Applied Food Research 6 (2026) 101829

Contents lists available at ScienceDirect

Applied Food Research

journal homepage: www.elsevier.com/locate/afres

Subcritical water extraction (SWE) for valorizing fish waste into collagen: Current state and future prospects

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ARTICLE INFO

Keywords:
Food waste
Sustainable extraction
Protein
Seafood
Green technology

ABSTRACT

The seafood industry generates significant waste, much of which is often discarded, contributing to environmental concerns. While conventional collagen extraction methods rely heavily on chemicals and extensive processing, these approaches pose environmental challenges due to excessive wastewater production. Subcritical water extraction (SWE) has recently emerged as a sustainable alternative, offering an efficient and eco-friendly method for recovering collagen and other bioactive compounds from fish waste. This study explores the potential of SWE as a green technology, highlighting its advantages in reducing environmental impact and enhancing resource utilization. It covers the types of collagens, their sources, and various applications, followed by a detailed discussion on the principles of SWE and the factors affecting its efficiency. The review also summarizes the findings of past studies on the use of SWE for extracting collagen from different fish waste sources, highlighting the advantages over traditional extraction methods in terms of yield, quality, and environmental sustainability. Furthermore, the paper addresses the challenges and limitations associated with the widespread adoption of SWE, such as the need for further optimization of process parameters and the integration of SWE with other green technologies. This review paper aims to provide a comprehensive understanding of the current state and future potential of using SWE for valorizing fish waste into high-value collagen, thereby addressing the environmental challenges faced by the seafood industry and promoting the development of a more circular and sustainable bioeconomy.

1. Introduction

Currently, the seafood industry produces a considerable amount of waste, including fish skins, bones, scales, and viscera, which are frequently underutilized or discarded, creating environmental issues. These by-products from fish processing, however, are rich in high-quality proteins like collagen up to 75% (Gaikwad & Kim, 2024), which have numerous applications in the food, cosmetic, and biomedical sectors. Conventional collagen extraction methods from fish waste often involve the use of chemicals such as sodium hydroxide (NaOH) and acetic acid (CH_3COOH) (Nirmala & Karthika, 2022), extended processing durations up to 200 h (Farooq et al., 2024), and result in significant wastewater production, making them environmentally unsustainable. In recent years, subcritical water extraction (SWE) has gained attention as an effective green technology for the efficient and eco-friendly recovery of collagen and other valuable compounds from fish waste. SWE utilizes water in a unique state between its boiling point (100°C) and critical point (374°C), where it exhibits enhanced solvating properties and the ability to disrupt molecular interactions, facilitating the extraction of target compounds. By carefully controlling the temperature and pressure, SWE can selectively extract collagen while minimizing the risk of protein denaturation.

Few studies have demonstrated the effectiveness of SWE in extracting collagen from a variety of fish waste sources, including skins, bones, and scales. Compared to traditional extraction methods, SWE has been shown to yield higher collagen recovery rates, with the added benefits of reduced processing time, lower energy consumption, and the elimination of harsh chemicals. Moreover, the collagen extracted using SWE has

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https://doi.org/10.1016/j.afres.2026.101829
Received 17 July 2024; Received in revised form 25 September 2025; Accepted 23 February 2026
Available online 24 February 2026

been found to possess desirable physicochemical and functional properties, making it suitable for various applications. Despite the promising results, the widespread adoption of SWE for collagen extraction from fish waste is still in its early stages. Ongoing research is focused on optimizing the SWE process parameters, such as temperature, pressure, and residence time, to further improve the yield and quality of the extracted collagen. Additionally, the integration of SWE with other green technologies, such as ultrasound-assisted extraction and microwave-assisted extraction, is being explored to enhance the efficiency and sustainability of the overall valorization process. The use of subcritical water extraction for the valorization of fish waste into high-value collagen represents a significant opportunity to address the environmental challenges faced by the seafood industry while unlocking new avenues for the development of innovative, sustainable, and eco-friendly products. As research in this field continues to evolve, the future prospects for SWE-based collagen extraction are promising, with the potential to transform waste into a valuable resource.

2. Fish waste

According to a report by FAO (2022), global fisheries and aquaculture production reached a record of 214 million tonnes in 2020, comprising 178 million tonnes of aquatic animals and 36 million tonnes of algae. This production comes from both capture fisheries (90 million tonnes) and aquaculture (122.6 million tonnes). However, the fisheries and aquaculture sectors also generate significant amounts of waste. Approximately 70% of fish and seafood undergo processing before reaching the market, which involves steps like decapitation, skin removal, gut removal, fin and scale separation, and bone removal (Rajabimashhadi et al., 2023). The main causes of fish waste include bycatch, discards at sea, poor handling and storage, and waste at the retail and consumer levels. It is estimated that the average annual discard from 2000 to 2018 was around 8.5 million tons, approximately 10% of the total catch (Zhao et al., 2022).

The fish canning industry is the second largest source of by-products (Aboudamia et al., 2021). For example, most sardines are used as bait, immediate feeding, canning, drying, flavoring, or decomposition to produce fish food or lipids (Muthumari, Anand & Maruthupandy, 2016). Sardine processing generates waste such as internal organs, tails, bones, scales, and heads, accounting for 60-70% of the total live weight (Rathod et al., 2024). Meanwhile, fish filleting produces waste like internal organs, heads, spines, vertebral fins, meat, skin, and scales.

This waste is often disposed of through methods such as burial, incineration, dumping in landfills, or discharge into the marine environment, all of which can have negative impacts on the surrounding ecosystems (Cooney et al., 2023; Rajabimashhadi et al., 2023). Fish waste has a high organic content and is costly to dispose of (Kannan et al., 2018). Much of the waste and low-value catch is used for fishmeal and fish oil production (Suuronen & Gilman, 2020; Vázquez et al., 2020), which can indirectly lead to human consumption when reused in aquaculture and animal husbandry.

By 2030, total fish production is expected to reach 202 million tons, with aquaculture contributing 106 million tons (D'agaro et al., 2022). Considering the fishing industry's potential to generate up to 75% waste, the amount of organic waste produced is substantial and often underestimated (Alvarado-Ramírez et al., 2023). Despite efforts to reduce fish waste, quantities continue to increase annually. This waste could instead be utilized for beneficial purposes. The European Commission's "Blue Growth" initiative aims to preserve the environment from industrial pollution while optimizing the use of fish resources, such as obtaining gelatin or collagen from solid waste in surimi processing (Li et al., 2024). Efforts to reduce fish waste are crucial for improving sustainability, mitigating environmental impact, and ensuring food security for the billions who depend on fish as a source of nutrition.

