| Threonine (Thr)      | 2.63                                       | 2.49                         | 3.18                     | 48.9                 | 21.3                 | 21.2                 | 22.0                                      | 26.6              |  |                   |   |              |   |              |
| Serine (Ser)         | 3.35                                       | 3.98                         | 13.72                    | 33.2                 | 28.8                 | 31.1                 | 35.0                                      | 38.5              |  |                   |   |              |   |              |
| Glutamic acid (Glu)  | 10.97                                      | 8.16                         | 38.21                    | 85.3                 | 50.4                 | 49.1                 | 74.0                                      | 74.0              |  |                   |   |              |   |              |
| Glycine (Gly)        | 17.38                                      | 9.78                         | 1.92                     | 330.2                | 400.2                | 399.6                | 307.0                                     | 318.2             |  |                   |   |              |   |              |
| Alanine (Ala)        | 6.68                                       | 5.51                         | 0.14                     | 131.18               | 131.8                | 128.0                | 89.0                                      | 119.0             |  |                   |   |              |   |              |
| Cysteine (Cys)       | 0.11                                       | 0.22                         | 0.13                     | -                    | -                    | -                    | 2.0                                       | 1.5               |  |                   |   |              |   |              |
| Valine (Val)         | -  | 2.79                         | 1.53                     | 20.1                 | 15.1                 | 17.4                 | 26.0                                      | 24.1              |  |                   |   |              |   |              |
| Methionine (Met)     | 2.24                                       | 2.06                         | 1.36                     | 12.3                 | 11.6                 | 12.6                 | 12.0                                      | 12.8              |  |                   |   |              |   |              |
| Isoleucine (Ile)     | 2.06                                       | 2.72                         | 0.27                     | 8.3                  | 5.5                  | 7.5                  | 9.0                                       | 13.3              |  |                   |   |              |   |              |
| Leucine (Leu)        | 2.84                                       | 4.49                         | 0.24                     | 20.4                 | 16.3                 | 15.9                 | 28.0                                      | 26.8              |  |                   |   |              |   |              |
| Tyrosine (Tyr)       | 0.85                                       | 1.49                         | 0.78                     | 1.02                 | 2.2                  | 2.1                  | 5.0                                       | 5.6               |  |                   |   |              |   |              |
| Phenylalanine (Phe)  | 2.44                                       | 2.21                         | 0.87                     | 11.3                 | 11.1                 | 11.5                 | 18.0                                      | 14.4              |  |                   |   |              |   |              |
| Hydrolysine (Hyl)    | 7  | -                            | -                        | -                    | -                    | -                    | 6.0                                       | -                 |  |                   |   |              |   |              |
| Lysine (Lys)         | 4.38                                       | 4.82                         | 2.05                     | 30.1                 | 21.5                 | 21.6                 | 31.0                                      | 29.4              |  |                   |   |              |   |              |
| Histidine (His)      | 1.48                                       | 1.54                         | 3.95                     | 9.1                  | 3.8                  | 5.3                  | 6.0                                       | 10.7              |  |                   |   |              |   |              |
| Arginine (Arg)       | 7.01                                       | 4.79                         | 2.68                     | 5.6                  | 57.1                 | 56.0                 | 56.0                                      | 51.7              |  |                   |   |              |   |              |
| Hydroxyproline (Hyp) | 4.74                                       | 2.31                         | 18.06                    | 6.6                  | 48.9                 | 46.8                 | 94.0                                      | 70.2              |  |                   |   |              |   |              |
| Proline (Pro)        | 6.21                                       | 4.88                         | 0.13                     | 120.3                | 146.0                | 144.8                | 126.0                                     | 109.9             |  |                   |   |              |   |              |
| <b>References</b>    | Abuine et al., 2019                        |                              | León-López et al., 2019a |                      | Kodous, 2020         |                      | Son et al., 2022                          |                   | Truong et al., 2021                        |                   | Rýglóvá et al., 2023                      |              |   |              |

