Trends in Food Science & Technology 152 (2024) 104684

Contents lists available at ScienceDirect

Trends in Food Science & Technology

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

Recent advances in the use of antarctic krill (*Euphausia superba*) as a sustainable source of high-quality protein: A comprehensive review

Shiying Tang [a], Jing Jing Wang [e], Yufeng Li [f], Pradeep K. Malakar [a], Yong Zhao [a,b,c,d,*]

[a] College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
[b] Laboratory of Quality & Safety Risk Assessment for Aquatic Products on Storage and Preservation (Shanghai), Ministry of Agriculture and Rural Affairs, Shanghai 201306, China
[c] Marine Biomedical Science and Technology Innovation Platform of Lin-gang Special Area, Shanghai 201306, China
[d] Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, Shanghai 201306, China
[e] Guangdong Provincial Key Laboratory of Intelligent Food Manufacturing, Foshan University, Foshan 528225, China
[f] College of Biological and Food Engineering, Anhui Polytechnic University, Wuhu 241000, China

ARTICLE INFO

Handling Editor: Dr. R.Y. Yada

Keywords:
Marine protein
Extraction process
Amino acids
Protein modification
Bioactivity
Edible safety

ABSTRACT

Background: With rapid population growth and improved quality of life, the global demand for protein is expected to double by 2050. This increasing awareness has created an urgent need for the food industry to explore more food-based protein sources. Among marine foods, Antarctic krill emerges as a promising and abundant species. Recently, this underutilized protein source has garnered significant attention.

Scope and approach: This review focuses on the current status of research on Antarctic krill as a potential protein source. First, a method for extracting Antarctic krill proteins (AKPs) is introduced, and the functional properties and modification techniques of AKPs are described. Then, the biological activities and mechanisms of action of AKP peptides are elucidated. Finally, the current challenges faced in the application of AKPs as a protein source in the human diet, as well as potential strategies to overcome these challenges, are described.

Key findings and conclusions: The isoelectric solubilization precipitation method is currently the most commonly used method to obtain AKPs, and the amino acid composition of the isolated protein meets the needs of the human body. Endogenous enzymes in krill destroy the functional properties of proteins (solubility, gelation, emulsification, etc.), but the functional properties of proteins can be improved using appropriate modification methods. Active peptides with multiple biological activities that can be obtained from AKP through incubation with enzyme preparations are food nutrients with broad application prospects. Overcoming the limitations of the fluoride residues in AKPs and AKP allergenicity represents a major challenge in incorporating AKPs into food applications. Therefore, AKP has enormous potential for future human consumption and utilization.

1. Introduction

The rapid increase in the global population has led to a situation where limited food resources are unable to meet people's demands for protein, energy, and nutrition, posing a major challenge in the 21st century (Yuan, Ye, Hou, & Chen, 2023; Zhang, Boateng, & Xu, 2023). It is estimated that by 2050, the world population will increase to a staggering 10 billion. Global food production needs to increase by 50% to meet nutritional needs (Hadidi, Palacios, McClements, Mahfouzi, & Moreno, 2023). For the past half-century, animals and plants have been the primary sources of edible protein (Hadidi et al., 2023; Wu et al., 2023; Zhang et al., 2023). However, traditional livestock industries are placing increasing pressure on land, freshwater, and resources. Plant seeds contain antinutritional compounds (such as lectins and protease inhibitors) that hinder protein digestion (Zhang, Ahmmed, Regenstein, & Wu, 2024). Therefore, continuing to explore new sustainable and renewable protein sources to reduce the ecological burden caused by the livestock industry, meet human nutritional needs, and increase public acceptance has become an unstoppable trend.

Marine foods, also known as "blue foods," primarily include marine fish, vertebrates, invertebrates, algae, and aquatic plants (Yu et al., 2023; Zhang et al., 2024). In recent years, marine food has attracted

* Corresponding author. College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China.
E-mail addresses: tangshiying1996@163.com (S. Tang), w_j2010@126.com (J.J. Wang), lyfahpu2018@126.com (Y. Li), pkmalakar@gmail.com (P.K. Malakar), yzhao@shou.edu.cn (Y. Zhao).

https://doi.org/10.1016/j.tifs.2024.104684
Received 31 January 2024; Received in revised form 20 August 2024; Accepted 23 August 2024
Available online 31 August 2024

great interest in the food and nutrition sectors. Antarctic krill (*Euphausia superba*) is one of the most abundant crustaceans in the Southern Ocean, with a total biomass of approximately 300–500 million tons (Shao et al., 2023). The enormous biomass of Antarctic krill serves as a crucial link in the Southern Ocean food chain between primary producers (phytoplankton) and top consumers (such as penguins and seals) (Zeng et al., 2024). To prevent disruption of the ecological balance of the Southern Ocean and to maintain the sustainability of the krill fishery, the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) has set a catch limit of 620,000 tons for Antarctic krill. In 2022, the global catch of Antarctic krill was only approximately 420,000 tons; thus, nearly 33% of the allowable catch was underutilized (CCAMLR Secretariat, 2023). Previous studies have indicated that Antarctic krill contains 77.9%–83.1% moisture, 0.4%–3.6% lipids, 11.9%–15.4% protein, and approximately 2% chitin and glucides (Suzuki & Shibata, 1990). Currently, a significant portion of Antarctic krill is primarily utilized for low-value products, such as animal feed and bait (Kaur, Kortner, Benitez-Santana, & Burri, 2022). Within the food industry, Antarctic krill is processed to produce krill oil, known for its health benefits, while the remaining krill biomass post-oil extraction is typically discarded (Gao, Ding, Liu, & Xu, 2024). Notably, Antarctic krill contains 65% protein content based on dry weight, and the residual byproduct after defatting also retains more than 65% protein (Li et al., 2021; Yao et al., 2023). The biological value of krill protein has been reported to be higher than that of other meat proteins and milk proteins, specifically casein (Chen, Tou, & Jaczynski, 2009). Antarctic krill proteins (AKPs) exhibit a balanced amino acid composition with an amino acid score ranging from 0.85 to 1.00. Although this score is lower than that of egg protein (1.21), the essential amino acids provided by AKP surpass the requirements for healthy adults (Tou, Jaczynski, & Chen, 2007). Additionally, chemical analysis further supports the high quality of AKPs, indicating that they contain all nine essential amino acids (EAAs), and the ratio of EAAs to total amino acids in AKP exceeds 40%, with the ratio of EAAs to non-essential amino acids (NEAAs) exceeding 60% (Chen et al., 2009; Zheng et al., 2021). The amino acid composition aligns with the ideal model proposed by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), and the United Nations University (UNU) (FAO/WHO/UNU, 2007). Based on animal feeding trials, the digestibility, net protein utilization, and protein efficiency ratio of AKPs are comparable to those of casein (Gigliotti, Jaczynski, & Tou, 2008). Moreover, in a study where adult men were fed boiled krill or whole eggs for 21 days, the net protein utilization values were found to be 55% and 61%, respectively, with no significant differences in their digestibility (Suzuki & Shibata, 1990). These advancements suggest that proteins derived from Antarctic krill may increasingly serve as viable substitutes for proteins sourced from terrestrial sources, including land animals and plants.

Both the whole Antarctic krill and the residual biomass after oil extraction can serve as high-quality raw materials for extracting AKPs (Hu, Wu, et al., 2021; Li et al., 2020). Currently, isoelectric solubilization precipitation (ISP) extraction methods have been widely applied for AKP extraction (Li et al., 2023). Although physical extraction methods have also been incorporated into AKP extraction, these are typically performed in conjunction with the ISP method (Li et al., 2022; Yao et al., 2023). Similar to other food-derived proteins, AKPs possess functional properties, including solubility, gelation, and emulsification (Li et al., 2020; Hu, Wu, et al., 2021). However, AKPs often exhibit poor solubility due to the rich endogenous protease system in krill, which can degrade their functional properties (Wang et al., 2022; Zeng et al., 2024). To enhance the functional properties of AKPs, chemical and physical modification methods can be applied to broaden their applications in food (Wang et al., 2022; Zeng et al., 2024). In terms of bioactivity, protease digestion of AKPs releases specific active peptide sequences with effects such as antioxidant, hypoglycemic, and antihypertensive activities (Zhang et al., 2021; Zheng et al., 2024). Krill shells contain high levels of fluoride, which transfer to the muscle post-mortem, resulting in significant fluoride residues in the protein. Excessive fluoride intake can have negative health effects (Peng et al., 2024; Yao et al., 2023). Additionally, the allergenicity of AKPs is a major reason why they are not tolerated by certain populations (Lin, Chi, Ni, Zhang, & Liu, 2023; Wang et al., 2023). Therefore, fluoride residues and allergenicity are the main risks of AKPs in human health and food industry production. Despite the numerous studies conducted on AKPs, to the best of our knowledge, no comprehensive systematic review of AKPs has been published to date.

Based on this background, this review systematically revisits the recent research status of AKPs. This review primarily introduces the advantages and disadvantages of existing and emerging AKP extraction technologies. Additionally, the functional characteristics of AKPs and the development prospects of modified AKPs for nutritional health and commercial value are analyzed. The bioactivity of AKP peptides and their potential mechanisms of action are further described. Finally, this review systematically analyzes the risks of AKPs as a dietary protein source in human health and the food industry. The results of this review help us further understand the current research status of AKPs and the challenges they face, providing new directions for the application of AKPs in food science and business. However, the main purpose of this review is to emphasize that Antarctic krill is a reliable and potential alternative source of protein for human nutrition and global protein supply development.

2. AKP extraction

Proteins from Antarctic krill must undergo appropriate separation and purification methods to obtain pure components with well-defined compositions and structural and functional properties. This process involves removing nonprotein substances from Antarctic krill to isolate complete AKPs. Various methods, such as chemical, physical, or biological means, can be employed for this purpose. Furthermore, the preprocessing of Antarctic krill after harvesting is crucial. Krill contain a significant number of endogenous proteases and should be rapidly frozen after harvesting. Thawing krill at low temperatures before protein extraction is necessary to prevent endogenous proteases from compromising protein integrity (Zheng, Beamer, Matak, & Jaczynski, 2019). Alternatively, defatted Antarctic krill powder can be utilized as a raw material for protein extraction. However, the protein in defatted krill powder may undergo severe denaturation during hot air drying and organic solvent extraction, posing a significant challenge for protein recovery (Shi et al., 2023). To date, various techniques have been used for the extraction of AKPs, including isoelectric solubilization precipitation extraction, ultrasound-assisted extraction, and enzymatic extraction (Fig. 1). Additionally, this chapter also explores green protein extraction technologies to provide insights for future AKP extraction processes.

2.1. Isoelectric solubilization precipitation

Isoelectric solubilization precipitation (ISP) is the most commonly used method for extracting proteins from Antarctic krill. This method separates proteins based on their solubility at different pH levels (Zheng et al., 2019, 2021). The specific steps begin with homogenizing Antarctic krill or its defatted byproducts in an alkaline solution (pH 11 to 13). The purpose of this process is to dissolve the myofibrillar and sarcoplasmic proteins in the krill. Subsequently, nonprotein impurities, such as the krill shell and lipids are removed through centrifugation or filtration. The pH of the solution is then adjusted to the isoelectric point (pI) of the protein using a dilute acid solution, which can precipitate up to 90% of the dissolved protein (Sasidharan & Venugopal, 2020; Zheng et al., 2021). The AKP extraction rate is significantly affected by different pH environments, typically showing the lowest solubility near the protein's pI and increasing as the pH moves away from the pI (Qi, Liao, Wang, Lin, & Xue, 2016). This phenomenon occurs because the

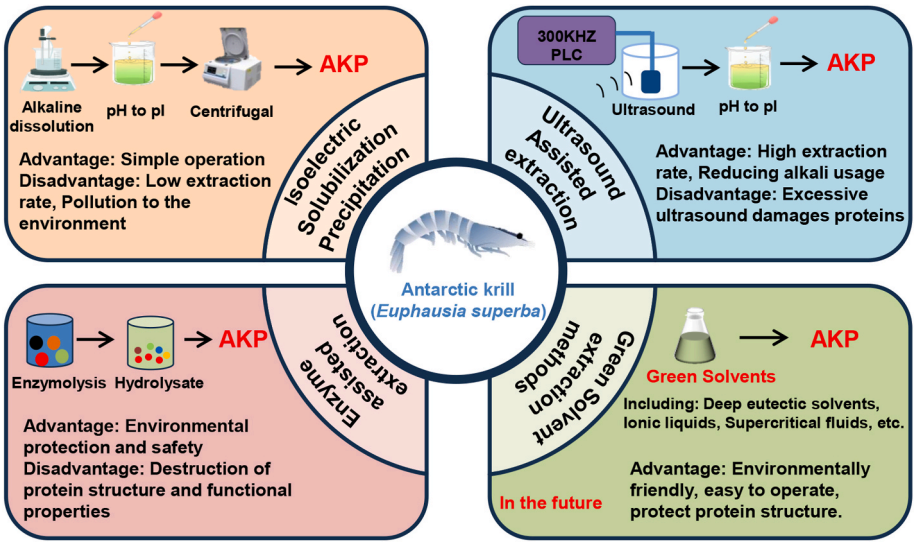

Fig. 1. Different extraction methods of Antarctic krill proteins and their advantages and disadvantages.