In recent years, alternatives to extract bioactive substances from fish industry waste have been seen to provide health benefits such as antioxidant, antimicrobial, neuroprotective, antihyperglycemic, and anti-inflammatory. The extraction carried out is aimed at obtaining important bioactive molecules such as collagen.

3. Collagen

Collagen is a triple helix structure that consists of three spiral fibrous polypeptide chains, namely two α1 and α2 chains and one β chain that has around 1000 amino acid residues (Hou & Chen, 2023). In this context, tropocollagen is the building block of collagen. Tropocollagen is cylindrical, has a diameter of 14 to 15 Å, and has a length of 2800 Å (Tiwari et al., 2023). Tropocollagen also consists of three polypeptide units which are also known as α chains. As shown in Fig. 1, the three chains are twisted to the right to form a triple helix structure, and each α chain is coiled to the left of the helix with three residues in each turn. Hydrogen bonds between adjacent -CO and -NH groups hold all three chains together. The total molecular mass of collagen is also about 300,000 Da, and each α chain has a molecular mass of about 100,000 Da.

In the extracellular space, collagen molecules arrange themselves into microfibrils that have a quarter-stepped arrangement. The cross-linking process begins and fibrils with a larger diameter are produced either through the addition of microfibrils or through fusion with other fibrils. In the case of cross-link formation, enzymatic oxidation of amino groups ε belonging to certain peptidyl residues for lysine and hydroxylysine to the corresponding aldehyde ω in the NH_2- and COOH- ends of non-helical peptides occurs, followed by exchange to aldol and aldimine and stabilization through addition reduction or oxidation reactions (Benjakul, Nalinanon & Shahidi, 2012). The intermolecular cross-links limited to the end-overlap region involve the aldehyde lysine in the telopeptide of one chain and the hydroxylysine of the adjacent chain. Cross-linking gives collagen fibrils mechanical strength. Stable hydrogen bonds between and within collagen molecules make collagen fibrils insoluble in water (Gaikwad & Kim, 2024). In terms of amino acid composition, collagen is generally rich in glycine, proline, and alanine, but contains little or no cysteine and tryptophan. Collagen also has a lower content of tyrosine and histidine. Hydroxylysine can also be found in collagen. Only collagen is a type of protein-rich in hydroxyproline, so hydroxyproline content is generally used to measure collagen content in food. In more depth, the collagen triple helix structure results from the combination of a specific polypeptide chain, namely the α chain, with the repetition of the Glycine-X-Y domain, where X and Y are occupied by different amino acids (Oliveira et al., 2021; Rajabimashhadi et al., 2023). In this regard, proline (Pro) and hydroxyproline (Hyp) are frequently found at the X and Y positions, respectively, which contribute to 10.5% of the total tripeptide (Jafari et al., 2020).

Glycine (Gly) represents almost one-third of the total residues and is uniformly distributed at every third position along the collagen molecule, except for 14 amino acid residues starting from the N-terminus and 10 residues starting from the C-terminus. Both ends are also called telopeptides. Glycine does not contain a side chain that allows it to fit into the center of the superhelix without any steric hindrance to form a compact structure. The distribution of polar and nonpolar residues at the X position determines the orderly aggregation of molecules into fibrils. Segments of polypeptide chains consisting of repeating triplets with amino acid residues are nonpolar regions, while segments containing glycine-X-Y triplets without most amino acids are polar. The mechanical and thermal properties are also highly dependent on the content of hydroxyproline in the collagen structure because it can facilitate the combination of molecules through hydrogen bonds in the triple helix structure (Ahn et al., 2021). In addition to amino acids, collagen also contains carbohydrates, such as glucose and galactose, which are attached to hydroxylysine residues in the peptide chain through O-glycosidic bonds.

In terms of opportunities in collagen regeneration, Rajabimashhadi et al. (2023) stated that approximately 30 to 40% of the total weight of valuable protein can be extracted from fishery by-products.

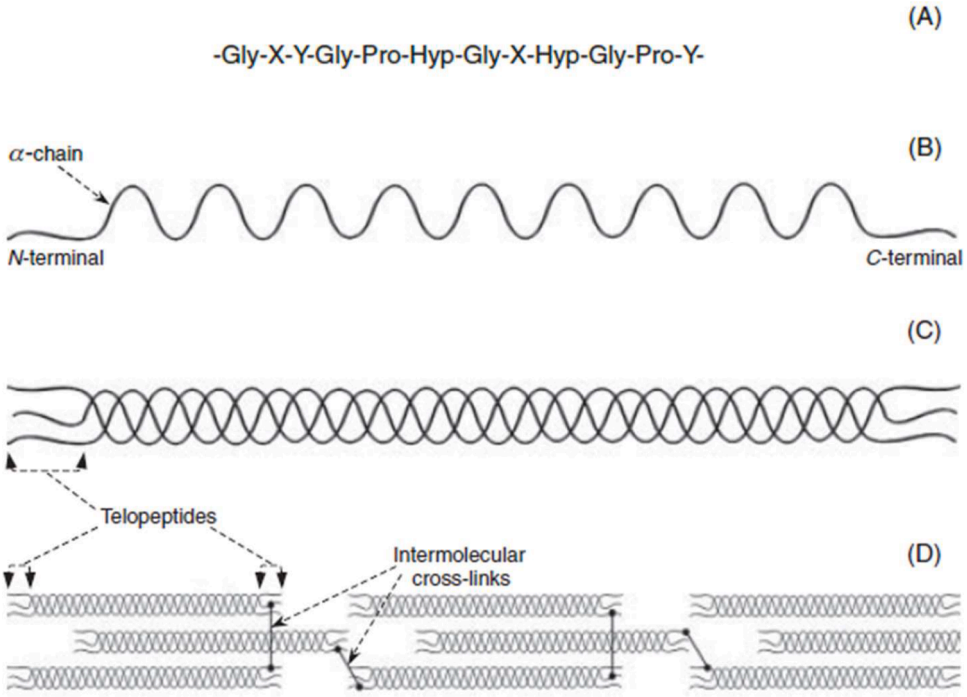

Fig. 1. The arrangement of collagen fibril in collagen fiber. (A) amino acid sequence of a collagen polypeptide. (B) Collagen polypeptide. (C) Tropocollagen. (D) Collagen fibril. (Benjakul et al., 2012).