**Table 5. Physicochemical properties of different sources of collagen hydrolysate.**

| Sources  | Body part | Hydrolysis method    | Physicochemical properties |                   |            | References                 |
|--|-----------|----------------------|----------------------------|-------------------|------------|----------------------------|
|  |           |                      | Viscosity                  | Thermal stability | Solubility |                            |
| Brownstripe red snapper<br>( <i>Lutjanus vitta</i> ) | Skin      | Chemical hydrolysis  | ND                         | 31.52°C           | 32.5%      | Jongjareonrak et al., 2005 |
| Sheep  | Hides     | Enzymatic hydrolysis | 4,576 cp                   | 137.91°C          | ND         | León-López et al., 2019a   |
| Chicken  | Feet      | Enzymatic hydrolysis | 5.14 mpa.s                 | ND                | 75.12%     | Kodous, 2020               |

ND = no data presented by the researcher.

hydrolysates from tuna and squid skins were produced using *Bifidobacterium animalis* subsp., *Lactis* and *Lactobacillus acidophilus*. These collagen hydrolysates (of 1–10 kDa and <1 kDa) were highly effective at a concentration of 0.2 mg/mL against Gram-positive and Gram-negative bacteria, including *Shewanella putrefaciens* and *Photobacterium phosphoreum* (Gomez-Guillen et al., 2011). Tuna hydrolysate fractions demonstrated greater activity than squid fractions against *Lactobacillus acidophilus* (at 0.2 mg/mL) as well as *Salmonella choleraesuis* and *Pseudomonas aeruginosa* (at 2 mg/mL). However, squid fractions were more effective against *Aeromonas hydrophila* at a concentration of 2 mg/mL. Collagens exhibited greater antibacterial efficacy compared to their hydrolysates, requiring lower concentrations for effective inhibition. Hydrolyzed collagens from lamb and sheep showed significant antimicrobial activity against both Gram-positive bacteria (*Listeria monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli*, *Salmonella choleraesuis*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*) (Vidal et al., 2022). The lowest molecular weight fractions, particularly in squid samples, had the most potent antibacterial activity. Peptides derived from fish gelatine contain repeating motifs of Gly-Pro-Ala triplets in their structure (Senadheera et al., 2020). Their hydrophobic nature allows peptides to pass through membranes, while their positive charge facilitates interaction with the negatively charged bacterial surface, resulting in pore formation (Aleman and Martinez-Alvarez, 2013).

### Antioxidant activity

Antioxidants are the chemicals that help the body combat free radicals. Oxidation is critical for aerobic species, such as vertebrates and humans. However, the overproduction of reactive oxygen species (ROS) in the human body can have damaging and deadly cellular consequences by oxidizing lipids, proteins, enzymes, and DNA (Banerjee et al., 2015). ROS production is linked to heart disease, stroke, diabetes, arteriosclerosis,

cancer, and neurological and inflammatory illnesses (Lee et al., 2011). Antioxidants help to mitigate these issues and can extend life expectancy. Hydrolysate peptides with antioxidant bioactivity safeguard the human body by neutralizing ROS, commonly referred to as free radicals, which cause oxidative damage at cellular level (Tawalbeh et al., 2025). The antioxidant properties of peptides are studied extensively for their ability to prevent lipid peroxidation in dietary proteins (Banerjee et al., 2015).

Some research on collagen peptides or hydrolysates has exhibited antioxidant properties, but fewer experiments on poultry and mammalian sources are found. Antioxidant properties are found in peptides derived from the enzymatic hydrolysis of fish processing wastes (Sila and Bougateg, 2016). Collagen hydrolysates (17%) from the skins of snakehead murels (*Channa striata*) showed substantial antioxidant activity, compared to butylated hydroxytoluene, vitamin C, and vitamin E (Sinaga et al., 2020). Collagen peptides from abalone foot muscles with 49.18% and 83.83% yield showed antioxidant properties in both *in vitro* and *in vivo* studies (Zhou et al., 2012). Similarly, salmon skin collagen hydrolysate, processed using free or immobilized extracellular protease from *Vibrio* sp. SQS2-3, exhibited enhanced antioxidant activity, particularly in peptides smaller than 3 kDa (Wu et al., 2018). A bioactive peptide derived from cow tendon collagen protects cells against oxidative stress (Banerjee et al., 2015). Royal jelly-derived collagen peptide also possesses anti-aging properties and protects against oxidative stress (Qiu et al., 2020). Moreover, two collagen hydrolysates derived from lamb and sheep demonstrated antioxidant activity through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, achieving 13.4% activity at a concentration of 0.0002% and 13.1% activity at a concentration of 0.0005% (Vidal et al., 2022). In another study, three glycosylated bone collagen hydrolysates derived from chicken, porcine, and bovine sources demonstrated significant antioxidant properties, as evidenced by DPPH, ABTS•+ radical cation (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid), and hydroxyl radical scavenging activities as well as ferrous

ion reducing power (Qi *et al.*, 2024). The hydroxyl radical scavenging activity showed a concentration-dependent increase, ranging from 0.25 mg/mL to 4.00 mg/mL. Collagen peptides are potent antioxidants that should be further investigated.