intermolecular forces in the solution weaken at the pI, causing the protein molecules aggregate and settle due to the lack of repulsion from like charges. When the pH deviates from the pI, the protein molecules carry a positive or negative charge depending on the acidity or basicity, and the electrostatic repulsion between the molecules helps dissolve AKPs (Zheng et al., 2019, 2021). Previous studies have shown that the solubility of AKPs tends to first decrease and then increase across pH levels from 1 to 13. At a pH of 4.6, only 0.6% of AKPs are dissolved, further indicating that the pI of AKPs is approximately 4.6 (Qi et al., 2016; Wang, Xue, Wang, & Yang, 2011). Moreover, the solubility of AKPs gradually increases as the pH moves away from the pI. Notably, AKPs are almost completely dissolved under alkaline conditions (pH 13), which can be attributed to the interaction between the charged protein molecules and the dipolar water molecules, leading to increased solubility (Zheng et al., 2021). Therefore, most studies currently use an alkaline solution to dissolve AKPs when extracting it using the ISP method. After precipitation at pH 4.6, the protein is obtained through centrifugation (Li et al., 2021; Zeng et al., 2024). It is essential to note that endogenous proteases in the krill body post-mortem can severely affect the integrity of the protein as the pH shifts. Thus, it is necessary to effectively control the activity of endogenous proteases. Methods to inhibit endogenous enzymes include heat treatment, low-temperature treatment, the addition of inhibitors, and ultra-high-pressure processing (Li et al., 2023; Zheng et al., 2021). Zheng et al. (2021) revealed that heat treatment of Antarctic krill can effectively prevent protein hydrolysis by endogenous proteases during the ISP process. However, this method significantly alters the properties of the extracted protein and is time-consuming and labor-intensive. Therefore, most studies use low-temperature methods to slow endogenous enzyme activity. Specifically, the extraction of AKPs is strictly controlled at temperatures below 4 °C (Zheng, Ping, Xu, Li, & Guo, 2022).

Although the ISP method is simple to use, the protein extraction rate is not high. Wang et al. (2011) reported that the protein extraction rate from Antarctic krill using the ISP method was only 52.68%. In addition, this traditional method consumes a large amount of acid and alkaline solutions to adjust the pH of the reaction system during the extraction process, potentially leading to resource waste and potential environmental pollution. To reduce the use of acids and bases, researchers have adopted a multistage countercurrent method based on the ISP method to extract AKPs (Qi, Liao, Zhao, Regenstein, & Mao, 2018). The multistage countercurrent extraction method is an engineering technique that combines dynamic cycle extraction with continuous countercurrent extraction. This technique can maintain the advantages of multiple extractions while further improving extraction efficiency and reducing solvent usage (Li et al., 2021). Studies have shown that using a three-stage countercurrent method with an extraction solution pH of 12.5 and a krill-to-water ratio of 1:10 (w/v) can extract 95.01% of the protein, and the protein recovery rate can be increased to 67.90% by precipitating the protein at pH 4.5 (Qi et al., 2018). This technique not only improves the extraction rate but also saves a large amount of acid and alkaline solutions during the extraction process. Although the ISP technique can extract and separate proteins from whole Antarctic krill, it primarily targets myofibrillar and sarcoplasmic proteins, relying heavily on ionic strength. Environmental concerns regarding the acidity and alkalinity of the extraction solutions should not be overlooked (Sasidharan & Venugopal, 2020).

2.2. *Ultrasound-assisted extraction method*

Ultrasound-assisted extraction (UAE) is a widely used method for pretreating extracts and assisting in protein extraction. UAE has been extensively applied in the extraction of marine animal and plant proteins, such as seaweed protein, fish protein, and marine byproduct protein (Yu et al., 2023). Researchers have discovered that combining ultrasonic treatment with the ISP method can increase the purity of AKPs. An ultrasound intensity of 0.08 W/mL combined with the ISP method improved the purity of AKPs to 88.41%, surpassing the purity achieved using the ISP method alone (77.70%). Additionally, sonication during the extraction process modifies the protein's three-dimensional structure, exposing sulfhydryl and hydrophobic groups, which ultimately enhances the emulsifying properties of these proteins (Yao et al., 2023). However, it is important to note that ultrasonic treatment can increase the exposure of certain active substances or functional groups to air, potentially leading to protein oxidation (Li et al., 2021). Although this combined extraction method promotes the purity and functional properties of the protein, it presents challenges in adjusting the pH of the extraction solution to the desired level, which often requires excessive alkali solution. This requirement not only increases economic investment but also has negative consequences for the environment, imposing a significant financial burden. To address these issues, researchers have explored the use of basic electrolyzed water (BEW) for extracting marine proteins (Li et al., 2021). BEW, a byproduct of the production of acidic electrolyzed water, has high utilization value and has wide applications in food, agriculture, aquaculture, and animal husbandry (Rebezov et al., 2022). BEW exhibits a high pH and redox potential (ORP, −800 to −900 mV) and possesses broad-spectrum antibacterial and

enzyme-inactivating capabilities, effectively preventing the degradation of active substances (Lemos et al., 2022). Li et al. (2021) demonstrated that the use of an alkaline electrolyzed aqueous solution generated by electrolysis of 0.5% (w/v) sodium chloride combined with an ultrasound-assisted extraction method improved the AKP extraction rate by 9.4% compared to that of an ultrasound-assisted sodium hydroxide solution. Moreover, the amount of NaOH used during the extraction process was reduced by 30.9%. Additionally, BEW safeguards the protein's active groups from oxidation during ultrasonic extraction (Rebezov et al., 2022). Importantly, all the physicochemical, structural, and functional properties of AKPs extracted using the ultrasound-assisted BEW method are comparable to those obtained using the traditional ultrasound-assisted deionized water extraction method (Li et al., 2021). Therefore, the ultrasound-assisted BEW extraction method has emerged as the most promising approach for AKP extraction.

2.3. Enzyme-assisted extraction

Enzyme-assisted extraction is a green and safe protein extraction method. Antarctic krill contain highly active endogenous proteases (proteases, carboxypeptidases, nucleases, phospholipases, etc.). After rigor mortis, these endogenous proteases are released into the surrounding tissues, causing the autolysis of Antarctic krill (Zheng et al., 2021). However, the characteristics of autolysis can be controlled to extract proteins. As early as 1980, Kolakowski, Gajowiecki, Szybowicz, and Chodorska (1980) proposed a method for extracting protein based on the autolysis of Antarctic krill. However, the proteins obtained using the autolysis method often exhibit minimal functional properties, such as poor gel formation and water retention. Therefore, proteins obtained through this method are typically used only as food additives in water-soluble foods rather than as functional ingredients in reconstituted foods that require strong gel formation and water retention. With the advancement of science and technology, researchers have developed more effective methods for protein extraction. Currently, the autolysis method is rarely used to extract AKPs due to difficulties in controlling the reaction process and the potential for damaging the integrity of the proteins. However, numerous studies are currently using protease preparations to extract biologically active proteins from Antarctic krill (Yuan et al., 2023). Most researchers use protease preparations to hydrolyze AKPs to produce active peptides because proteases are powerful tools for producing peptides. The biological activity of AKP hydrolysates depends on many factors, including protein type, enzyme specificity and hydrolysis conditions (pH, time, temperature, solid-liquid ratio, and enzyme-to-substrate ratio). The specificity of the enzyme is crucial, and the cleavage sites of different enzyme preparations are different. Therefore, the hydrolysis conditions and specificity affect the amino acid sequence and size of the peptides produced, which is crucial to the biological activity of the hydrolysate (Ji, Zhang, Song, & Ji, 2021b; Zhang et al., 2021).

2.4. Green solvent extraction method

At present, the most commonly used methods for extracting food proteins are alkali dissolution and acid separation. Although these methods are simple to perform, they may corrode the industrial equipment used and generate a significant amount of industrial wastewater due to the use of large quantities of corrosive acids and alkalis during the extraction process. In efforts to maintain environmental balance, researchers are diligently seeking a greener and more sustainable method to replace the current protein extraction techniques (Bowen et al., 2022). Various environmentally friendly protein extraction solvents, such as deep eutectic solvents (DESs), ionic liquids (ILs), and supercritical fluids, have been discovered and are now widely utilized (Bharmoria et al., 2024). Researchers have experimented with treating Antarctic krill using a mixture of acetic acid and ethylene glycol in a 2:1 M ratio, resulting in proteins with high crystallinity and molecular weight. The proteins extracted through this method offer advantages in composite applications for biomedical materials (Xu et al., 2023). Among these green solvents, DESs and ILs are considered the most promising media for protein extraction (Boateng., 2023; Zhou et al., 2022; Bharmoria et al., 2024). ILs are nonflammable, have low vapor pressure, and exhibit high stability. However, due to their high production cost and cumbersome recovery and purification, the use of these methods is limited to the laboratory scale (Bowen et al., 2022). DESs are mainly composed of hydrogen bond donors (HBDs: polyols, urea and carboxylic acids) and hydrogen bond acceptors (HBAs: quaternary ammonium salts, such as choline chloride) that form binary and ternary eutectic solvents (Zhou et al., 2022). The principle of DES extraction is that proteins are easily distributed in the DES-rich phase. It is currently believed that the formation of aggregates is the primary reason why proteins are easily distributed in DESs. Researchers have used UV–visible spectroscopy, Fourier transform infrared spectroscopy, circular dichroism spectroscopy, dynamic light scattering spectroscopy, conductivity measurements, transmission electron microscopy and other methods to study the mechanism of DES extraction from bovine serum albumin (BSA). The results showed no interaction between the DES and BSA molecules or the formation of new bonds, and the secondary structure of BSA was well preserved (Zhang et al., 2016). Moreover, DESs have been utilized in food protein extraction. Liu et al. (2017) devised a novel method for extracting pumpkin seed protein by combining DES with ultrasonic-microwave synergistic extraction. PEG200 was selected as the HBD, and ChCl was selected as the HBA. The optimized extraction conditions included a DES concentration of 28% (w/w), a solid-liquid ratio of 28 g/mL, a microwave power of 140W, and an extraction temperature of 43 °C. Under these conditions, the actual extraction rate was 93.95% after a 4-min reaction. Compared with single microwave-assisted or ultrasound-assisted technology, this method overcomes the shortcomings of microwave-induced uneven heating and long-term high-power ultrasound treatment, which may change the natural state of proteins or peptides (Liu et al., 2017). Furthermore, in comparison to the traditional alkali-acid separation method, the DES-based approach offers higher extraction efficiency while maintaining the activity of the extract (Bharmoria et al., 2024). Although no studies have yet explored the use of green solvents for extracting AKPs, green solvent extraction stands out as the most promising method for future food protein extraction endeavors.

3. Amino acid composition of AKPs

The application of proteins in the food industry is largely dependent on their amino acid composition. A favorable amino acid profile is crucial for human metabolism, as it is essential for the synthesis of biomolecules that play key roles in growth, maintenance, and metabolic activities (Wang et al., 2022). Various sources of AKP contain all EAAs and various NEAAs (Table 1) (Yao et al., 2023; Zheng et al., 2021). The content of each EAA meets the recommended limits for all amino acids for adults and infants set by the FAO/WHO/UNU (2007). The amino acid ratio indicates that EAAs constitute more than 40% of the total amino acids in AKP, with the ratio of EAAs to NEAAs exceeding 60%. These characteristics make AKP an ideal choice for supplementing the amino acid deficiencies of other food proteins. Furthermore, the hydrophobic amino acids in AKPs comprise more than 40% of the total amino acids, suggesting a greater tendency for globule formation (Li et al., 2021; Yao et al., 2023).

4. Functional characteristics

The functional properties of AKPs play a crucial role in the research and development of this potential food source and include solubility, emulsibility, gelling properties, and water and oil holding capacity. These properties hold significant importance for the practical utilization

Table 1
Amino acid composition of Antarctic krill proteins from different sources.