Furthermore, fishery by-products are also now easier to obtain. Fig. 2 illustrates the parts of fish byproducts that can be utilized for collagen recovery. Furthermore, fish collagen can be purified and extracted with simpler extraction methods. Moreover, fish and humans have great ontogenetic differences. Therefore, fish collagen is free from hygiene restrictions. This means that fish collagen has a low risk of spreading bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), foot and mouth disease (FMD), avian influenza (AI), and also allergic reactions (Asaduzzaman et al., 2020; Liao et al., 2018; Song et al., 2019). Unlike collagen that comes from cows and pigs, fish collagen is free from religious and cultural restrictions, especially for Muslims, Hindus, and Jews (Melgosa et al., 2021; Menezes et al., 2020). The properties of collagen, such as low viscosity, non-toxicity, reasonable homeostatic properties, bio-resorbabilities, as well as more adaptability and metabolic compatibility, open opportunities in collagen regeneration (Oliveira et al., 2021; Rajabimashhadi et al., 2023). Furthermore, collagen also provides a minimal inflammatory response.

Based on the source of collagen, there are two types of collagen, namely mammalian collagen and fish collagen. Fish collagen and mammalian collagen share similar physicochemical properties, but the chemical composition between the two types of collagen has slight differences. In this context, fish collagen contains fewer amino acids that refer to proline and hydroxyproline when compared to mammalian collagen. In the deeper aspect of fish collagen, fish that live in cold water have a lower amino acid content than fish that live in warm water. Fish collagen is generally extracted from skin, bones, and scales, which make up about 30% of fish processing waste because those parts have a high collagen content (Tiwari et al., 2023). Muscle collagen, skin, bone, and fish scales have a significantly higher content of seven amino acids,

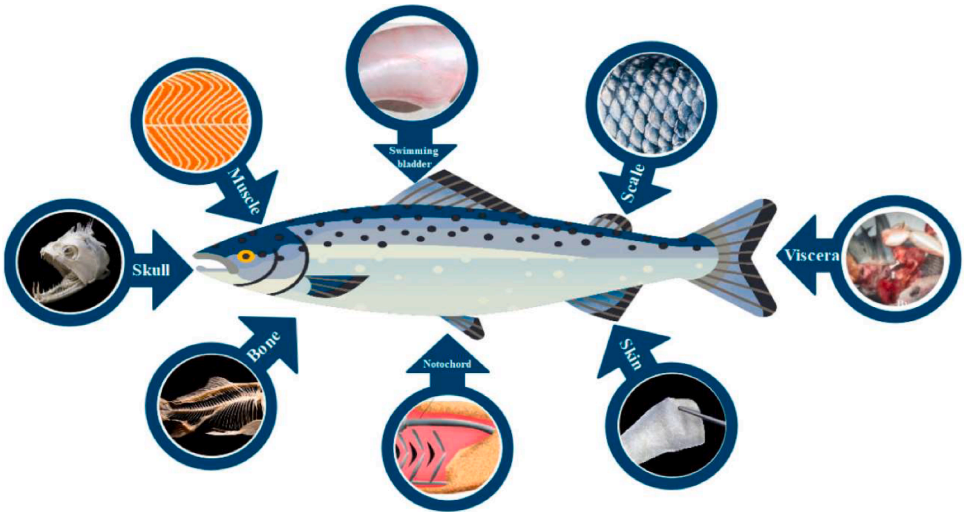

Fig. 2. Different parts of fish can be the source for collagen extraction. (Rajabimashhadi et al., 2023).

including alanine, arginine, aspartic acid or asparagine, glutamic acid or glutamine, glycine, hydroxyproline, and proline. Some fish collagens also contain small amounts of arabinose, xylose, and ribose residues. According to Gaikwad and Kim (2024), 1.25% of pepsin-soluble collagen and 57.1% of collagen were extracted from gold pomace and bighead carp bones, respectively. Based on Jafari et al. (2020), tilapia (*Oreochromis mossambicus*) bone produced 3.5% acid-soluble collagen and 6.0% pepsin-soluble collagen. Additionally, 4.535% collagen was extracted from the bones of Lutjanus sp. The combination of 1% pepsin and a pulsed electric field produced 16.13 mg/mL of collagen from the bones of *Aristichthys nobilis*. 0.1% acid-soluble collagen and 2.6% pepsin-soluble collagen were isolated from tuna bones and bigeye tuna bones, respectively.

29 types of collagens have been described and identified according to Liao et al. (2018) and Song et al. (2021). Each type of collagen is characterized by having a triple helix motif as part of its structure. Among all types of collagen, type I is the most commonly found type of collagen (Hou & Chen, 2023). According to Jafari et al. (2020) on the other hand, 80 to 90% of collagen in the body consists of types I, II, and III, which can be divided into four categories based on structure, namely fibril-forming collagen, basal membrane collagen, short-chain collagen and collagen with multiple interruptions. In this regard, collagen types I, II, III, and V are known as fibril-forming collagens and have a large proportion of homologous sequences. Type IV collagen, also known as basement membrane collagen, has a disordered three-helical conformation with large non-helical domains and short non-helical peptide disruptions. Based on the macromolecular structure, collagen can also be categorized into three main groups, namely striated fibrous collagen which includes type I, II, and III collagen, nonfibrous collagen which contains type IV and microfibrillar collagen) which includes types VI and VII (matrix microfibrils), types V, IX, and X (pericellular collagen) as well as types VIII and XI that have not yet been classified (Tiwari et al., 2023). Collagen associated with fibrils usually has small chains containing non-helical domains.