### Antihypertensive/Angiotensin-Converting Enzyme (ACE) Inhibitory Activity

Angiotensin-converting enzyme (ACE) inhibitors reduce blood pressure by relaxing the veins and arteries (Chen *et al.*, 2020). These inhibitors prevent the release of angiotensin II, a chemical that narrows blood arteries, leading to high blood pressure. Angiotensin II also stimulates the production of hormones that raise blood pressure. ACE inhibitors are used to prevent and treat high blood pressure (hypertension), coronary artery disease, heart failure, heart attack, diabetes, certain chronic kidney diseases, scleroderma (hardening of the skin and connective tissues), and migraine (Chen *et al.*, 2020). ACE inhibitory activity is found in the collagen peptides of the skin of milkfish (*Chanos chanos*) (Baehaki *et al.*, 2016). Pig femoral bone peptides also exhibit antihypertensive and ACE-inhibitory properties (Liu *et al.*, 2011).

### Anti-aging properties

Anti-aging peptides, whether natural or synthetic, have emerged as revolutionary products (Jhawar *et al.*, 2020). Collagen peptides, a key component in food supplements and cosmetics, have the potential to address skin issues, as suggested by researchers (Li *et al.*, 2022). Most of the research in this field has been directed toward the prevention and treatment of skin aging (Bolke *et al.*, 2019), instilling hope for the future of skincare.

Collagen peptides from various sources have distinct effects on the skin. For example, they can reduce the activity of collagenase enzymes (matrix metalloproteinases: MMP-3 and MMP-13, and gelatinase enzymes: MMP-2 and MMP-9), thereby regulating skin hydration, reducing loss of skin moisture, minimizing wrinkling, improving skin elasticity, and repairing collagen degradation and elastic fiber damage caused by ultraviolet (UV) radiation (Pyun *et al.*, 2012). This was proved by Kang *et al.* (2018) in a study where hairless mice exposed to UV radiation were administered 1,000 mg/kg collagen peptide for 9 weeks. In addition, collagen peptides regulate the expression of hyaluronic acid synthase mRNA and the skin moisturizing factor filaggrin, increase hyaluronic acid levels in skin tissue, and decrease the expression of hyaluronidase (HYAL-1 and HYAL-2) mRNA. In other words, collagen peptide may help to prevent loss of skin moisture caused by UV exposure (UV-B). In a recent

study, an orally disintegrating collagen film derived from tilapia fish scales was shown to reduce skin wrinkle depth while significantly enhancing skin elasticity and density, highlighting its potential as an effective anti-aging agent (Lee *et al.*, 2022).

### Antifreeze properties

Antifreeze is a situation that prevents the formation of crystals while maintaining a delicate texture. Specific collagen peptides inhibit ice crystal development. In industry, antifreeze activity is a common problem (Chen *et al.*, 2017). The structure, texture, and quality of frozen meals can be preserved using an antifreeze ingredient. For example, in sucrose model situations, chicken collagen peptides hinder ice crystal formation (Du and Betti, 2016).

The enzymatic supramolecular assembly significantly enhanced the antifreeze activity of tilapia skin-collagen peptides, highlighting their potential for development as novel antifreeze additives in the food industry (Ouyang *et al.*, 2024). Similarly, antifreeze peptides derived from squid skin collagen demonstrated superior cryoprotective activity, showing potential as innovative antifreeze agents. These peptides also exhibited an inhibitory effect on the denaturation and structural changes of myofibrillar proteins during freeze-thaw cycles and partially retained their water-binding capacity (Cao *et al.*, 2023b). Additionally, hydrolysates obtained from salmon skin collagen displayed notable anti-freezing activity, particularly in peptide fractions >3 kDa (Wu *et al.*, 2018).