Essential amino acids	Antarctic krill protein (mg/g) (Zheng et al., 2021)	Defatted Antarctic krill powder protein (mg/g) (Yao et al., 2023)	[c] FAO/WHO/ UNU (2007) adult (infant) (mg/g protein)
Histidine	16.6	24.8	15 (16)
[a]Isoleucine	53	58.7	30 (31)
[a]Leucine	76.2	91.2	59 (61)
Lysine	59.8	67.4	45 (48)
[a]Methionine + [b]cysteine	69.9	34.4	22 (24)
[a]Phenylalanine + [b]tyrosine	86.3	108.4	38 (41)
[b]Threonine	49.8	35.7	23 (25)
Tryptophan	15.8	12.3	6 (6.6)
[a]Valine	88.2	54.6	39 (40)
Total EAA	515.6	487.5	277 (292.6)
Nonessential amino acids			
[b]Cysteine	32.9		
[b]Tyrosine	38.4		
Glutamate	136.7	142.5	
Aspartate	107.4	136.9	
[a]Alanine	68.7	53.7	
Arginine	47.3	47.1	
[b]Serine	42.2	46.7	
[a]Glycine	36.6	44.5	
[a]Proline	35.2	41.2	
Total NEAA	545.4	512.6	

[a] Hydrophobic amino acid.
[b] Hydrophilic amino acid.
[c] FAO (Food and Agriculture Organization of the United Nations), WHO (World Health Organization) and UNU (United Nations University) recommend amounts of all essential amino acids in protein for adults and infants (mg/g protein).

of AKPs.

4.1. Solubility

Solubility is a crucial functional property of proteins because it directly impacts their use as food ingredients in liquid foods (Khan et al., 2022; Sasidharan & Venugopal, 2020). The pI plays a significant role in APKs. The lowest solubility is observed near the pI, with increased solubility noted when deviating from it. The pI of AKPs is approximately pH 4.6. At this pH, the weakened intermolecular forces in the solution cause protein molecules to coalesce and settle due to the absence of repulsion between similarly charged molecules. Consequently, the solubility of AKPs is the lowest, measuring only 0.61%. However, within the pH ranges of 2.6–4.6 and 4.6 to 6.6, the solubility of AKPs increases remarkably. This phenomenon occurs because under acidic or alkaline conditions, protein molecules acquire positive or negative charges via acid-base reactions, leading to intermolecular electrostatic repulsion that enhances AKP solubilization. In fact, AKPs exhibit 90% solubility when the pH of the solution exceeds 10 (Qi et al., 2016; Wang et al., 2011, 2015a). Additionally, hydrophobic and ionic interactions significantly influence protein solubility. Hydrophobic interactions decrease solubility by promoting protein-protein interactions, whereas ionic interactions support protein-water interactions, thereby increasing solubility. AKP solubility may be limited by various factors. The relationship between the solubility of AKPs and pH indicates that surface hydrophobicity plays a significant role, but it is not the sole determinant of protein solubility (Yao et al., 2023). However, AKPs can only be dissolved at 20% in aqueous solution, which greatly limits the application of this potentially important food source (Zeng et al., 2024; Wang et al., 2022).

4.2. Emulsibility

Emulsifying properties, including the emulsifying activity index, emulsifying ability, and emulsifying stability, are crucial for various food applications (Khan et al., 2022; Sasidharan & Venugopal, 2020). Proteins, with their amphoteric structures, are considered excellent emulsifiers due to their absorption at the oil-water interface (Hu, Wu, et al., 2021; Li et al., 2020). Emulsifiers can protect dispersed fine particles by forming a thin layer at the interface or reducing interfacial tension (Sasidharan & Venugopal, 2020). The emulsification index measures a protein's ability to induce the formation of new dispersed particles in an emulsion. On the other hand, the emulsification stability quantifies the amount of cream or oil that separates from the emulsion over time and under specific conditions (Khan et al., 2022; Sasidharan & Venugopal, 2020). APK, which contains more essential amino acids than vegetable protein, shows promise as a food-grade emulsifier resource (Hu, Wu, et al., 2021; Li et al., 2020). Studies have shown that at pH 7, the emulsifying activity index of AKPs is greater than that of commercial soy protein isolates (SPIs) (Yao et al., 2023). The hydrophobic interactions, disulfide bonds, and β-sheet structure of APKs play crucial roles in the formation and stability of protein aggregates. When Antarctic krill are subjected to ultrasonic treatment, the α-helical structure disappears, leading to increased protein flexibility. This feature subsequently enhances the interactions between proteins and lipids and promotes the rearrangement of proteins at the oil–water interface, resulting in the increased emulsification of APKs (Hu, Wu, et al., 2021; Yao et al., 2023). However, excessive sonication reduces the surface hydrophobicity and water solubility of AKPs, thereby weakening their emulsifying ability. The emulsification index of AKPs is influenced by the pI of AKPs. When the pH deviates significantly from the pI, the increase in intermolecular repulsion impedes protein aggregation, resulting in smaller and more flexible molecules. These modifications subsequently facilitate the adsorption of proteins at the oil-water interface (Hu, Wu, et al., 2021; Li et al., 2020). On this basis, researchers have developed a shrimp protein-stabilized emulsifier with particle sizes less than 20 μm. Compared to other protein- or biopolymer-stabilized emulsifiers, this emulsifier demonstrates superior stability even after 30 days of storage (Li et al., 2020).

4.3. Gelling property

Gelling is a significant functional property of proteins in food applications (Khan et al., 2022; Sasidharan & Venugopal, 2020). The gelation properties of Antarctic krill myosin play a crucial role in this process. At low temperatures, myosin dissolves and unfolds when exposed to salt, exposing hydrophobic sites. Subsequently, myosin forms a rigid network through intermolecular association and aggregation during thermal induction (Wang, Wang, Chang, et al., 2015). The minimum gel concentration of AKPs is 8% (w/v), which was superior to that of commercial soybean isolate protein (10%). This difference can be attributed to the higher content of myosin heavy chain and actin in AKPs, which are favorable for gel formation (Yao et al., 2023). APKs can form a gel without the need for salt or other additives, even at concentrations exceeding 100 g/L. This gel exhibits thermoreversible behavior when subjected to repeated heating and cooling cycles. When heat-treated, APKs can also form fluid gels within a pH range of 5.5–8.6. Notably, the gel strength is significantly greater at pH 5.5 and 7.2 than at other pH values. Moreover, at pH 7.2, the liquid gel demonstrates a notably greater apparent viscosity than gels formed under different pH conditions (Wang et al., 2016). However, AKP gelation is susceptible to endogenous proteases during the isolation process. The strong activity of endogenous proteases in AKPs can easily lead to self-hydrolysis, resulting in autolysis and the loss of functional properties, particularly gelation properties (Zheng et al., 2019). This poses challenges to the development of commercial processing and recombinant krill protein products (e.g., shrimp sliders) using Antarctic krill.

4.4. Water and oil holding capacity

Water holding capacity is a crucial functional property of AKPs and plays a significant role in the appearance, color, and taste of food products made from Antarctic krill (Lin, Liu, Liu, & Qi, 2020). Although the water absorption ability of AKPs is relatively weak, its ability to absorb oil is superior to that of whey protein concentrate and soy protein isolate. This difference can be attributed to the composition of groups present on the surface of the protein molecules (Li et al., 2021; Yao et al., 2023). Notably, the water absorption of krill protein is at its lowest at the pI primarily due to protein aggregation and precipitation, resulting in the weakest water absorption ability (Qi et al., 2016).

5. Modified processes for AKP recovery

Although AKPs possess specific physicochemical and functional properties, most AKPs exhibit poor solubility (Zeng et al., 2024; Wang et al., 2022). Furthermore, endogenous proteases, such as serine proteases, in Antarctic krill remain highly active during the rigor mortis. The hydrolysis caused by these endogenous enzymes results in a significant loss of the functional properties of AKPs. These limitations severely restrict the application of AKPs in the food industry (Zheng et al., 2019, 2022). Therefore, modifying AKPs is crucial. Current studies primarily employ chemical, physical, and biological methods to enhance the functionality of AKPs and broaden the application of this potentially important food source.

5.1. Chemical modification

Numerous research applications on the chemical modifications of AKPs have been reported. The method is simple, effective and easy to industrialize. Chemical modification mainly focuses on changing the solubility, gel properties, emulsification properties, and texture properties of AKPs and further broadens their commercial and nutritional health value.

5.1.1. Improving AKP solubility

Studies have shown that some basic physicochemical properties of AKPs are poor, especially their solubility. The content of insoluble protein accounts for 80.0% of total AKPs, which is much greater than that of plant protein. Generally, the solubility of food protein is one of its most important physicochemical properties and also serves as the basis of other functional properties (Zeng et al., 2024; Wang et al., 2022). Researchers have attempted to modify AKPs with succinylation to improve AKP solubility. When the ratio of succinic anhydride to protein was 0.5 g/g, the solubility of the succinylated protein was greatest. During the succinylation reaction, a large number of hydrophilic amide groups were introduced to AKPs, which greatly improved the hydrophilicity of the proteins (Wang et al., 2022). In addition, the number of hydrophobic amino groups exposed on the surface of the proteins decreased, and the electrostatic force increased, thereby reducing protein aggregation and greatly reducing the particle size. These features jointly promoted the solubility of the succinylated proteins. However, it is worth noting that the reagent used for the succinylation of modified proteins is succinic anhydride, which is an organic compound that is slightly toxic. Therefore, after the reaction, the succinic anhydride that did not participate in the reaction must be removed using dialysis or other methods. Only in this way can the modified protein be used in food. In addition, Zeng et al. (2024) successfully glycosylated AKPs with inulin and found that the solubility of the modified AKPs increased significantly. The combination of glycosylated AKPs with a photosensitizer (curcumin) can effectively extend the shelf life of salmon through photodynamic inactivation (PDI) technology. This has greatly improved the commercial application of AKPs.

5.1.2. Improving the gel and texture properties of AKPs

Antarctic krill contain large amounts of endogenous proteases. Post rigor-mortis, the muscle is rapidly autolyzed under the influence of endogenous proteases, thus affecting the gel properties of AKP. The gel properties formed by heating AKPs extracted using ISP are very poor (Zheng et al., 2019). The addition of κ-carrageenan significantly improved the gel properties of AKPs and further improved the textural properties and water holding capacity (Zheng et al., 2019). In addition, κ-carrageenan can induce the unfolding of Antarctic krill myofibrillar proteins, resulting in changes in the spatial conformation of the protein, exposure of side chain groups, and enhanced intramolecular or intermolecular interactions in the gel system, further increasing the degree of cross-linking of the κ-carrageenan and AKP composite gel network. A higher degree of cross-linking is conducive to the formation of a denser network structure with higher energy storage and loss modulus, ultimately improving the gel quality (Zheng et al., 2019). In addition, directly adding 4% κ-carrageenan to Antarctic krill surimi products can effectively improve the gel properties, texture properties and water holding capacity of the surimi. On the other hand, κ-carrageenan has good thermal stability and can effectively inhibit damage to the Antarctic krill surimi gel structure after heating (Chen et al., 2024; Li et al., 2024). In addition, exogenous basic amino acids can affect the interactions between protein molecules, thereby affecting the gel properties of the proteins. Man et al. showed that adding L-lysine and L-arginine to Antarctic krill surimi improved the gel strength and water-holding capacity of low-salt gels. Lys and Arg play a role in enhancing hydrogen bonds and disulfide bonds in low-salt gel systems, promoting the dissolution of myofibrillar proteins and thereby increasing the number of myofibrillar protein molecules involved in gel formation (Man, Sun, Lin, Ren, & Li, 2024). These results indicate that modified AKPs can be used in ready-to-eat foods and reconstituted foods.

5.1.3. Improving the emulsification properties of AKPs

AKPs contain more essential amino acids than plant protein and is a promising food-grade emulsifier (Li et al., 2021). However, due to the poor solubility of AKPs, the emulsification properties are also reduced (Zeng et al., 2023). Researchers used succinic anhydride to succinylate AKPs. The succinylation reaction reduced the particle size and hydrophobicity of AKPs but increased the surface charge, greatly improving protein solubility and emulsification (Wang et al., 2022). Moreover, succinylated AKPs showed an excellent ability to coat curcumin to prepare emulsions and endowed the emulsions with good physical properties to resist photothermal treatment, salt and different temperature stresses. In addition, the emulsion prepared by loading curcumin with succinylated AKPs can effectively inactivate bacteria in salmon and extend its shelf life through the PDI system, which plays a role in food preservation (Zeng et al., 2023). In addition, polyphenols can covalently modify AKPs to improve its functional properties. The combination of rutin with soluble AKPs (SAKPs) under alkaline conditions significantly improved their emulsifying properties. Compared with those of native SAKPs, the formed SAKP–rutin conjugates contain fewer β-sheets but more α-helices and β-turns. More importantly, the SAKP–rutin conjugates prepared into high internal phase emulsions have excellent physical properties and oxidative stability (Li et al., 2023). The emulsifying properties of chemically modified AKPs significantly improved, increasing the application of AKPs in food and increasing the commercial value.