According to Benjakul et al. (2012), the distribution of α1, α2, and α3 chains in collagen molecules differs based on specific genetic variants. Different α chains in the same type of collagen may differ in their amino acid composition. For example, collagen types IV and III contain oxidizable cysteine residues and are rich in hydroxyproline and hydroxylysine. Although type V is also rich in hydroxyproline and hydroxylysine, no cysteine can be found in it. Type I collagen, the most common protein found in the human body, is also abundant in fish. In more detail, fish bone collagen exhibits the characteristics of type I collagen, which has two α1 and one α2 chains. This can be proven by Benjakul et al. (2012) who stated that collagen type I was extracted from both the bones of carp (*C. carpio*) and bigeye carp (*P. tayenus*). In the study of Nur et al. (2021), type I collagen was isolated from the bones of Lamuru fish (*Caranx ignobilis*). Type I collagen has been produced from the bones of tilapia (*Oreochromis mossambicus*), Lutjanus sp., and Aristichthys nobilis based on the review paper of Jafari et al. (2020). Type I collagen was also successfully extracted from the backbone of Baltic cod (*Gadus morhua*). According to Ferraro et al. (2013) on the other hand, the scales and bones of pelagic fish, such as sardines and mackerel fish, contain type I collagen. In addition to type I collagen, Tiwari et al. (2023) stated that type II collagen was extracted from the skeleton and head bones of the silvertip shark (*Carcharhinus albimarginatus*).

However, a lack of awareness of the recycling of these byproducts can cause losses in biomass, energy, and nutrients and become an obstacle to collagen regeneration. The characteristics of collagen, such as low molecular weight, low denaturation temperature, low gelation temperature, low melting temperature, high degradation rate, and low mechanical properties, make collagen difficult to extract in its original and complete form. This is said to be so because differences in the distribution of amino acids, such as a reduction in the amount of proline and hydroxyproline and an increase in serine, threonine, methionine, and hydroxylysine lead to labile cross-linking and high heat sensitivity of fish collagen (Song et al., 2019). Because of this, fish collagen is more easily denatured than mammalian collagen. During collagen denaturation, the hydrogen bonds that support the helical structure are partially or destroyed. As an implication, collagen loses its structural role and conformation-related biological activity and then turns into gelatin.

Acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) are widely recognized as the primary methods for collagen extraction, as highlighted by Menezes et al. (2020). Both ASC and PSC methods fall under the broader category of solvent extraction techniques, which remain the most used conventional approaches due to their effectiveness in isolating collagen from various sources. The ASC method involves treating collagen-rich materials with diluted acids, such as acetic acid, to solubilize collagen by disrupting non-covalent interactions and some cross-links within the collagen fibers. For instance, a study successfully extracted ASC from salmon skin using 0.5 M acetic acid, yielding a significant amount of collagen (Oslan et al. 2022). This method is particularly effective for extracting collagen from fish skins, scales, and other soft tissues. In contrast, the PSC method employs proteolytic enzymes like pepsin to cleave specific peptide bonds in the telopeptide regions of collagen, enhancing its solubility without compromising the integrity of the triple-helical structure. This approach has been effective in extracting collagen from various fish by-products, including scales and skins (Wu et al., 2019). Both methods have been widely adopted due to their ability to produce high-quality collagen suitable for applications in food, cosmetics, and biomedical fields. However, they also present challenges, such as the use of large volumes of acids or enzymes and extended extraction times, prompting ongoing research into more sustainable and efficient extraction techniques (Gaikwad & Kim, 2024). Some of the past studies utilizing conventional methods from fish are summarized in Table 1.

However, conventional extraction methods are complicated (Huang et al., 2016) because they require many steps to extract target components. This means longer times, larger quantities of reagents, and higher costs are involved. Besides, these methods employ toxic solvents and cause the loss of amino acids, especially when using acids or bases. As people nowadays are more and more interested in conserving the environment, a combination of environmentally friendly solvents and green techniques have been adopted for the extraction of peptides with high purity. Reducing fish waste and loss is an important part of transforming aquatic food systems to be more sustainable, productive, and equitable. Innovative technologies like subcritical water extraction can help convert fish waste into valuable products like collagen.

4. Subcritical water extraction

Subcritical water extraction (SWE) is an advanced green extraction technique that utilizes water at high temperatures and controlled pressures below the critical point (374.15°C, 22.1 MPa) to maintain the liquid state of water (Cheng et al., 2021; Mohd Thani et al., 2019). This approach offers several advantages over conventional solvent extraction methods. Water is an environmentally friendly, inexpensive, and readily available solvent that can have its physicochemical properties tuned by adjusting the temperature and pressure.

As the temperature of water increases above the boiling point of 100°C under high pressure, the hydrogen bonds between water molecules begin to dissociate. This leads to a decrease in the dielectric constant of water, causing it to behave more similarly to moderately polar organic solvents like methanol, ethanol, and acetone. Simultaneously, the viscosity and surface tension of water decrease, while the diffusion coefficient increases, enhancing the mass transfer of target compounds. Additionally, the ionic product of water (Kw) rises significantly at elevated temperatures, increasing the concentration of hydronium and hydroxide ions, which can catalyze certain extraction reactions (Mohd Thani et al., 2019; Pinto et al., 2021; Cheng et al., 2021).

These unique properties of subcritical water allow it to effectively extract a wide range of bioactive compounds, including polar and

Table 1
Summary of studies on traditional collagen extraction methods.