Besides its potential utility in food preservation, collagen peptides with antifreeze and cryoprotection activity also protect cells and tissues from freezing (Nguyen *et al.*, 2018). They interact with cell membrane phospholipids during freeze-drying, providing antifreeze effects, such as those observed in pig skin collagen peptides on freeze-dried *Streptococcus thermophiles* (Wang *et al.*, 2016). *Lactobacillus bulgaricus* is protected from hypothermia by a shark skin collagen hydrolysates peptide. Alternative antifreeze proteins may be found in collagen hydrolysates, with antifreeze proteins (AFP) and antifreeze glycoproteins (AFGP) being the most studied components (Chen *et al.*, 2020).

In addition, these proteins by attaching to microscopic ice crystals not only inhibit ice nucleation but also reduce ice crystal development and alter ice crystal habits. The ice-binding face of these antifreeze proteins is flat, with the distance between oxygen atoms nearly matching the distance in ice nuclei that of about 4.52 Å (Kumar *et al.*, 2015).

## Application of Hydrolyzed Collagen

### Food industry

Collagen hydrolysates have the potential to attach calcium ions, increasing their bioavailability. As a result, collagen hydrolysates are used in functional food components to treat mineral shortages (Pal and Suresh, 2016). Hydrolyzed collagen works as an anticoagulant and helps to reduce the damage caused by low temperatures in cells and tissues. Furthermore, the versatility of marine collagen has broadened its utilization across the food industry because of its stabilizing properties. Specifically, it finds applications as a biodegradable film-forming material, colloid stabilizer, foaming agent, and a microencapsulating agent (Farooq *et al.*, 2024). However, it might benefit foods stored at cold or freezing temperatures (Cao *et al.*, 2016). Collagen hydrolysates are utilized for selecting products, such as meats, drinks, soups, and much more, as summarized in Table 6. It helps to improve and maintain their sensory and physico-chemical properties.

In processed food, collagen hydrolysates are utilized to substitute pork fat in sausages at a 50% substitution level, resulting in higher water-holding capacity, better after-cooking stability, and enhanced texture (chewiness and hardness) (Sousa *et al.*, 2017). In buffalo patties, fish-hydrolyzed collagen increased protein content, reduced fat content, and maintained sensory acceptance and superior texture, compared to patties without hydrolyzed collagen (Ibrahim *et al.*, 2020). Bovine skin collagen hydrolysates combined with modified starch and guar gum in ham production resulted in lower syneresis with a final concentration of 2.0% collagen hydrolysates (Prestes, 2015). Adding hydrolyzed collagen from fish to beverages, such as orange juice (2.5%), enhanced nutritional and functional qualities, including higher protein content, bioavailability, low viscosity, and high-water solubility (Bilek and Bayram, 2015). Hydrolyzed collagen may also be added to soup, affecting viscosity and functional characteristics. It has strong radical scavenging abilities for ABTS•+ radical cation and DPPH (Benjakul *et al.*, 2012). When added to chrysanthemum beverages, pig skin-hydrolyzed collagen improved clarity, sensory quality, and storage stability in a smaller quantity than other commercial clarifiers. Hydrolyzed collagen is also employed in developing physicochemical and functional qualities in various food items without affecting sensory characteristics, making it a promising functional substance.

### Cosmetics and nutraceuticals

Skin aging is a continuous biological process characterized by a progressive decline in its physiological

functions, including collagen and elastin production, thermoregulation, hydration, and sebum production (Al-Atif, 2022). Collagen is critical in cosmetic formulations due to hydrating, rejuvenating, and film-forming characteristics. It binds water considerably, maintaining adequate skin hydration throughout the day, which softens and moisturizes the skin (Jridi *et al.*, 2015). Collagen's film-forming ability reduces transepidermal water loss in addition to being a natural humectant. Peptide occlusion prevents mechanical deficiencies from causing harm to the skin and hair. Furthermore, occlusion improves appearance of the skin by making it more radiant, lighted, and smooth (Sionkowska *et al.*, 2020). Collagen is utilized in various oral and topical cosmetic products, such as creams, gels, serums, and masks (Jadach *et al.*, 2024).