5.2. Physical modification

In recent years, physical modification has gained popularity as a means to enhance protein functional properties given its eco-friendly nature, safety, and cost-effectiveness (Rahman & Lamsal, 2021). Ultrasonic treatment can alter the functional properties of AKPs by modifying the spatial structure, exposing hydrophilic groups, and enhancing

solubility and water absorption (Li et al., 2022; Yao et al., 2023). This treatment also improves the emulsifying properties of AKPs. In the absence of ultrasound assistance, a stable emulsion containing 2% AKPs exhibited noticeable stratification after being stored at 4 °C for 30 days (Li et al., 2021). In contrast, the emulsion prepared after 30 min of ultrasonic treatment with AKPs showed less delamination after 30 days of storage. The creaming index (CI) of AKPs after ultrasonic treatment decreased by 56% compared with that without ultrasonic treatment. Generally, the lower the CI is, the stronger the emulsion stability. Moreover, the disordered and irregular structures formed by ultrasound-induced AKP emulsions contribute to emulsion stability (Hu, Wu, et al., 2021; Li et al., 2022). Ultrasound treatment also modifies the surface hydrophobicity of krill proteins, facilitating the unfolding of protein molecules at the water–air interface and the formation of interfacial films. This process increases the solubility, reduces the particle size, and promotes the formation of α-helices and β-sheets. These transformations enhance the formation of surface hydrophobic groups and disulfide bonds in krill protein, thereby improving its foaming ability and foam stability (Hu, Wu, et al., 2021; Li et al., 2022). However, excessive ultrasound exposure may lead to AKP aggregation, reducing the surface hydrophobicity and water solubility of AKPs and consequently weakening their emulsifying ability (Li et al., 2022).

5.3. Enzymatically hydrolysis

The controlled application of proteolytic to alter the structural properties of a protein constitutes the enzymatic modification techniques (Batish et al., 2020). Depending on the functionality of interest, enzymatic modification can be applied to break down or build up a protein structure to achieve the desired functionality (Gao et al., 2021). Proteolytic enzymes (such as pepsin, papain, trypsin, and alcalase) are used to cleave peptide bonds within the primary amino acid sequence of proteins, thereby altering their functionality during hydrolysis (Cunha & Pintado, 2022). The various factors that influence enzymatic hydrolysis of proteins include the type of enzyme, nature of the protein substrate, the enzyme to substrate volume ratio, process conditions (pH, temperature, and pressure), and availability/absence of proteolytic inhibitors (Batish et al., 2020). Generally, because of the size reduction of the

Table 2
Bioactive peptides derived from Antarctic Krill.

Extract source	Enzyme	Peptide sequence	Molecular weight (Da)	Bioactive property	Biological effect	Reference
Antarctic Krill	Pepsin and trypsin	LKPGN	527.62	Antioxidant	Scavenged hydroxyl (EC_{50} of 0.72 mM), DPPH (EC_{50} of 1.18 mM), superoxide anion (EC_{50} of 0.75 mM). Protected against H_2O_2- induced DNA damage.	Wang, Zhou, and Lin (2021b)
		LQP	356.42	Antioxidant	Scavenged hydroxyl (EC50 of 0.84 mM), DPPH (EC_{50} of 1.41 mM), superoxide anion (EC_{50} of 0.76 mM) Protected against H_2O_2- induced DNA damage.	
Antarctic krill powder	Alcalase	SLPY	478.80	Antioxidant	Scavenged hydroxyl (EC_{50} of 0.826 mg/mL), DPPH (EC_{50} of 1.181 mg/mL), superoxide anion (EC_{50} of 0.789 mg/mL). Protected against H_2O_2- induced DNA damage.	Zhang et al. (2021)
		OYPPMOY	926.00		Scavenged hydroxyl (EC_{50} of 1.022 mg/mL), DPPH (EC_{50} of 1.574 mg/mL), superoxide anion (EC_{50} of 0.913 mg/mL). Protected against H_2O_2- induced DNA damage.	
		EYEA	510.60		Scavenged hydroxyl (EC_{50} of 0.946 mg/mL), DPPH (EC_{50} of 1.372 mg/mL), superoxide anion (EC_{50} of 0.793 mg/mL). Protected against H_2O_2- induced DNA damage.	
Antarctic krill	Animal proteolytic enzyme	AP	186	Antidiabetic	DPP-IV inhibitory activity (IC_{50} of 0.053 mg/mL).	Ji et al. (2017a)
		IPA	299	Antidiabetic	DPP-IV inhibitory activity (IC_{50} of 0.037 mg/mL).	
Antarctic krill defatted powder	Trypsin	WF	351.47	Antihypertensive	ACE inhibitory activity (IC_{50} of 0.318 mg/ml)	Zhao et al. (2019)
		FAS	323.35		ACE inhibitory activity (IC_{50} of 0.152 mg/ml)	
Antarctic krill powder	Endonuclease, exonuclease, and flavor protease	FAGDDAPR	847.87	Antidiabetic	DPP-IV inhibitory activity (IC_{50} of 0.297 mg/mL).	Lang et al. (2021)
		LAPPRGSL	809.95		DPP-IV inhibitory activity (IC_{50} of 0.374 mg/mL).	
Antarctic krill	Corolase PP	KVEPLP	682.39	Antihypertensive and Antidiabetic	DPP-IV inhibitory activity (IC_{50} of 0.73 mg/mL). ACE inhibitory activity (IC_{50} of 0.93 mg/mL)	Ji et al., (2017b)
		PAL	299.39		DPP-IV inhibitory activity (IC_{50} of 0.88 mg/mL). ACE inhibitory activity (IC_{50} of 0.64 mg/mL)	
Antarctic krill defatted powder	Neutral proteinase		200–2000	Anti-osteoporosis	Ameliorate senile osteoporosis	Wang et al. (2017)
Antarctic krill defatted powder	Neutral proteinase		400–2000	Anti-osteoporosis	Increased longitudinal bone growth and improved bone strength	Dai et al., (2021)
Antarctic krill powder	Neutral proteinase		200–2000	Anti-inflammatory activities	Improves osteoarthritis	Wang et al. (2019)
Antarctic krill	Protamex		245–709	Antibacterial	Effectively inhibit the propagation of *Staphylococcus aureus*	Zhao et al. (2013)
Antarctic krill defatted powder	Protamex		600–2000	Antifreeze	Protects *Lactobacillus rhamnosus* ATCC7469 from freezing damage	Liu, Yu, et al. (2022)

protein polypeptides, enzymatic hydrolysis has been reported to increase certain functionality of food proteins such as solubility, emulsifying, and foam properties (Wu et al., 2020). During the enzymatic modification process, the degree of hydrolysis (DH) of AKP continuously increases, leading to the production of small peptides with exposed polar groups. These polar groups can form hydrogen bonds with water, thereby enhancing hydrophilicity, solubility, and water absorption (Park, Je, & Ahn, 2016). Concurrently, enzymatic hydrolysis reduces the molecular weight of the protein, improving its solubility and flexibility, which in turn contributes to better foaming ability and stability. However, it is important to note that hydrolysates with high DH exhibit poor emulsifying activity index and emulsifying stability index. This is because small peptides, although they rapidly move to and adsorb at the interface, are less efficient at reducing interfacial tension. Unlike larger protein molecules, they cannot unfold and reorient at the interface to stabilize the emulsion effectively (Park et al., 2016).

In addition, appropriate hydrolysis of Antarctic krill proteins using single or composite protease preparations can yield Antarctic krill protein peptides (AKPPs) with various biological activities (Table 2). The biological activities of AKPPs are typically related to enzymatic hydrolysis conditions (such as the type of enzyme and hydrolysis time), as well as the molecular weight and amino acid composition of the peptides (Ge et al., 2023; Zheng et al., 2024). Current research has shown that the biological activities of AKPPs mainly include antioxidant and anti-osteoporosis activities as well as lowering blood pressure, inducing hypoglycemia, and promoting mineral absorption and others (Fig. 2).

5.3.1. *Antioxidant activity*

Various environmental factors like cigarettes, alcohol, unhealthy eating habits, and late nights are increasingly becoming part of people's lives. Consequently, a growing number of free radicals, including hydroxyl radicals (-OH), superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen (O_2), hypochlorite (ClO-), nitrogen radicals (-NO), and peroxynitrite radicals (ONO_2^-), are damaging our bodies (Cunha & Pintado, 2022). These unstable and highly reactive free radicals can cause cellular damage and affect molecules such as lipids, proteins, carbohydrates, and DNA (Wu et al., 2023). Moreover, free radicals contribute to various human diseases such as cancer, cardiovascular diseases, neurological disorders, pulmonary disorders, rheumatoid arthritis, liver diseases, and obesity (Escamilla Rosales et al., 2023). Therefore, developing antioxidants is crucial because they can prevent free radical formation and protect the body from oxidative damage.

Numerous studies have highlighted the antioxidant potential of AKP hydrolysates and peptides derived. Studies have shown that among the five commercial proteases (alcalase, trypsin, neutrase, pepsin, and papain), AKPPs produced by alcalase exhibit the highest hydroxyl radical (-OH) scavenging activity, as well as the highest 1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2-azobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging abilities (Lan, Zhao, Li, & Wang, 2019). Additionally, AKPPs effectively increase the activities of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), thereby reducing H_2O_2-induced oxidative stress and lipid oxidation (Fernando et al., 2020; Zhang et al., 2021). AKPPs demonstrated a concentration-dependent ability to increase the levels of antioxidant enzymes (SOD and GSH-PX) for scavenging excess ROS, enhancing mitochondrial membrane potential, and reducing DNA damage and the MDA content. This protective effect was also observed in Chang hepatocytes exposed to H_2O_2-induced oxidative stress (Wang, Zhou, & Lin, 2021b). Different types of amino acids are associated with antioxidant peptide potential (Cunha & Pintado, 2022; Gao et al., 2021). Hydrophobic amino acids play a major role in scavenging free radicals, whereas polar amino acids contribute to the reduction and chelation of metal ions. It is widely accepted that hydrophobic amino acid residues, including tyrosine, tryptophan, phenylalanine, leucine, isoleucine, and alanine, act as hydrogen donors for free radical peroxidation on the side chains of aromatic residues (Escamilla Rosales et al., 2023). Additionally, certain combinations of amino acids seem to have synergistic effects, resulting in increased antioxidant activity. For example, glutamine-proline (Gln-Pro) and Pro-Tyr exhibit increased antioxidant activity (Zou, He, Li, Tang, & Xia, 2016). Several antioxidant peptides, such as AMVDAIAR, SKASAAAGASIKKK, and FSIIKDSR, have been

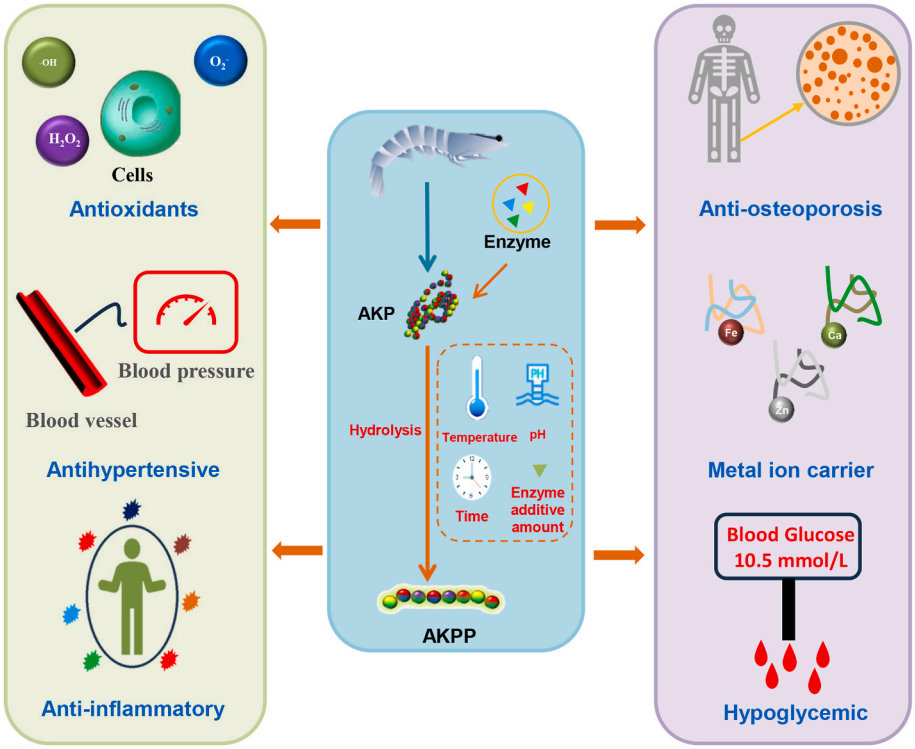

Fig. 2. An overview of the biological activities of Antarctic krill proteins.