No	Species	Extraction Method	Process Condition	Yield (%)	Reference
1	*Oreochromis niloticus*	ASC extraction	Solvent: 0.5 M acetic acid Time: 48 h Temperature: 4°C	Red: 19.04 ± 0.59 Grey: 20.06 ± 2.20 F1: 20.28 ± 1.86	Reátegui-Pinedo et al., 2022
		PSC extraction	Solvent: 0.5 M acetic acid with 0.1% pepsin Time: 48 h Temperature: 4°C	Red: 21.10 ± 1.59 Grey: 21.21 ± 1.14 F1: 21.10 ± 1.59	
2	*Oreochromis niloticus*	ASC extraction	Fermentation pre-treatment	Solvent: 0.5 M acetic acid Solid: solvent ratio: 1:60 (w/v) Time: 48 h Temperature: 4°C	4.76 ± 0.06 (wet weight)	Song et al., 2021
			Chemical pre-treatment		4.27 ± 0.09 (wet weight)	
		PSC extraction	Fermentation pre-treatment	Solvent: 0.5 M acetic acid with 5% pepsin (w/w) Solid: solvent ratio: 1:80 (w/v) Time: 24 h Temperature: 4°C	8.14 ± 0.60 (wet weight)	
			Chemical pre-treatment		7.60 ± 0.06 (wet weight)	
3	*Sardinella longiceps*	ASC extraction	Solvent: 0.5 M acetic acid Time: 4 days Temperature: 4°C	Scale: 35.24 (wet weight) Skin: 47.48 (wet weight) Muscle: 48.45 (wet weight)	Srinivasan & Durairaj, 2021	
		PSC extraction	Solvent: 0.5 M acetic acid with 1.5% pepsin Solid: solvent ratio: 1: 15 (w/v) Time: 4 days Temperature: 4°C	Scale: 58.87 (wet weight) Skin: 66.24 (wet weight) Muscle: 69.09 (wet weight)		
4	*Oreochromis niloticus*	Acetic acid method with ultrafine bubbles	Solvent: 5 L 0.1 M / 0.5 M acetic acid Mass of scale: 125 g Type of gas bubbles: O2, CO2 / O3 Gas flow rate: 3 L/min Time: 6 h Temperature: 25°C	0.1 M acetic acid, CO2, 5 h: 1.58 (dry weight)	Kuwahara, 2021	
5	*Sardinella longiceps*	ASC extraction	-	2.50 (dry weight)	Bailore et al., 2020	
6	Nile tilapia (*Oreochromis niloticus*)	ASC extraction	Acetic acid concentration: 0.35/ 1.05 mol L-1 Temperature: 4/ 20°C Time: 31, 45/ 65 h Sample: solution ratio: 1:25	0.35 mol L-1, 4°C, 31 h: 15.3 (wet weight) 1.05 mol L-1, 4°C, 31 h: 13.5 (wet weight) 0.35 mol L-1, 20°C, 31 h: 15.0 (wet weight) 1.05 mol L-1, 20°C, 31 h: 14.5 (wet weight) 0.35 mol L-1, 4°C, 65 h: 15.2 (wet weight) 1.05 mol L-1, 4°C, 65 h: 13.7 (wet weight) 0.35 mol L-1, 20°C, 65 h: 19.0 (wet weight) 1.05 mol L-1, 20°C, 65 h: 18.3 (wet weight)	Menezes et al., 2020	
7	Tilapia	Acetic acid method	Solvent: 1000 mL 0.5 M acetic acid Mass of skin: 50 g Time: 24 h Temperature: Room temperature	11.7 (wet weight)	Bi et al., 2019	
		Hot water method	Solvent: 1000 mL ultrapure water Mass of skin: 50 g Time: 6 h Temperature: 80°C Rotation speed (oscillator water bath shaker incubator): 10 rpm	10.7 (wet weight)		
		Sodium hydroxide method	Solvent: 1000 mL 0.12 M NaOH Mass of skin: 50 g Time: 24 h Temperature: Room temperature	8.5 (wet weight)		
8	*Oreochromis niloticus*	ASC extraction	Solvent: 0.5 M acetic acid Sample: solution ratio: 1: 50 (w/v) Time: 48 h Temperature: 4°C	19.07 (dry weight)	Song et al., 2019	
		PSC extraction	Solvent: 0.5 M acetic acid with 0.1% (w/v) pepsin Sample: solution ratio: 1: 50 (w/v) Time: 48 h Temperature: 4°C	19.61 (dry weight)		
9	*Oreochromis niloticus*	ASC extraction	First extraction Solvent: 0.5 M acetic acid Sample: solution ratio: 1: 50 (w/v) Time: 48 h Temperature: < 10°C Second extraction Solvent: 0.5 M acetic acid Sample: solution ratio: 1: 10 (w/v) Time: 18 h Temperature: < 10°C	19.80 (dry weight)	Li et al., 2018	
		PSC extraction	First extraction Solvent: 0.5 M acetic acid with 0.1% (w/v) pepsin Sample: solution ratio: 1: 50 (w/v) Time: 48 h; Temperature: < 10°C Second extraction 0.5 M acetic acid with 0.1% (w/v) pepsin Time: 48 h	20.03 (dry weight)		

(*continued on next page*)

Table 1 (*continued*)

No	Species	Extraction Method	Process Condition	Yield (%)	Reference	
10	*Oreochromis niloticus*	PSC extraction	-	52.6 ± 6.1 (dry weight)	Liao et al., 2018	
	Lates calcarifer			47.3 ± 3.7 (dry weight)		
11	*Oreochromis aureus*	ASC extraction	Solvent: 0.5 M acetic acid Sample: solution ratio: 1: 50 (w/v) Time: 3 days	11.37 ± 0.88 (dry weight)	Valenzuela-Rojo et al., 2018	
12	*Oreochromis niloticus*	ASC extraction	Solvent: 0.5 mol/L acetic acid Time: 24 h	Scale: 3.2 (dry weight)	Chen et al., 2016	
				Skin: 27.2 (dry weight)		
13	*Oreochromis mossambicus*	ASC extraction	Decalcification (pre-treatment) 0.5 mol/L EDTA (pH 7.4, 4°C), 9 days / 0.6 mol/L HCl (4°C), 9 h	Extraction (2 times) Solvent: 200 mL 0.5 mol/L acetic acid Sample: solution ratio: 1: 20 g/mL; Time: 24 h	EDTA: 2.5 (dry weight)	Liu & Huang, 2016
		PSC extraction		Extraction Solvent: 200 mL 0.5 mol/L acetic acid + 1000 U pepsin Temperature: 4°C Time: 96 h	EDTA: 7.3 (dry weight)	
					HCl: 0.5 (dry weight)	
14	*Sardinella longiceps*	ASC extraction	Solvent: 0.5 M acetic acid Time: 4 days Temperature: 4°C	1.25 (dry weight)	Muthumari, Anand & Maruthupandy, 2016	
		PSC extraction	Solvent: 0.5 M acetic acid with pepsin (40 unit/ g waste) Solid: solvent ratio: 1: 15 (w/v) Time: 4 days Temperature: 4°C	3.00 (dry weight)		
15	*Oreochromis niloticus*	ASC extraction	Solvent: 60 vol. lactic acid (pH 2.3) Time: 46 h; Temperature: 4°C	25.6 ± 5.0 (dry weight)	Zhang et al., 2016	
		PSC extraction	Solvent: 50 vol. lactic acid (pH 1.8) with pepsin (50 U/ g skin) Time: 17 h; Temperature: 4°C	21.8 ± 2.8 (dry weight)		
	Ictalurus punctatus	ASC extraction	Solvent: 60 vol. lactic acid (pH 2.3) Time: 46 h; Temperature: 4°C	25.9 ± 2.7 (dry weight)		
		PSC extraction	Solvent: 50 vol. lactic acid (pH 1.8) with pepsin (50 U/ g skin) Time: 17 h; Temperature: 4°C	28.8 ± 4.6 (dry weight)		
16	*Sardinops melanostictus* (Sardine)	PSC extraction	Solvent: 0.5 M acetic acid with 10% (w/w) pepsin Scale mass: 5 g Time: 24 h Temperature: 4°C	50.9 (dry weight)	Nagai et al., 2004	
	Pagrus major			37.5 (dry weight)		