Types I, III, and V collagen predominantly make up skin tissues. Marine collagen is rich in type I collagen, as it is the most prevalent component of the skin (Sionkowska *et al.*, 2020). Furthermore, anti-aging, anti-wrinkle, UV radiation defense, and wound-healing capabilities are found in marine collagen, which has led to incorporation of collagen in cosmetic formulations (Avila *et al.*, 2018). As a result, various cosmetic products, such as the Etude House Moist-Full Collagen Cream, 3W Clinic Collagen Eye Cream, and Bio-Essence Collagen Essence Cream, were available on the market, with collagen in their compositions (Loh *et al.*, 2021). In addition, collagen cosmetic masks are found to decrease effectively wrinkles and expression lines in the neck, face, and neckline. The cosmetics industry has done various tests on using collagen in cosmetics, and the results are patented by Sionkowska *et al.* (2020). Therefore, more information on using collagen in cosmetics is needed in the public domain.

Nutraceuticals, or oral collagen supplementation, are oral-based natural health products containing targeted nutrients and mixed antioxidant elixirs that have a prophylactic or therapeutic impact on the skin, hair, and nails. Nutraceuticals are evolved from the nutraceutical and cosmeceutical sectors (Faria-Silva *et al.*, 2020). Nutraceuticals have gained popularity as anti-aging products, with hydrolyzed collagen oral supplements improving skin hydration, wrinkle reduction, elasticity, firmness, and skin rejuvenation by reaching to deeper layers of the skin (Table 6) (Usman and Bharadvaja, 2023).

Human skin consists of the epidermis, dermis, and subcutaneous tissue. Collagen and elastin in the dermis keep the skin's structure and suppleness (Ganceviciene *et al.*, 2012). Type I collagen, making up to 80% of dermal collagen, is crucial for skin strength (Abedin *et al.*, 2014). Collagen production decreases with age, resulting in worsening of dermis structure (Ganceviciene *et al.*, 2012). Collagen is commonly included in skin's anti-aging treatments for its inherent moisturizing, softening,

Table 6. Application of hydrolysed collagen in multiple industries

| Industry   | Product   | Sources                       | Hydrolyzed collagen   | Application  | References                       |
|--|---|-------------------------------|---|--|----------------------------------|
| Food industry  | Tilapia muscle (preserved)                              | Nile tilapia                  | 1.1%  | Antimicrobial and maintenance of freshness (pH and hardness)   | Song <i>et al.</i> , 2022        |
|  | Butter and chocolate sauce                              | Pacu skin and Tilapia bones   | 1%  | Collagen is able to retain the emulsion properties of butter and chocolate sauce for 25 weeks and does not show any cytotoxic effect on leukocytes and Vero cells. (EA [R = 0.832], ESI [R=0.842]).  | Dey <i>et al.</i> , 2021         |
| Cosmetics and Nutri-cosmetics  | Probiotic fermented Milk                                | Not defined by the author     | 3%  | Collagen protein hydrolysate acts as additive and favorably stimulated the survival of bifidobacterium Bb-12 during 21 days of storage.  | Znamirowska <i>et al.</i> , 2020 |
|  | CSC-film  | Wastes from leather industry  | 15%   | The packaging material is used in very hot and humid environments without any deterioration of physical properties.  | Mailha <i>et al.</i> , 2024      |
|  | Meat balls  | Fish                          | 3%  | Greatly inhibited the lipid oxidation and improved the antioxidant activity of product (thiobarbituric acid [TBA] value $0.78 \pm 0.24$ mg MDA/kg).  | Palamutoğlu and Kasnak, 2019     |
|  | Chicken patties   | Not defined by the author     | 20%   | Improves the quality characteristics of reduced-fat chicken patties.   | Kim <i>et al.</i> , 2018b        |
|  | HC in sausages to replace pork backfat                  | Germina®                      | 11.25%  | Sausages with greater water-holding capacity ( $80.49 \pm 0.54\%$ ) and improved texture in terms of hardness ( $0.694 \pm 0.090$ ), cohesiveness ( $0.459 \pm 0.032$ ), gumminess ( $0.304 \pm 0.025$ ), and chewiness ( $0.248 \pm 0.065$ ). | Sousa <i>et al.</i> , 2017       |
|  | Fish HC in buffalo patties                              | Az-Zahrah Sdn.Bhd®            | 10%   | Products with higher protein content ( $26.68 \pm 0.86\%$ ), lower fat content ( $3.11 \pm 0.09\%$ ).  | Ibrahim <i>et al.</i> , 2020     |
|  | Dairy beverage with HC, açai pulp, and cheese           | Tovani Benzaquen ingredients® | 1%  | High sensory acceptability, and good physicochemical and microbiological parameters.   | Mata-Rigoto <i>et al.</i> , 2019 |
|  | Chrysanthemum beverage                                  | Pigskin                       | 0.1%  | Excellent clarification effect and better sensory quality and storage stability.   | Zhang <i>et al.</i> , 2018       |
|  | Oral supplementation with vitamins A, C, and E and zinc | Not defined by the author     | 90%   | Improved the hydration, texture, elasticity, and firmness of the skin in women aged between 40 and 50 years after 28 days of treatment.  | Maia <i>et al.</i> , 2019        |
|  | Beverage  | Nitta Gelatin Inc®            | 15%   | Changes in periorbital wrinkles, facial skin hydration, and skin elasticity in healthy women aged 30–60 years.   | Koizumi <i>et al.</i> , 2018     |
| Drinking ampoules with acerola fruit extract, vitamins C and E, zinc, and biotin | ELASTEN®  | 10%                           | 12 weeks of oral supplementation improved skin properties, such as hydration ( $35.0 \pm 4.8$ AU), elasticity ( $R2=0.69 \pm 0.05$ ), roughness ( $161.6 \pm 11.4 \mu\text{m}$ ), and density ( $35.7 \pm 7.2 \mu\text{m}$ ). | Bolke <i>et al.</i> , 2019   |                                  |
| Fish   | Milkfish scales   | Not provided                  | Excellent water-holding capacity, moisture absorption, retention and anti-skin aging, and anti-melanogenic capacities.  | Chen <i>et al.</i> , 2018  |                                  |
| Fish   | Pure Gold Collagen®                                     | 10%                           | Reduction of dryness of the skin, wrinkles, and nasolabial depth, as well as increase in skin firmness and collagen density.  | Borumand and Sibilla, 2014   |                                  |
| HC peptide   | CollaSel Pro®   | >90%                          | Effectively improves dermal health and the appearance of sagging and ameliorates the signs of the aging process.  | Demir-Dora <i>et al.</i> , 2024  |                                  |