detected in AKPs. Notably, the AMVDAIAR peptide has an IC_{50} of 0.87 mM for scavenging DPPH and an oxygen radical absorbance capacity (ORAC) of 1.56 mM TE/mM peptide (Fernando et al., 2020). Three peptide sequences (SLPY, QYPPMQY, and EYEA) were isolated from AKPs using ultrafiltration and chromatographic purification after a single alcalase digestion. The effects of SLPY, QYPPMQY, and EYEA on the expression of DPPH (with EC_{50} values of 1.18, 1.54, and 1.37 mg/mL, respectively), -HO (with EC_{50} values of 0.82, 1.02, and 0.94 mg/mL, respectively), and superoxide anion radicals (with EC_{50} values of 0.78, 0.91, and 0.79 mg/mL, respectively) were investigated. Furthermore, SLPY, QYPPMQY, and EYEA exhibited strong reducing power, resistance to lipid peroxidation, and good stability during simulated gastrointestinal digestion (Zhang et al., 2021). Phosphorylated AKPPs also exhibit antioxidant activity. In fact, these peptides demonstrate stronger free radical scavenging activity (specifically, DPPH and -OH scavenging activity) than nonphosphorylated antioxidant peptides (Wang, Zhou, & Lin, 2021a). Phosphorylation leads to the exposure of previously buried hydrophobic residues on the peptide surface due to repulsion from the negative charges of the added phosphate groups. Consequently, the enhanced antioxidant properties can be attributed to the electrostatic interaction between the negatively charged phosphate groups introduced into P-AKPPs and the increased number of exposed hydrophobic groups following phosphorylation (Wang, Zhou, & Lin, 2021a). These studies indicate that AKPPs exert potent antioxidant activity, both in vivo and in vitro, highlighting their potential application to combat oxidative stress-associated diseases.

5.3.2. Promotion of mineral absorption

Iron, zinc, and calcium are essential minerals crucial for good health. Deficiencies in these minerals can lead to various health issues (Hou et al., 2018; Hu, Lin, et al., 2021a; Sun et al., 2021). Iron is vital for metabolic pathways related to energy utilization and is a key component of myoglobin, hemoglobin, cytochromes, and enzymes responsible for functions like oxygen transport and electron transfer (Wang et al., 2020). Zinc, the second most abundant metal in the body, is essential for over 200 metalloenzymes involved in the metabolic processes of carbohydrates, proteins, lipids, and nucleic acids (Sun et al., 2021). Calcium is critical for bone health and is involved in nerve transmission, muscle contraction, and blood coagulation (Ge et al., 2023). Peptides are known for their effectiveness as metal-binding ligands. Peptides possess coordination sites that allow for the formation of stable complexes through covalent bonding and coordination with metal ions (Caetano-Silva, Netto, Bertoldo-Pacheco, Alegría, & Cilla, 2021). Various food peptides have been shown to bind metals and enhance mineral bioavailability. Previous studies have demonstrated that peptides bind metal ions through amino and carboxyl groups, such as aspartic acid (Asp), glutamic acid (Glu), and histidine (His) (Ashaolu, Lee, Ashaolu, Pourjafar, & Jafari, 2023). Three peptides were extracted from AKPs: DELEDSLER, EEEFDATR, and DTDSEEEIR. The binding of each peptide to $FeSO_4$ was examined, revealing that DTDSEEEIR, which contains three consecutive Glu residues in its sequence, exhibited a greater capacity for iron binding and release than EEEFDATR, which has three consecutive Glu residues at its N-terminal end. On the other hand, DELEDSLER, which has three noncontiguous Glu residues, displayed the lowest iron binding capacity and iron release potential. The digestive stability test indicated that EEEFDATR was significantly less stable than DTDSEEEIR (Hu, Wu, et al., 2021). However, the iron solubilization and uptake effects of EEEFDATR were comparable to those of DTDSEEEIR and superior to those of DELEDSLER and $FeSO_4$. Therefore, peptides with consecutive Glu residues have the potential to serve as effective iron carriers (Hu, Wu, et al., 2021). Furthermore, defatted Antarctic krill meal mixed with trypsin exhibited increased iron chelating activity with longer enzyme digestion times, peaking at 180 min. This finding suggests that excessive hydrolysis reduces the iron-binding capacity of the hydrolysis products (Wang et al., 2020). Over hydrolysis may result in the production of more free amino acids and smaller peptides, which can disrupt iron binding sites and lead to reduced iron binding. The content of negatively charged amino acids (Asp and Glu) on AKPs was positively correlated with iron binding activity, whereas positively charged amino acids showed a negative correlation. Additionally, His, Ser, and Thr on AKPs are also involved in iron binding activity (Sun et al., 2020; Wang et al., 2020). Calcium-binding peptides are considered a class of calcium-enhancing agents that form soluble peptide-calcium complexes under specific conditions, improving the efficiency of calcium ion utilization. Antarctic krill was incubated with trypsin obtain a calcium-chelating peptide with the sequence VLGYIQIR. The chelation site of calcium ions may involve the carbon or amino groups of the Gln, Ile, and Arg residues. Compared with amino nitrogen atoms and water molecules, calcium ions tend to form complexes with negatively charged carboxylic acid groups (Hou et al., 2018; Liu, Yu, et al., 2022a). Zinc can chelate functional groups, such as carboxyl oxygen, amino nitrogen, and carbonyl groups, in AKPPs. This chelation process disrupts the dense structure of the peptide surface. As a result, the particle size of the peptide-zinc complex increases, and the zeta potential also increases. Furthermore, even after gastric digestion, the solubility of the peptide–zinc complex remains at its highest level. These findings demonstrate the excellent stability of the Antarctic krill peptide–zinc complex during gastrointestinal digestion (Sun et al., 2021).

5.3.3. Improving bone resorption

Osteoporosis (OP) is a significant public health concern due to the aging population. It can be classified into primary OP, including postmenopausal and senile osteoporosis, and secondary osteoporosis induced by glucocorticoids (GIOP) (Han et al., 2018). Osteoblasts are crucial for bone formation, but their deficiency leads to osteoporosis, with many patients showing osteogenic dysfunction (Wang et al., 2017). Approximately 9.6% of fractures annually are due to osteoporosis. Glucocorticoids are effective therapies for chronic inflammatory diseases, but GIOP results from impaired osteoblast activity and decreased osteoblast numbers as bone marrow stem cells differentiate into adipocytes rather than osteoblasts (Han et al., 2018). Current treatments include bisphosphonates, parathyroid hormone, and estrogen analogs, but they have limitations and potential side effects. Expensive osteogenesis-promoting drugs like teriparatide are used for GIOP but can cause long-term adverse effects (Han et al., 2018; Liu, Yu, et al., 2022). Therefore, there is interest in identifying new bioactive ingredients from natural products for preventing and treating osteoporosis. In recent years, there has been growing interest in researching food proteins and peptides due to their significant role in bone metabolism. A specific octapeptide, EEEFDATR, was discovered in AKP hydrolysate, and its effects on the proliferation, differentiation, and mineralization of MC3T3-E1 osteoblastic cells were investigated. Interestingly, when combined with $CaCl_2$, EEEFDATR showed a remarkable value-added rate of 70.94% for MC3T3-E1 osteoblastic cell proliferation and mineralization, which was 2.07 and 1.74 times greater than that of $CaCl_2$ and EEEFDATR alone, respectively (Liu, Yu, et al., 2022). Additionally, Antarctic krill peptide improves osteoporosis in aged accelerated P6 (SAMP6) mice by activating the bone morphogenetic protein 2 (Smads)/Smads and Wnt/β-catenin molecular pathways, leading to enhanced bone formation (Dai et al., 2021; Wang et al., 2017). Moreover, the phosphorylation of proteins enhances their physiological functions, suggesting that phosphorylated Antarctic krill peptides may have even greater potential in ameliorating primary osteoporosis (Han et al., 2018; Wang, Wang, Wang, et al., 2015; Xia et al., 2015). Phosphorylated AKPPs with P-O and P=O bonds administered at a dose of 800 mg/kg (body weight) exhibit the potential to ameliorate osteoporotic features in a Sprague−Dawley rat model of osteoporosis established by ovariectomy by increasing bone density and trabecular and cortical bone strength (Wang, Wang, Wang, et al., 2015). Moreover, phosphorylated AKPPs promote the healing of tibial fractures in ovariectomized-induced osteoporotic mice by facilitating the transformation of cartilage into osteochondral tissue and the remodeling of

osteochondral scaffolds, as well as by regulating the expression of genes related to ossification in cartilage (Xia et al., 2015). Furthermore, these peptides have ameliorative effects on dexamethasone-induced secondary osteoporosis, accelerating bone formation, improving the quality of bone trabeculae, and reducing bone loss and microstructural degradation in mice. They enhance the expression of osteogenic nuclear transcription factors Runx2 and OSX and activate downstream osteogenesis-related signaling pathways, thereby improving overall bone turnover (Han et al., 2018). These findings provide a theoretical basis for the potential use of the bioactive peptide of Antarctic krill as a natural alternative to improve bone metabolism.

5.3.4. Antidiabetic and antihypertensive activity

Type 2 diabetes and hypertension are two common chronic diseases that often coexist and influence each other. Currently, the development of angiotensin-converting enzyme (ACE) inhibitors and dipeptidyl peptidase-IV (DPP-IV) inhibitors holds great potential for the treatment of diabetes and hypertension (Khan et al., 2022). Although synthetic inhibitors of ACE and DPP-IV are available, their potential adverse side effects should not be overlooked. Therefore, there is a growing need to seek natural compounds for the treatment of type 2 diabetes and hypertension (Gao et al., 2021; Ji, Zhang, & Ji, 2017a). Numerous studies have demonstrated the potent inhibitory effects of bioactive peptides derived from marine proteins on ACE and DPP-IV. Specifically, three DPP-IV inhibitory peptides, namely, Ala-Pro (AP), Ile-Pro-Ala (IPA), and Ile-Pro-Ala-Val-Phe (IPAVF), were successfully purified from AKP hydrolysate using ultrafiltration and chromatographic separation techniques. These three peptides have shown significant effectiveness in reducing DPP-IV activity, as well as blood glucose, triglyceride, and cholesterol levels, in diabetic zebrafish. Moreover, these peptides increase insa gene expression levels and decrease glucagon and pck1 gene expression levels (Ji, Zhang, et al., 2021). Another study by Lang et al. (2021) isolated two DPP-IV inhibitory peptides (FAGDDAPR and LAPPRGSL) from AKP hydrolysate. The IC_{50} values of the two peptides were 349.70 μM and 461.14 μM, respectively. Molecular modeling of DPP-IV and the two inhibitory peptides revealed that the inhibitory activities of FAGDDAPR and LAPPRGSL were primarily attributed to the formation of strong hydrophobic interactions and hydrogen bonds with specific amino acids of DPP-IV. Similarly, Ji et al. (2017a) used AKP hydrolysate to sequentially purify a dipeptide and tripeptide (AP and IPA, respectively) using ultrafiltration, gel filtration chromatography, and reversed-phase high-performance liquid chromatography (RP-HPLC). The IC_{50} values for the inhibitory activity of AP and IPA against DPP-IV were 0.0530 mg/mL and 0.0370 mg/mL, respectively. Furthermore, molecular docking analysis indicated that the inhibitory effects of AP and IPA on DPP-IV were primarily due to the formation of strong interaction surface forces with specific amino acids (Asp, Try, Thr, and Phe) of DPP-IV. Some studies showed that short-chain polypeptides with low molecular masses exhibited high ACE-inhibitory activity (Cunha & Pintado, 2022). Eight ACE inhibitory peptides were isolated from Antarctic krill hydrolysate, and it was found that the small molecular weight peptides Trp-Phe (351 Da) and Phe-Ala-Ser (323 Da) demonstrated higher ACE inhibitory activity compared to other isolated peptides (Zhao, Zhang, Tao, Chi, & Wang, 2019). Park et al. (2016) conducted research on active peptides derived from AKP hydrolysate through ultrafiltration and found that active peptides with a molecular weight less than 1 kDa exhibited stronger inhibitory activity against ACE. Furthermore, Ji, Zhang, and Ji (2017b) isolated two peptides, Lys-Val-Glu-Pro-Leu-Pro and Pro-Ala-Leu, from AKP hydrolysate using ultrafiltration and chromatography. These peptides demonstrated dual inhibitory effects on ACE and DPP-IV and have the potential to regulate blood pressure and blood glucose levels. Consequently, AKP hydrolysates can be utilized for the development of functional products aimed at reducing blood pressure and controlling blood glucose.