moderately polar substances, from various natural sources. Compared to traditional extraction techniques, SWE is a greener, faster, and more efficient method that can be optimized to target specific classes of compounds based on their polarity. The versatility and environmental benefits of SWE make it a promising technique for the isolation of valuable bioactive ingredients from renewable natural sources. Subcritical water extraction of collagen can be performed using either batch or continuous systems. In a batch system, the setup includes a stainless-steel reactor (Fig. 3) and a salt or oil bath, a heating coil, and a K-type thermocouple for precise temperature control. The reactor is made from stainless steel, generally 2 cm in diameter, 17 cm in length, and has a maximum internal capacity of 5 mL. Its compact size ensures efficient heat transfer and pressure control, while the tightly sealed cap prevents vapor loss and maintains system integrity during extraction.

Meanwhile for the heating system (Fig. 4), the choice between an oil bath and a salt bath depends largely on the targeted temperature range. The oil bath system is commonly used for SWE processes operating between 100°C and 200°C. Silicone oil is often selected as the heating medium due to its excellent heat transfer properties and stability within this temperature range. As for higher temperatures, typically ranging between 200°C and 374°C, a salt bath system is more suitable. The salt bath relies on a mixture of potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$) in a 1:1 ratio due to its superior heat retention and stable thermal properties at elevated temperatures.

The SWE process itself follows a structured workflow. First, fish waste materials such as skins, scales, or bones are prepared by cutting them into small pieces and weighing them to ensure consistency. The prepared sample is then placed inside the stainless-steel reactor, along with a measured volume of distilled water. The reactor is securely sealed and immersed in the appropriate heating bath. The heating coil and thermocouple system maintain the desired temperature throughout the process. Once the extraction is complete, the reactor is removed and allowed to cool before the pressure is carefully released. The extracted solution is then filtered to separate the collagen from any insoluble residues. In some cases, additional concentration or freeze-drying steps may be carried out to obtain purified collagen powder (Mohd Thani et al., 2019).

The efficiency of subcritical water extraction is affected by many factors, but according to Basak and Annapure (2022), the main variables consist of temperature, pressure, liquid-to-solid ratio, and extraction time. The first parameter that affects the extraction efficiency is the extraction temperature. In subcritical water extraction (SWE), water is maintained in a liquid state at temperatures between 100°C and 374°C under pressurized conditions (0.1 – 22 MPa). As the temperature increases within this range, several physicochemical properties of water change significantly. However, subcritical water extraction must be done at the highest temperature allowed because according to Rodrigues et al. (2021), the temperature has the most significant effect on the

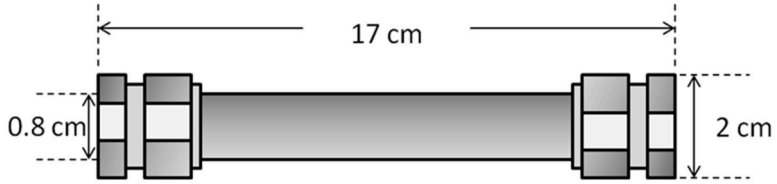

Fig. 3. The stainless-steel reactor (Mohd Thani et al., 2019).

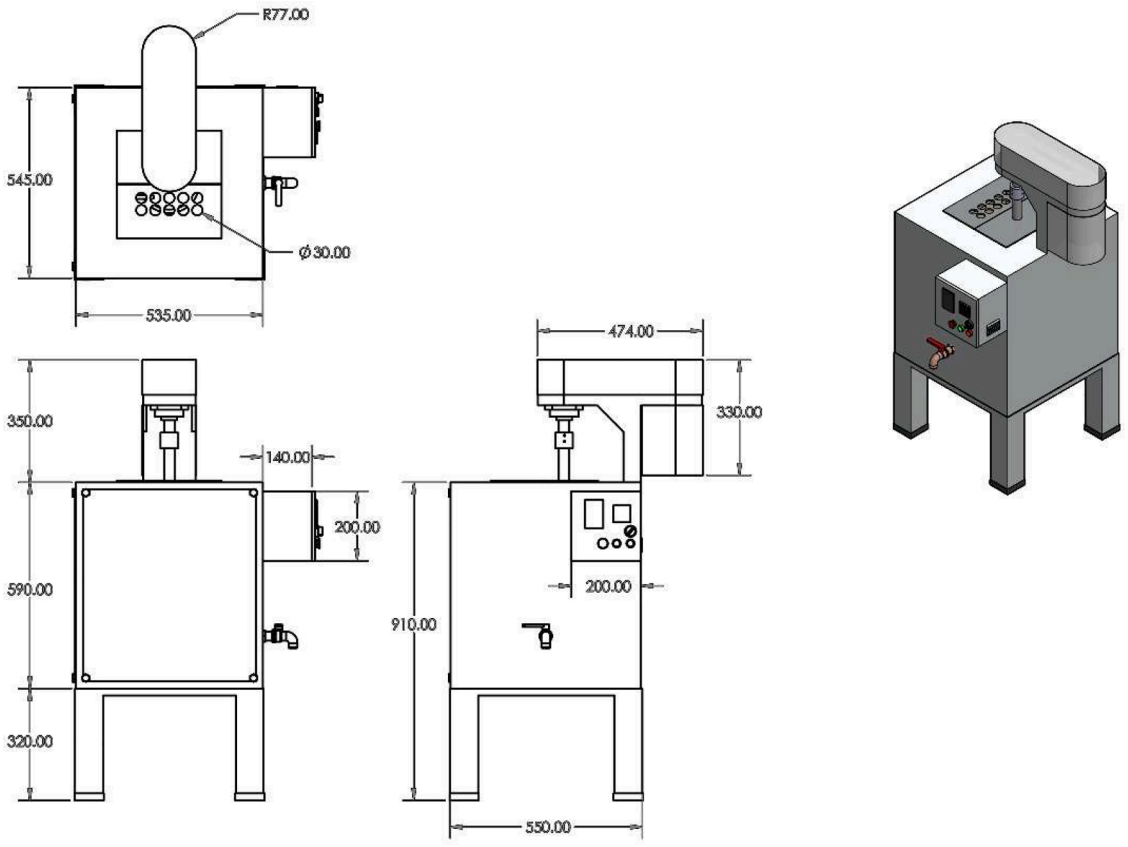

Fig. 4. The salt or oil bath for subcritical water extraction (Mohd Thani et al., 2022).

efficiency of subcritical water extraction, so the temperature can be optimized. However, increasing the extraction temperature beyond the optimum value leads to degradation of the target components. So, the maximum allowable extraction temperature must be obtained from experiments for different compounds. Based on Cheng et al. (2021), pressure has no significant effect on the efficiency of subcritical water extraction to reproduce compounds from natural products. However, pressure is still one of the variables that cannot be neglected because the pressure needs to be ensured high enough, which is usually from 10 to 80 bar (Zhang et al., 2020), to keep water in liquid form.