|              |   |                              |       |   |                       |
|--------------|---|------------------------------|-------|---|-----------------------|
| Bio-material | HC-chitosan hydrogels                       | Tilapia fish                 | 2%    | Antibacterial activity against <i>E. coli</i> and <i>Staphylococcus aureus</i>  | Ouyang et al., 2018   |
|              | Nanofibrous scaffold functionalized with HC | Tilapia fish                 | 1.88% | Antimicrobial property against <i>E. coli</i> and <i>Pseudomonas aeruginosa</i>   | Ramadass et al., 2019 |
|              | Collagen–HC films                           | Wastes from leather industry | 8%    | Excellent barrier properties against UV light.  | Ocak, 2018            |
|              | Chitosan-fucoidan cryogels                  | Jellyfish                    | 3%    | It is biocompatible and its porous nature supports cell infiltration and repair processes in wound-healing process with non-cytotoxic nature. | Carvalho et al., 2020 |
|              | Collagen cream                              | Snakehead fish               | 3%    | Promoting faster wound recovery by improving skin elasticity and reduces the size and severity of burn wounds.                                | Hasri et al., 2020    |
|              | HC film                                     | Skin waste of squid          | 0.5%  | Improves dermal wound-healing process with promising biocompatibility and better biodegradability.  | Veeruraj et al., 2019 |

Note: HC = hydrolyzed collagen; MDA: malondialdehyde.

and luminous properties (Alves *et al.*, 2017). Hydrolyzed collagen is preferred in cosmetics due to its higher solubility at neutral pH, ease of dermis penetration, and water-binding characteristics (Valenzuela-Rojo *et al.*, 2018).

*In vivo* studies using hydrolyzed collagen oral supplementation in women aged 40–60 years resulted in a substantial increase in skin hydration, wrinkling, and agility after 12 weeks (Kim *et al.*, 2018a). Women aged 35–65 years experienced improved dermal thickness, firmness, and elasticity after 3 months of hydrolyzed collagen supplementation (Addor *et al.*, 2018). In another research with 120 patients, regular oral supplementation of fish-hydrolyzed collagen improved skin texture and elasticity over 90 days. In addition, there was evidence of a preventive impact on joint health (Czajka *et al.*, 2018). Nutricosmetics resulted in a substantial increase in skin hydration and collagen density at the dermis level in women aged 40–59 years. However, after 4 weeks of supplementation, the dermal collagen network fragmentation was dramatically reduced, and these benefits lasted for another 12 weeks (Asserin *et al.*, 2015).