5.3.5. Other activities

Protein peptides derived from Antarctic krill exhibit a protective effect on osteoarthritic cartilage. These peptides effectively inhibit cartilage matrix loss in osteoarthritis induced by medial meniscus destabilization. They contribute to the maintenance of normal bone homeostasis by reducing the level of hypoxia-inducible factor (HIF)-2α in articular cartilage (Wang et al., 2019). Furthermore, they regulate downstream catabolic genes, decrease the expression of proinflammatory genes, and activate anabolic genes in articular cartilage. AKPPs prevent chondrocyte apoptosis through two mechanisms. Firstly, they obstruct the Fas-FasL signaling pathway in articular chondrocytes, thereby inhibiting abnormal apoptosis. Secondly, they modulate the expression of key genes in the DR3-DR3L apoptosis signaling pathway. These discoveries suggest that AKPPs could serve as functional foods to ameliorate osteoarthritis and provide a novel approach for the clinical treatment of this disease (Wang et al., 2019). Additionally, AKPPs demonstrate antimicrobial efficacy against *S. aureus* by inhibiting its cell division and altering the osmotic pressure of its cell wall (Zhao, Yin, Liu, & Cao, 2013). Furthermore, AKPPs provide antifreeze properties for cryopreservation, with the Antarctic krill antifreeze peptide can delay the reduction of viability in *Lactobacillus rhamnosus*. The antifreeze effect of the Antarctic krill peptide surpasses that of current commercial antifreeze agents like sucrose, skim milk, and glycerol, as it provides better protection for cell integrity (Liu, Yu, et al., 2022).

6. Potential risks of AKPs

Although AKPs exhibit strong functional properties and biological activity, the safety and potential risks of AKPs must be evaluated before Antarctic krill can be included as a source of new food proteins. This information can provide new insights into AKPs in human health and the food industry.

6.1. Fluoride residue

Antarctic krill serve as a natural protein source for humans, yet their high fluoride content makes them primarily utilized in aquaculture feed production (Kaur et al., 2022). Excessive fluoride ingestion can result in dental fluorosis, chronic toxicity, and other health complications (Peng, Ji, Zhang, Ji, & Liu, 2019). In live Antarctic krill, approximately 99% of fluoride is concentrated within the krill cuticle, with minimal amounts found in muscle tissue, indicating stable binding within the cuticle (Peng et al., 2024). In postmortem conditions, fluoride migration from the cuticle to soft tissues is likely regulated by endogenous enzymes in krill. Specifically, the actions of chitinase and trypsin promote the migration of fluoride into proteins, thereby facilitating autolysis (Peng et al., 2024; Ji, Peng, & Ji, 2021a). The migration of fluoride severely hinders the application of AKPs in food production. Therefore, removing residual fluoride from AKPs is necessary. Research has shown that washing AKPs with a dilute acid solution (citric acid, phosphoric acid, and hydrochloric acid) at pH 4.6 effectively reduces protein fluoride levels. This finding demonstrates the efficacy of acidic washing for fluoride removal from AKPs (Jung et al., 2013; Yao et al., 2023). However, this method faces challenges in large-scale industrial production due to the substantial amount of acid solution needed. Researchers have developed a multistage countercurrent washing technology to remove fluoride from AKPs, achieving an excellent fluoride removal rate of 98.58% (Qi et al., 2018). However, given the mechanism of fluoride transfer in Antarctic krill, washing may not be the optimal method for fluoride removal. In the future, new technologies for removing fluoride, such as rapid shelling of krill after fishing or methods for effectively inhibiting endogenous enzyme activity after the death of Antarctic krill, should be developed. This can further prevent fluoride from transferring to the muscles, thereby reducing the levels of residual fluoride in AKPs. Therefore, after the removal of fluoride from AKPs, it could serve as a novel food source or additive for human consumption. In addition, the

development of new, efficient methods for fluoride removal could enable large-scale production of functional AKP products in the future.

6.2. Allergy

Shrimp are the most common offender in the human diet and are often associated with allergic reactions ranging from mild to life-threatening. According to the allergic foods proposed by the FAO of the United Nations, crustacean aquatic products such as shrimp and crab are the most common foods that cause food allergies worldwide (Dong & Raghavan, 2022). Studies using the serum of crustacean allergic patients and immunoblotting have shown that tropomyosin (TM) is the main allergen of Antarctic krill (Lin et al., 2023; Wang et al., 2023). The separation and purification of Antarctic krill TM and the in vitro simulated digestion experiment revealed that although Antarctic krill TM was degraded, hydrogen bonds were broken after digestion with pepsin and trypsin. However, through the positioning of the TM antigenic epitopes, multiple indigestible linear epitopes were retained after digestion, which was considered to be the main reason for the high allergenicity of Antarctic krill TM (Lin et al., 2023). In vivo experiments, Antarctic krill TM was administered to BALB/c mice by gavage or intraperitoneal injection. Both administration methods induced severe allergic reactions in mice, produced high levels of specific antibodies and caused mast cell degranulation (Wang et al., 2023). Therefore, Antarctic krill TM is a potential cross-reactive allergen. Moreover, AKPs still have great potential to harm the health of allergic people. Due to the allergenic potential of Antarctic krill TM, reducing or eliminating the allergenicity of AKPs is particularly important. Existing research indicates that the allergenicity of crustacean TMs can be reduced using enzymatic hydrolysis, microwave treatment, glycosylation reactions, and various nonthermal processing techniques (Dong & Raghavan, 2022). Researchers have used ribose, lactose oligomers, and chitooligosaccharides for glycosylation reactions to eliminate the allergenicity of shrimp TM by inducing conformational changes from α-helical to β-folded structures, thereby reducing allergenicity (Cheng, Wang, & Sun, 2022). However, most current studies typically employ combined methods, which may demonstrate better effectiveness in reducing allergenicity. For example, high-pressure cooking (0.14 MPa, 121 °C, 20 min) significantly reduced TM allergenicity after shrimp treatment. Glycosylation reactions (shrimp meat with lactose) combined with high-temperature high-pressure (0.08 MPa, 115 °C, 6 min) treatment significantly reduced TM binding activity with IgE, and this effect was attributed to the modification of lysine, arginine, and cysteine residues within antigenic epitopes (Dong & Raghavan, 2022). However, there is limited research on reducing the allergenicity of Antarctic krill tropomyosin, necessitating further investigation.

7. Conclusions and future perspectives

Antarctic krill exhibits high protein content and a balanced amino acid profile. The ISP method is the most commonly used technique for extracting AKP. Although this method is straightforward, the use of acid and alkaline solutions during extraction poses challenges to industrial equipment and the environment. Therefore, it is essential to develop new technologies for the extraction of AKPs in the future. Post-mortem autolysis in krill and the low water solubility of proteins weaken the functional properties of AKPs. Reasonable modification techniques can enhance the functional properties and commercial value of AKPs; however, the safety of these modified proteins for human consumption requires further evaluation. Fluoride residues and allergenicity are major obstacles to the application of AKPs. Efficient processes need to be developed to remove fluoride, and novel desensitization techniques should be researched to mitigate the risks to human health and industrial production. Enzymatic hydrolysis of AKPs can produce various bioactive peptides. However, current methods for producing well-defined AKPPs are limited, necessitating the development of more extensive and economically feasible processes for commercial production. Additionally, in-depth in vivo studies are needed to determine the relationship between the structure of bioactive peptides and their biological activities. In summary, the integration of alternative proteins from Antarctic krill sources has the potential to improve global food security and sustainability. The rational development of the vast resources of the Antarctic Ocean can diversify protein sources, reduce environmental impacts, and ensure a more resilient food supply for the growing global population.

Author contributions

Shiying Tang: Writing - original draft, Investigation, Data curation. **Jing Jing Wang**: Supervision, Validation, Writing - review & editing. **Yufeng Li**: Investigation. **Pradeep K. Malakar**: Editing-original draft. **Yong Zhao**: Conceptualization, Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by Program of Shanghai Academic Research Leader (21XD1401200).

References

Ashaolu, T. J., Lee, C. C., Ashaolu, J. O., Pourjafar, H., & Jafari, S. M. (2023). Metal-binding peptides and their potential to enhance the absorption and bioavailability of minerals. *Food Chemistry, 136678*. https://doi.org/10.1016/j.foodchem.2023.136678

Batish, I., Brits, D., Valencia, P., Miyai, C., Rafeeq, S., Xu, Y., … Ovissipour, R. (2020). Effects of enzymatic hydrolysis on the functional properties, antioxidant activity and protein structure of black soldier fly (*Hermetia illucens*) protein. *Insects, 11*(12), 876. https://doi.org/10.3390/insects11120876

Bharmoria, P., Tietze, A. A., Mondal, D., Kang, T. S., Kumar, A., & Freire, M. G. (2024). Do ionic liquids exhibit the required characteristics to dissolve, extract, stabilize, and purify proteins? Past-Present-Future assessment. *Chemical Reviews, 124*(6), 3037–3084. https://doi.org/10.1021/acs.chemrev.3c00551

Boateng, I. D. (2023). Evaluating the status quo of deep eutectic solvent in food chemistry. Potentials and limitations. *Food Chemistry, 406*, Article 135079. https://doi.org/10.1016/j.foodchem.2022.135079

Bowen, H., Durrani, R., Delavault, A., Durand, E., Chenyu, J., Yiyang, L., … Fei, G. (2022). Application of deep eutectic solvents in protein extraction and purification. *Frontiers in Chemistry, 10*, Article 912411. https://doi.org/10.3389/fchem.2022.912411

Caetano-Silva, M. E., Netto, F. M., Bertoldo-Pacheco, M. T., Alegría, A., & Cilla, A. (2021). Peptide-metal complexes: Obtention and role in increasing bioavailability and decreasing the pro-oxidant effect of minerals. *Critical Reviews in Food Science and Nutrition, 61*(9), 1470–1489. https://doi.org/10.1080/10408398.2020.1761770

CCAMLR Secretariat. (2023). Fishery report 2022: Euphausia superba inArea 48. https://fishdocs.ccamlr.org/FishRep_48_KRI_2022.html.

Chen, J., Hu, Y., Gao, P., Jiang, Q., Yu, P., Yang, F., … Xia, W. (2024). Effect of κ-carrageenan on the physicochemical and structural characteristics of ready-to-eat Antarctic Krill surimi gel. *International Journal of Food Science and Technology, 59*(6), 3711–3722. https://doi.org/10.1111/ijfs.17113

Chen, Y. C., Tou, J. C., & Jaczynski, J. (2009). Amino acid and mineral composition of protein and other components and their recovery yields from whole Antarctic krill (Eup hausia superba) using isoelectric solubilization/precipitation. *Journal of Food Science, 74*(2), H31–H39. https://doi.org/10.1111/j.1750-3841.2008.01026.x

Cheng, J. H., Wang, H., & Sun, D. W. (2022). An overview of tropomyosin as an important seafood allergen: Structure, cross-reactivity, epitopes, allergenicity, and processing modifications. *Comprehensive Reviews in Food Science and Food Safety, 21*(1), 127–147. https://doi.org/10.1111/1541-4337.12889

Cunha, S. A., & Pintado, M. E. (2022). Bioactive peptides derived from marine sources: Biological and functional properties. *Trends in Food Science & Technology, 119*, 348–370. https://doi.org/10.1016/j.tifs.2021.08.017

Dai, Y., Li, Z., Fu, M., Li, Y., Xue, C., & Wang, J. (2021). Peptides from Euphausia superba promote longitudinal bone growth by accelerating growth plate chondrocyte

proliferation and hypertrophy. *Current Pharmaceutical Biotechnology, 22*(14), 1866–1877. https://doi.org/10.2174/1381612826666200612170316

Dong, X., & Raghavan, V. (2022). Recent advances of selected novel processing techniques on shrimp allergenicity: A review. *Trends in Food Science & Technology, 124*, 334–344. https://doi.org/10.1016/j.tifs.2022.04.024

Escamilla Rosales, M. F., Olvera Rosales, L., Jara Gutiérrez, C. E., Jaimez Ordaz, J., Santana Sepúlveda, P. A., & González Olivares, L. G. (2023). Proteins of milk, egg and fish as a source of antioxidant peptides: Production, mechanism of action and health benefits. *Food Reviews International*, 1–21. https://doi.org/10.1080/87559129.2023.2227974

Fernando, I. P. S., Park, S. Y., Han, E. J., Kim, H. S., Kang, D. S., Je, J. Y., … Ahn, G. (2020). Isolation of an antioxidant peptide from krill protein hydrolysates as a novel agent with potential hepatoprotective effects. *Journal of Functional Foods, 67*, Article 103889. https://doi.org/10.1016/j.jff.2020.103889