The liquid-to-solid ratio, or solid loading, is a crucial parameter in subcritical water extraction (SWE) as it directly influences the efficiency and yield of the process. An optimal ratio ensures adequate interaction between the solvent and the solute, facilitating effective extraction. According to Ravber, Knez, and Škerget (2015), a ratio of 1:15 g/mL achieved the highest extraction yield (15.8%), highlighting this as an optimal condition for maximizing efficiency. They also reported that higher liquid-to-solid ratios require more water to be compressed and heated, resulting in increased energy consumption and operational costs. Despite this, some studies have adopted significantly higher ratios, such as 1:200 (w/v), as seen in research by Asaduzzaman et al. (2020), Haq et al. (2019), and Ahmed and Chun (2018). These higher ratios may be chosen to enhance the solubility of certain compounds or to reduce issues related to sample agglomeration. However, excessively high ratios may lead to diluted solutions, ultimately reducing extraction efficiency. Given these varying approaches, selecting an appropriate liquid-to-solid ratio is essential to achieving maximum extraction yield while balancing resource efficiency and cost-effectiveness. Optimizing this parameter requires consideration of both the target compound's solubility and the economic feasibility of the extraction process.

The next major variable is extraction time. In this context, an extraction time that is too long to exceed the optimal time can damage the structure of the target component (Zhang et al., 2020). As such, an optimal extraction time is required. Another parameter is the water flow rate. The extraction rate will be faster when a higher flow rate is used. An increase in flow rate causes an increase in superficial velocity leading to faster mass transfer. However, the use of a higher water flow rate increases the extract volume leading to a lower final extract concentration. Therefore, the best flow rate must be selected considering two important factors, including the extraction time and the final extract concentration. Shorter extraction times and more concentrated extracts are usually desirable. In addition to the main parameters and water flow rate, particle size can also affect efficiency and extraction results. For small-sized particles, the extraction yield is higher, and the extraction time is shorter. This is said to be so because the small particle size reduces the diffusion distance for components in the matrix (Zhang et al., 2020). Not only that, the contact area between the sample matrix and the extractor increases. As an implication, the extraction efficiency is increased. In brief, various parameters need to be well controlled to obtain the most high-quality extraction results.

Previous studies have demonstrated the application of subcritical water for hydrolysis and extraction processes. Specifically, Ahmed and Chun (2018), Asaduzzaman et al. (2020), Haq et al. (2019), and Melgosa et al. (2021) utilized subcritical water hydrolysis to produce protein hydrolysates. These studies focused on extracting collagen or protein components from aquaculture sources using enzymatic or chemical methods. In the context of subcritical water extraction, research conducted by Cardeira et al. (2022), Hao et al. (2019), Melgosa et al. (2021), and Rodrigues et al. (2021b) successfully reproduced collagen or other protein components from aquaculture products. The results of these studies are summarized in Table 2, highlighting the four applications of subcritical water extraction. Moreover, the studies listed in Table 2 employed different samples and process conditions for subcritical water extraction, except for the works of Ahmed and Chun (2018) and Haq

Table 2
Summary of studies on collagen extraction using subcritical water.

No	Species	Subcritical Water Extraction Process Condition	Yield (%)	Reference
1	*Sardina pilchardus* (Sardin)	Mass of sample: 60 g Flow rate: 10 mL/ min Temperature: 190 & 250°C Pressure: 100 bar Time: 30 min	45.7 ± 2.8 (190°C, remove fat) 58.5 ± 0.4 (250°C) 61.7 ± 2.0 (250°C, remove fat)	Cardeira et al., 2022
2	*Gadus morhua* (Cod Fish)	Mass of sample: 60 g Flow rate: 10 mL/min Pressure: 100 bar Temperature: 90, 140, 190 & 250°C Holding time: 30 min	13.2 ± 0.5 (90°C) 27.7 ± 0.5 (140°C) 41.4 ± 0.5 (190°C) 53.9 ± 0.5 (250°C)	Melgosa et al., 2021
3	*Cancer pagurus*	Solid loading: 1:5, 1: 10 & 1: 15 g/mL Temperature: 150, 200 & 250°C Heating rate: 3 & 6°C/min Pressure: 100 bar	11.1 ± 0.11 (150°C, 1: 10 g/mL, 6°C/min) 14.2 ± 0.48 (200°C, 1: 10 g/mL, 6°C/min) 15.5 ± 0.11 (250°C, 1: 10 g/mL, 6°C/min) 10.6 ± 0.42 (200°C, 1: 15 g/mL, 6°C/min) 15.8 ± 0.11 (200°C, 1: 15 g/mL, 6°C/min) 15.0 ± 0.11 (200°C, 1: 10 g/mL, 3°C/min)	Rodrigues et al., 2021
4	*Scomber japonicus* (Mackerel)	Solid loading: 1:200 (w/v) Temperature: 200- 250°C Pressure: 30- 70 bar Time for reaching desirable temperature: 36- 54 min Holding time: 3 min	-	Asaduzzaman et al., 2020
5	*Haliotis discus hannai* Ino (Abalone)	Solid loading: 1: 15 (w/v) Combination of temperature and pressure: 110°C (0.15 MPa); 140°C (0.36 MPa); 170°C (0.80 MPa); 200°C (1.60 MPa); 230°C (2.90 MPa) Extraction time: 60 min	28 ± 1 (110°C) 34 ± 1 (140°C) 46 ± 2 (170°C) 41 ± 1 (200°C) 36 ± 2 (230°C)	Hao et al., 2019
6	*Bigeye tuna*	Solid loading: 1:200 (w/v) Temperature: 250°C Pressure: 50 bar Time for reaching desirable temperature: 45 min Holding time: 3 min	-	Haq et al., 2019
7	*Bigeye tuna*	Solid loading: 1:200 (w/v) for collagen; 1: 50 (w/v) for skin Temperature: 120 -300°C Pressure: 50 bar for 120-250°C, 80 bar for 280°C, 100 bar for 300°C Reaction time after reaching desirable temperature and pressure: 5 min	-	Ahmed and Chun, 2018