### Biomaterials

Collagen is highly considered for its biocompatibility and biodegradability, making it a safe and effective biomaterial. Recently, its use in tissue engineering and therapeutic applications has gained attraction for these properties (Ramadass *et al.*, 2014). Hydrolyzed collagen offers significant benefits over native collagen in terms of solubility and ease of extraction, as it does not require a complex multistep extraction process. However, due to the low molecular weight of peptides, hydrolyzed collagen cannot form scaffolds on its own, and is often combined with other biopolymers, such as cellulose and chitosan, to overcome this limitation (Ficai *et al.*, 2013).

Films made from a cellulose–hydrolyzed collagen mix exhibit vital transparency, excellent UV radiation absorption, and strong cell adhesion and proliferation support. High biocompatibility of these films suggests their potential utility as biomaterials, as shown in Table 6 (Pei *et al.*, 2013). Additionally, collagen–hydrolyzed collagen films from leather waste have demonstrated high transparency and effective UV light barrier qualities. Experiments using Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) revealed 100% miscibility between the two polymers (Ocak, 2018).

Furthermore, due to low molecular weight and amino acid content, hydrolyzed collagen is advantageous for treating bone and joint issues, as it enhances bioavailability and promotes collagen production, thereby improving

osteointegration (Ficai *et al.*, 2013). Chitosan sponges are an alternative biomaterial with hydrolyzed collagen. They are made using a sol-gel transition approach, and hydrolyzed collagen includes porous shape, better biostability, superior water absorption capacity, excellent biocompatibility, and antibacterial activity (Ramadass *et al.*, 2014).

Hydrolyzed collagen has been employed in producing hydrogels for administering pharmaceuticals, such as insulin and methylene blue, as it reduces water absorption. Drug release from these hydrogels is faster at pH 2, making it suitable for medications susceptible to degradation in acidic gastric fluids (Noppakundiligrat *et al.*, 2018). Hydrogels made with chitosan and fish-hydrolyzed collagen have demonstrated antibacterial efficacy against *E. coli* and *Staphylococcus aureus* and promote cell proliferation, migration, and wound healing (Ouyang *et al.*, 2018). Electrospinning can functionalize nanofibrous scaffolds with hydrolyzed collagen, providing a porous structure, good mechanical strength, biocompatibility, and antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* (Ramadass *et al.*, 2019).

## Conclusion

In conclusion, acid, alkali, and enzyme extraction methods are the standard approaches for collagen extraction with these methods being the most efficient and preferred. Advanced collagen extraction technologies, such as DES, SFE, and MAE, offer advantages such as improved yield, reduced protein denaturation, effective preservation of bioactivity, and enhanced environmental sustainability. However, these methods also face challenges, such as high initial equipment expenses, operational complexity, and the need for further optimization to ensure product stability across various biological sources. Hydrolyzed collagen provides significant functional properties due to its bioactivities, such as antifreeze, anticancer, antioxidant, and antihypertensive properties. In addition, hydrolyzed collagen has good biological effects in cosmetic applications, such as increasing cell proliferation, water-holding capacity, moisture absorption and retention, and anti-aging effects on the skin. In the food sector, collagen hydrolysate is widely used as a functional component, promoting water retention in meat products, enhancing sensory properties, and improving the chemical and physical qualities of beverages and dairy products. Furthermore, hydrolyzed collagen combined with cellulose or chitosan for creating scaffolds in the biomedical industry has aided collagen formation, bone and joint disease management, wound therapy, high biocompatibility, and antibacterial qualities. Thus, hydrolyzed collagen possesses a vast potential for applications in various industries.

## Data Availability Statement

Data are made available on request.

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## Author Contributions

Norizah Mhd. Sarbon: supervision, conceptualization, methodology, validation, and writing (review and editing). Assyarafanee Hiefnee and Mannur Ismail Shaik: methodology, writing (original draft, review, and editing), investigation, and visualization. Noraizah Mhd. Sarbon and Ismail Fitriy Mohammad Rashedi: Review and editing, investigation, and visualization. Anis Syafiqah Yusri: validation, review, and editing.

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The authors declared no conflict of interest or any competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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