Gao, Y., Ding, Z., Liu, Y., & Xu, Y. J. (2024). Advances in encapsulation systems of Antarctic krill oil: From extraction to encapsulation, and future direction. *Comprehensive Reviews in Food Science and Food Safety, 23*(3), Article e13332. https://doi.org/10.1111/1541-4337.13332

Gao, R., Yu, Q., Shen, Y., Chu, Q., Chen, G., Fen, S., … Sun, Q. (2021). Production, bioactive properties, and potential applications of fish protein hydrolysates: Developments and challenges. *Trends in Food Science & Technology, 110*, 687–699. https://doi.org/10.1016/j.tifs.2021.02.031

Ge, M. X., Chen, R. P., Zhang, L., Wang, Y. M., Chi, C. F., & Wang, B. (2023). Novel Ca-chelating peptides from protein hydrolysate of antarctic krill (*Euphausia superba*): Preparation, characterization, and calcium absorption efficiency in caco-2 cell monolayer model. *Marine Drugs, 21*(11), 579. https://doi.org/10.3390/md21110579

Gigliotti, J. C., Jaczynski, J., & Tou, J. C. (2008). Determination of the nutritional value, protein quality and safety of krill protein concentrate isolated using an isoelectric solubilization/precipitation technique. *Food Chemistry, 111*(1), 209–214. https://doi.org/10.1016/j.foodchem.2008.03.030

Hadidi, M., Palacios, J. C. O., McClements, D. J., Mahfouzi, M., & Moreno, A. (2023). Alfalfa as a sustainable source of plant-based food proteins. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2023.03.023

Han, L., Mao, X., Wang, K., Li, Y., Zhao, M., Wang, J., et al. (2018). Phosphorylated peptides from Antarctic krill (*Euphausia superba*) ameliorated osteoporosis by activation of osteogenesis-related MAPKs and PI3K/AKT/GSK-3β pathways in dexamethasone-treated mice. *Journal of Functional Foods, 47*, 447–456. https://doi.org/10.1016/j.jff.2018.06.004

Hou, H., Wang, S., Zhu, X., Li, Q., Fan, Y., Cheng, D., et al. (2018). A novel calcium-binding peptide from Antarctic krill protein hydrolysates and identification of binding sites of calcium-peptide complex. *Food Chemistry, 243*, 389–395. https://doi.org/10.1016/j.foodchem.2017.09.152

Hu, S., Lin, S., Wang, D., Zhang, S., & Sun, N. (2021a). Antarctic krill-derived peptides with consecutive Glu residues enhanced iron binding, solubility, and absorption. *Food & Function, 12*(18), 8615–8625. https://doi.org/10.1039/d1fo01405f

Hu, S., Wu, J., Zhu, B., Du, M., Wu, C., Yu, C., … Xu, X. (2021b). Low oil emulsion gel stabilized by defatted Antarctic krill (*Euphausia superba*) protein using high-intensity ultrasound. *Ultrasonics Sonochemistry, 70*, Article 105294. https://doi.org/10.1016/j.ultsonch.2020.105294

Ji, W., Peng, Y., & Ji, H. (2021a). Purification and characterization of Antarctic krill chitinase and its role on free fluoride release from Antarctic krill cuticle. *Food Science and Technology, 42*, Article e78021. https://doi.org/10.1590/fst.78021

Ji, W., Zhang, C., & Ji, H. (2017a). Purification, identification and molecular mechanism of two dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from Antarctic krill (*Euphausia superba*) protein hydrolysate. *Journal of Chromatography B, 1064*, 56–61. https://doi.org/10.1016/j.jchromb.2017.09.001

Ji, W., Zhang, C., & Ji, H. (2017b). Two novel bioactive peptides from Antarctic krill with dual angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory activities. *Journal of Food Science, 82*(7), 1742–1749. https://doi.org/10.1111/1750-3841.13735

Ji, W., Zhang, C., Song, C., & Ji, H. (2021b). Three DPP-IV inhibitory peptides from Antarctic krill protein hydrolysate improve glucose levels in the zebrafish model of diabetes. *Food Science and Technology, 42*, Article e58920. https://doi.org/10.1590/fst.58920

Joint Who/FAO/UNU Expert Consultation. (2007). Protein and amino acid requirements in human nutrition. *World Health Organ Tech Rep Ser, 935*, 1–265. http://www.ncbi.nlm.nih.gov/pubmed/18330140.

Jung, H. R., Kim, M. A., Seo, Y. S., Lee, Y. B., Chun, B. S., & Kim, S. B. (2013). Decreasing effect of fluoride content in Antarctic krill (*Euphausia superba*) by chemical treatments. *International Journal of Food Science and Technology, 48*(6), 1252–1259. https://doi.org/10.1111/ijfs.12084

Kaur, K., Kortner, T. M., Benitez-Santana, T., & Burri, L. (2022). Effects of Antarctic krill products on feed intake, growth performance, fillet quality, and health in salmonids. *Aquaculture Nutrition, 2022*. https://doi.org/10.1155/2022/3170854

Khan, S., Rehman, A., Shah, H., Aadil, R. M., Ali, A., Shehzad, Q., … Khaliq, A. (2022). Fish protein and its derivatives: The novel applications, bioactivities, and their functional significance in food products. *Food Reviews International, 38*(8), 1607–1634. https://doi.org/10.1080/87559129.2020.1828452

Kolakowski, E., Gajowiecki, L., Szybowicz, Z., & Chodorska, T. (1980). Application of partial autoproteolysis to extraction of protein from Antarctic krill (*Euphasia superba*) Part 1. Effect of pH on protein extraction intensity. *Food, 24*(6), 499–506. https://doi.org/10.1002/food.19800240603

Lan, C., Zhao, Y. Q., Li, X. R., & Wang, B. (2019). High Fischer ratio oligopeptides determination from Antartic krill: Preparation, peptides profiles, and in vitro antioxidant activity. *Journal of Food Biochemistry, 43*(5), Article e12827. https://doi.org/10.1111/jfbc.12827

Lang, M., Song, Y., Li, Y., Xiang, X., Ni, L., & Miao, J. (2021). Purification, identification, and molecular mechanism of DPP-IV inhibitory peptides from defatted Antarctic krill powder. *Journal of Food Biochemistry, 45*(9), Article e13872. https://doi.org/10.1111/jfbc.13872

Lemos, J. G., Stefanello, A., Garcia, M. V., Furian, A. F., Cichoski, A. J., & Copetti, M. V. (2022). Potential of electrolyzed water to inactivate bread and cheese spoilage fungi. *Food Research International, 162*, Article 111931. https://doi.org/10.1016/j.foodres.2022.111931

Li, S., Lin, S., Jiang, P., Qian, X., Wang, S., & Sun, N. (2024). κ-Carrageenan improves the gelation capacity of antarctic krill myofibrillar proteins through covalent and noncovalent interactions. *Food Hydrocolloids, 110220*. https://doi.org/10.1016/j.foodhyd.2024.110220

Li, Y., Tan, L., Liu, F., Li, M., Zeng, S., Gui, Y., … Wang, J. J. (2023). Effects of soluble Antarctic krill protein-curcumin complex combined with photodynamic inactivation on the storage quality of shrimp. *Food Chemistry, 403*, Article 134388. https://doi.org/10.1016/j.foodchem.2022.134388

Li, Y., Wang, J., Zhu, X., Tan, L., Xie, D., Xu, W., … Wang, J. J. (2022). Basic electrolyzed water coupled with ultrasonic treatment improves the functional properties and digestibility of Antarctic krill proteins. *Food Research International, 162*, Article 112201. https://doi.org/10.1016/j.foodres.2022.112201

Li, Y., Zeng, Q. H., Liu, G., Chen, X., Zhu, Y., Liu, H., … Wang, J. J. (2020). Food-grade emulsions stabilized by marine Antarctic krill (*Euphausia superba*) proteins with long-term physico-chemical stability. *Lebensmittel-Wissenschaft & Technologie, 128*, Article 109492. https://doi.org/10.1016/j.lwt.2020.109492

Li, Y., Zeng, Q. H., Liu, G., Peng, Z., Wang, Y., Zhu, Y., … Wang, J. J. (2021). Effects of ultrasound-assisted basic electrolyzed water (BEW) extraction on structural and functional properties of Antarctic krill (*Euphausia superba*) proteins. *Ultrasonics Sonochemistry, 71*, Article 105364. https://doi.org/10.1016/j.ultsonch.2020.105364

Lin, N., Chi, H., Ni, L., Zhang, H., & Liu, Z. (2023). Study on the sensitization and antigenic epitopes of tropomyosin from antarctic krill (*Euphausia superba*). *Journal of Agricultural and Food Chemistry, 71*(16), 6445–6457. https://doi.org/10.1021/acs.jafc.3c00159

Lin, N., Liu, B., Liu, Z., & Qi, T. (2020). Effects of different drying methods on the structures and functional properties of phosphorylated Antarctic krill protein. *Journal of Food Science, 85*(11), 3690–3699. https://doi.org/10.1111/1750-3841.15503

Liu, Y., Lin, S., Hu, S., Wang, D., Yao, H., & Sun, N. (2022a). Co-administration of antarctic krill peptide EEEFDATR and calcium shows superior osteogenetic activity. *Food Bioscience, 48*, Article 101728. https://doi.org/10.1016/j.fbio.2022.101728

Liu, R., Yu, P., Ge, X., Bai, X., Li, X., & Fu, Q. (2017). Establishment of an aqueous PEG 200-based deep eutectic solvent extraction and enrichment method for pumpkin (*Cucurbita moschata*) seed protein. *Food Analytical Methods, 10*(6), 1669–1680. https://doi.org/10.1007/s12161-016-0732-y

Liu, Y., Yu, X., Zhu, Y., Yang, W., Zeng, Y., Hu, Y., et al. (2022b). Preparation, characterization, and mechanism of antifreeze peptides from defatted antarctic krill (*Euphausia superba*) on *Lactobacillus rhamnosus*. *Molecules, 27*(9), 2771. https://doi.org/10.3390/molecules27092771

Man, H., Sun, P., Lin, J., Ren, X., & Li, D. (2024). Based on hydrogen and disulfide-mediated bonds, l-lysine and l-arginine enhanced the gel properties of low-salt mixed shrimp surimi (Antarctic krill and Pacific white shrimp). *Food Chemistry, 138735*. https://doi.org/10.1016/j.foodchem.2024.138735

Park, S., Je, J., & Ahn, C. (2016). Protein hydrolysates and ultrafiltration fractions obtained from krill (*Euphausia superba*): Nutritional, functional, antioxidant, and ACE-inhibitory characterization. *Journal of Aquatic Food Product Technology, 25*(8), 1266–1277. https://doi.org/10.1080/10498850.2015.1054539

Peng, Y., Ji, W., Ji, H., Han, Z., Zhang, S., & Wen, Y. (2024). Endogenous trypsin triggers free fluoride release from antarctic krill (*Euphausia superba*) cuticle. *Journal of Ocean University of China, 23*(3), 766–774. https://doi.org/10.1007/s11802-024-5738-2

Peng, Y., Ji, W., Zhang, D., Ji, H., & Liu, S. (2019). Composition and content analysis of fluoride in inorganic salts of the integument of Antarctic krill (*Euphausia superba*). *Scientific Reports, 9*(1), 7853. https://doi.org/10.1038/s41598-019-44337-6

Qi, X. M., Liao, E., Wang, L., Lin, H., & Xue, C. H. (2016). Extracting protein from antarctic krill (*Euphausia superba*). *Journal of Aquatic Food Product Technology, 25*(4), 597–606. https://doi.org/10.1080/10498850.2014.904461

Qi, X., Liao, E., Zhao, K., Regenstein, J. M., & Mao, X. (2018). Multi-stage countercurrent process for extracting protein from Antarctic Krill (*Euphausia superba*). *Journal of Food Science and Technology, 55*, 4450–4457. https://doi.org/10.1007/s13197-018-3368-7

Rahman, M., & Lamsal, B. (2021). Ultrasound-assisted extraction and modification of plant-based proteins: Impact on physicochemical, functional, and nutritional properties. *Comprehensive Reviews in Food Science and Food Safety, 20*(2), 1457–1480. https://doi.org/10.1111/1541-4337.12709

Rebezov, M., Saeed, K., Khaliq, A., Rahman, S. J. U., Sameed, N., Semenova, A., … Thiruvengadam, M. (2022). Application of electrolyzed water in the food industry: A review. *Applied Sciences, 12*(13), 6639. https://doi.org/10.3390/app12136639

Sasidharan, A., & Venugopal, V. (2020). Proteins and co-products from seafood processing discards: Their recovery, functional properties and applications. *Waste and Biomass Valorization, 11*, 5647–5663. https://doi.org/10.1007/s12649-019-00812-9