et al. (2019). Although these two studies share similarities in process parameters, Haq et al. introduced a combination of various catalysts, such as sodium chloride, acetic acid, and sodium bicarbonate, to enhance the hydrolysis process. These catalysts play distinct roles in facilitating the breakdown of complex biomolecules. Sodium chloride enhances the ionic strength of the solution, which can modify the solubility of organic compounds and improve the extraction of target molecules. Acetic acid, being a weak organic acid, reduces the pH of the extraction medium, which accelerates the hydrolysis of ester and glycosidic bonds. This acid-catalyzed process is particularly effective in breaking down complex carbohydrates and lipids, releasing valuable bioactive compounds. Meanwhile, sodium bicarbonate acts as a buffering agent, promoting an alkaline environment that enhances protein hydrolysis, facilitating the conversion of proteins into smaller peptides and amino acids (Barea et al., 2025).

In addition to Haq et al.'s findings, several other studies have explored the use of catalysts in SWE for fish waste hydrolysis. For instance, Asaduzzaman et al. (2020) evaluated various catalysts, including formic acid, acetic acid, sodium hydroxide, sodium bicarbonate, carbon dioxide, and nitrogen gas, for the hydrolysis of deoiled mackerel muscle. Their results indicated that sodium bicarbonate was particularly effective, achieving over 99% hydrolysis yield at 260°C and 70 bar pressure. The integration of catalysts such as sodium chloride, acetic acid, and sodium bicarbonate in SWE systems offers considerable potential for improving hydrolysis efficiency. By accelerating the breakdown of complex biomolecules, these catalysts enhance the release of valuable compounds, making the process more effective for fish waste valorization.

While subcritical water extraction (SWE) has shown promise for the recovery of collagen from various sources, several key advancements are needed to enhance its efficiency and enable widespread adoption. Careful optimization of the extraction parameters, including temperature, pressure, and time, is crucial to maximize the yield and quality of the extracted collagen while avoiding thermal degradation. Thorough characterization of the physicochemical, structural, and functional properties of the extracted collagen, such as molecular weight, amino acid composition, thermal stability, and bioactivity, is essential to ensure its suitability for diverse applications.

Scaling up the SWE process from the laboratory to the industrial scale requires innovations in equipment design, process automation, and cost-effective implementation (Zhang et al., 2020). More comprehensive comparative studies are needed to fully evaluate the advantages and limitations of SWE over conventional solvent-based extraction methods

in terms of extraction yield, purity, and cost-effectiveness. Additionally, the potential to extract collagen from various fish industry waste streams, such as skin, bones, and scales, using SWE should be further explored to improve the sustainability and economic viability of the process (Majeed et al., 2024). Finally, addressing the regulatory requirements for the use of SWE-extracted collagen in food, pharmaceutical, and cosmetic products is crucial for the widespread adoption of this technology. Overcoming these key challenges through continued research and development will help unlock the full potential of SWE for the sustainable and high-quality recovery of collagen from renewable sources.

5. Conclusion

The application of subcritical water extraction (SWE) for valorizing fish waste into high-value collagen presents a promising and sustainable approach to addressing environmental concerns in the seafood industry. This review has provided a comprehensive overview of the current state and future potential of SWE, highlighting its advantages over traditional collagen extraction methods. While SWE is often claimed to have lower energy consumption, this aspect requires further exploration, as detailed studies directly comparing the energy efficiency of SWE to conventional methods are currently limited. Future research should focus on quantifying energy usage across various SWE conditions to better support this claim.

Despite these gaps, ongoing research efforts are actively working to optimize SWE parameters, such as temperature, pressure, and catalyst selection, to improve extraction efficiency and product quality. Integrating SWE with other green technologies may further enhance its potential as a sustainable solution for fish waste valorization. As the seafood industry continues to generate substantial amounts of waste, SWE offers a significant opportunity to convert this underutilized resource into valuable bioactive compounds. With continued development and improved understanding of its energy efficiency and application potential, SWE could play a pivotal role in promoting a more circular and sustainable bioeconomy.

Ethical Statement

We, the authors of the manuscript titled " **Subcritical Water Extraction (SWE) for valorizing fish waste into collagen: Current state and future prospects**," hereby declare that this manuscript is an original work and has not been previously published, nor is it under consideration for publication elsewhere. We confirm that all authors have read and approved the final version of the manuscript, and have agreed to its submission to Applied Food Research.

We also declare that:

Data Integrity

The data presented in this manuscript are accurate and have been obtained following ethical guidelines. We have ensured that all data used in the research are authentic and have not been fabricated, falsified, or manipulated.

Human and Animal Rights

This research did not involve human participants or animals. Therefore, no ethical approval or informed consent was required.

Plagiarism

We confirm that the manuscript is free from plagiarism and that proper citations and references have been provided for all sources of information and previous work.

By submitting this manuscript, we accept responsibility for the integrity of the work and agree to comply with all ethical guidelines and policies of Applied Food Research.

Acknowledgments

This work was financially supported by the Geran Galakan Penyelidik Muda (GGPM) provided by Universiti Kebangsaan Malaysia (UKM) under Grant No. GGPM-2022-039. We gratefully thank the Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia for their support of this study.

CRediT authorship contribution statement

Nurfatimah Mohd Thani: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Angel Hui Zi Ling:** Writing – review & editing, Formal analysis, Data curation. **Ameera Khaireena Azmi:** Writing – review & editing, Formal analysis, Data curation. **Suryati Mohd Thani:** Writing – review & editing, Formal analysis.

Declaration of competing interest

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

Acknowledgment

This work was financially supported by the Geran Galakan Penyelidik Muda (GGPM) provided by Universiti Kebangsaan Malaysia (UKM) under Grant No. GGPM-2022-039. We gratefully thank the Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia for their support of this study.

Data availability

No data was used for the research described in the article.

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