Shao, C., Sun, S., Liu, K., Wang, J., Li, S., Liu, Q., … Wang, Q. (2023). The enormous repetitive Antarctic krill genome reveals environmental adaptations and population insights. *Cell, 186*(6), 1279–1294. e1219. https://doi.org/10.1016/j.cell.2023.02.005

Shi, J., Sun, X., Wang, Y., Yin, S., Liu, Y., & Xu, Y. J. (2023). Foodomics reveals altered lipid and protein profiles of Antarctic krill (*Euphausia superba*) under different processing. *Food Bioscience, 53*, Article 102565. https://doi.org/10.1016/j.fbio.2023.102565

Sun, R., Liu, X., Yu, Y., Miao, J., Leng, K., & Gao, H. (2021). Preparation process optimization, structural characterization and in vitro digestion stability analysis of Antarctic krill (*Euphausia superba*) peptides-zinc chelate. *Food Chemistry, 340*, Article 128056. https://doi.org/10.1016/j.foodchem.2020.128056

Sun, N., Wang, T., Wang, D., Cui, P., Hu, S., Jiang, P., et al. (2020). Antarctic krill derived nonapeptide as an effective iron-binding ligand for facilitating iron absorption via the small intestine. *Journal of Agricultural and Food Chemistry, 68*(40), 11290–11300. https://doi.org/10.1021/acs.jafc.0c03223

Suzuki, T., & Shibata, N. (1990). The utilization of Antarctic krill for human food. *Food Reviews International, 6*(1), 119–147. https://doi.org/10.1080/87559129009540863

Tou, J. C., Jaczynski, J., & Chen, Y. C. (2007). Krill for human consumption: Nutritional value and potential health benefits. *Nutrition Reviews, 65*(2), 63–77. https://doi.org/10.1111/j.1753-4887.2007.tb00283.x

Wang, Y., Chang, Y., Xue, Y., Li, Z., Wang, Y., & Xue, C. (2016). Rheology and microstructure of heat-induced fluid gels from Antarctic krill (*Euphausia superba*) protein: Effect of pH. *Food Hydrocolloids, 52*, 510–519. https://doi.org/10.1016/j.foodhyd.2015.07.032

Wang, K., Li, Y., Dai, Y., Han, L., Zhu, Y., Xue, C., … Wang, J. (2019). Peptides from Antarctic Krill (*Euphausia superba*) improve osteoarthritis via inhibiting HIF-2α-mediated death receptor apoptosis and metabolism regulation in osteoarthritic mice. *Journal of Agricultural and Food Chemistry, 67*(11), 3125–3133. https://doi.org/10.1021/acs.jafc.8b05841

Wang, T., Lin, S., Cui, P., Bao, Z., Liu, K., Jiang, P., … Sun, N. (2020). Antarctic krill derived peptide as a nanocarrier of iron through the gastrointestinal tract. *Food Bioscience, 36*, Article 100657. https://doi.org/10.1016/j.fbio.2020.100657

Wang, S., Lin, S., Liu, K., Liu, Y., Liu, Q., & Sun, N. (2023). Digestion-resistant linear epitopes as dominant contributors to strong allergenicity of tropomyosin in antarctic krill (euphausia superba). *Journal of Agricultural and Food Chemistry, 71*(44), 16739–16751. https://doi.org/10.1021/acs.jafc.3c04999

Wang, Y., Wang, R., Chang, Y., Gao, Y., Li, Z., & Xue, C. (2015). Preparation and thermo-reversible gelling properties of protein isolate from defatted Antarctic krill (*Euphausia superba*) byproducts. *Food Chemistry, 188*, 170–176. https://doi.org/10.1016/j.foodchem.2015.04.126

Wang, R., Wang, J. J., Guo, X., Li, Y., Wu, Y., Liu, H., et al. (2022). Physicochemical and functional properties of the Antarctic krill proteins modified by succinylation. *Lebensmittel-Wissenschaft & Technologie, 154*, Article 112832. https://doi.org/10.1016/j.lwt.2021.112832

Wang, Y., Wang, S., Wang, J., Xue, C., Chang, Y., & Xue, Y. (2015). Preparation and anti-osteoporotic activities in vivo of phosphorylated peptides from Antarctic krill (*Euphausia superba*). *Peptides, 68*, 239–245. https://doi.org/10.1016/j.peptides.2014.10.004

Wang, L., Xue, C., Wang, Y., & Yang, B. (2011). Extraction of proteins with low fluoride level from Antarctic krill (*Euphausia superba*) and their composition analysis. *Journal of Agricultural and Food Chemistry, 59*(11), 6108–6112. https://doi.org/10.1021/jf201009t

Wang, F., Zhao, Y., Liu, Y., Yu, P., Yu, Z., Wang, J., et al. (2017). Peptides from Antarctic krill (*Euphausia superba*) ameliorate senile osteoporosis via activating osteogenesis related bmp2/smads and wnt/β-catenin pathway. *Journal of Food Biochemistry, 41*(4), Article e12381. https://doi.org/10.1111/jfbc.12381

Wang, Y., Zhao, Y., Wang, Y., Zhao, W., Wang, P., Chi, C., et al. (2021a). Antioxidant peptides from Antarctic Krill (*Euphausia superba*) hydrolysate: Preparation, identification and cytoprotection on H_2O_2-induced oxidative stress. *Journal of Functional Foods, 86*, Article 104701. https://doi.org/10.1016/j.jff.2021.104701

Wang, S., Zhou, D., & Lin, H. (2021b). Ameliorative effects of phosphorylated peptide from Antarctic krill (*Euphausia superba*) against H_2O_2-induced damage in MC3T3-E1 cells. *Food Science and Technology, 42*, Article e64920. https://doi.org/10.1590/fst.64920

Wu, D., Tu, M., Wang, Z., Wu, C., Yu, C., Battino, M., … Du, M. (2020). Biological and conventional food processing modifications on food proteins: Structure, functionality, and bioactivity. *Biotechnology Advances, 40*, Article 107491. https://doi.org/10.1016/j.biotechadv.2019.107491

Wu, X., Yang, J., Mumby, W., Zhang, Y., Zhang, Y., Wang, C., … Song, J. (2023). Silkworm pupa protein and its peptides: Preparation, biological activity, applications in foods, and advantages. *Trends in Food Science & Technology*. , Article 104129. https://doi.org/10.1016/j.tifs.2023.104129

Xia, G., Zhao, Y., Yu, Z., Tian, Y., Wang, Y., Wang, S., … Xue, C. (2015). Phosphorylated peptides from Antarctic krill (*Euphausia superba*) prevent estrogen deficiency induced osteoporosis by inhibiting bone resorption in ovariectomized rats. *Journal of Agricultural and Food Chemistry, 63*(43), 9550–9557. https://doi.org/10.1021/acs.jafc.5b04263

Xu, Y., Guo, J., Guan, F., Yang, Q., & Zhang, X. (2023). Organic solvent extraction and characterization of Antarctic krill protein with high crystallinity and high molecular weight. *Process Biochemistry, 134*, 363–369. https://doi.org/10.1016/j.procbio.2023.10.014

Yao, M., Gai, X., Zhang, M., Liu, X., Cui, T., Liu, C., … Jia, A. (2023). Two proteins prepared from defatted Antarctic krill (*Euphausia superba*) powder: Composition, structure and functional properties. *Food Hydrocolloids, 109009*. https://doi.org/10.1016/j.foodhyd.2023.109009

Yu, Z., Gao, Y., Jia, X., Cui, S., Ma, L., Zheng, D., … Chen, Y. (2023). Recent advance in high-intensity ultrasound modification of blue food protein: Mechanisms, functional properties and structural alterations. *Trends in Food Science & Technology, 104271*. https://doi.org/10.1016/j.tifs.2023.104271

Yuan, Z., Ye, X., Hou, Z., & Chen, S. (2023). Sustainable utilization of proteins from fish processing by-products: Extraction, biological activities and applications. *Trends in Food Science & Technology, 104276*. https://doi.org/10.1016/j.tifs.2023.104276

Zeng, Q., Hu, D., Li, Y., Qi, P., Chen, L., Wang, R., et al. (2024). Conjugates of glycosylated antarctic krill proteins and curcumin: Maintaining the storage quality of salmon fillets coupling with photodynamic inactivation. *Food Hydrocolloids, 155*. https://doi.org/10.1016/j.foodhyd.2024.110191

Zeng, Q., Hu, D., Wang, R., Li, Y., Wen, Y., Xiao, H., … Wang, J. (2023). Curcumin-loaded emulsions stabilized by the succinylated Antarctic krill proteins: Establishment of photodynamic inactivation to preserve salmon. *Lebensmittel-Wissenschaft & Technologie, 178*, Article 114613. https://doi.org/10.1016/j.lwt.2023.114613

Zhang, J., Ahmmed, M. K., Regenstein, J. M., & Wu, H. (2024). Recent advances of recycling proteins from seafood by-products: Industrial applications, challenges, and breakthroughs. *Trends in Food Science & Technology, 149*. https://doi.org/10.1016/j.tifs.2024.104533

Zhang, W., Boateng, I. D., & Xu, J. (2023). Novel marine proteins as a global protein supply and human nutrition: Extraction, bioactivities, potential applications, safety assessment, and deodorization technologies. *Trends in Food Science & Technology, 104283*. https://doi.org/10.1016/j.tifs.2023.104283

Zhang, H., Wang, Y., Xu, K., Li, N., Wen, Q., Yang, Q., et al. (2016). Ternary and binary deep eutectic solvents as a novel extraction medium for protein partitioning. *Analytical Methods, 8*(46), 8196–8207. https://doi.org/10.1039/C6AY01860B

Zhang, S., Zhao, G., Suo, S., Wang, Y., Chi, F., & Wang, B. (2021). Purification, identification, activity evaluation, and stability of antioxidant peptides from alcalase hydrolysate of Antarctic Krill (*Euphausia superba*) proteins. *Marine Drugs, 19*(6), 347. https://doi.org/10.3390/md19060347

Zhao, L., Yin, B., Liu, Q., & Cao, R. (2013). Purification of antimicrobial peptide from Antarctic Krill (*Euphausia superba*) and its function mechanism. *Journal of Ocean University of China, 12*, 484–490. https://doi.org/10.1007/s11802-013-2180-2

Zhao, Y., Zhang, L., Tao, J., Chi, C., & Wang, B. (2019). Eight antihypertensive peptides from the protein hydrolysate of Antarctic krill (*Euphausia superba*): Isolation, identification, and activity evaluation on human umbilical vein endothelial cells (HUVECs). *Food Research International, 121*, 197–204. https://doi.org/10.1016/j.foodres.2019.03.035

Zheng, H., Beamer, S. K., Matak, K. E., & Jaczynski, J. (2019). Effect of κ-carrageenan on gelation and gel characteristics of Antarctic krill (*Euphausia superba*) protein isolated with isoelectric solubilization/precipitation. *Food Chemistry, 278*, 644–652. https://doi.org/10.1016/j.foodchem.2018.11.080

Zheng, Y., Ping, Z., Xu, Y., Li, X., & Guo, Q. (2022). Changes in the autolysis level, muscle stability and myofibrillar protein properties of Antarctic krill (*Euphausia superba*) during refrigerated storage. *International Journal of Food Science and Technology, 57*(12), 7829–7839. https://doi.org/10.1111/ijfs.16133

Zheng, H., Shiming, L., Gaozhan, X., Dingding, R., Wenhong, C., Chaohua, Z., et al. (2021). Effect of heat pretreatment before isoelectric solubilisation/precipitation on the characteristics of Pacific oyster (*Crassostrea hongkongensis*) and Antarctic krill (*Euphausia superba*) protein isolates. *International Journal of Food Science and Technology, 56*(2), 682–691. https://doi.org/10.1111/ijfs.14702

Zheng, K., Wu, Y., Dai, Q., Yan, X., Liu, Y., Sun, D., … Jiang, W. (2024). Extraction, identification, and molecular mechanisms of α-glucosidase inhibitory peptides from defatted Antarctic krill (*Euphausia superba*) powder hydrolysates. *International Journal of Biological Macromolecules, 266*, Article 131126. https://doi.org/10.1016/j.ijbiomac.2024.131126

Zhou, Y., Wu, W., Zhang, N., Soladoye, O. P., Zhang, Y., & Fu, Y. (2022). Deep eutectic solvents as new media for green extraction of food proteins: Opportunity and challenges. *Food Research International, 161*, Article 111842. https://doi.org/10.1016/j.foodres.2022.111842

Zou, T., He, T., Li, H., Tang, H., & Xia, E. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules, 21*(1), 72. https://doi.org/10.3390/molecules21010072