

Structure, function, action mechanism and application prospect of black garlic peptide

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Author contributions

Meng-Han Yang conceived the structure of this article and wrote the paper. Yuan-Yuan Wang collected data and analyzed relevant studies. Chun-Ming Dong directed the drawing and critically reviewed the article. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Peer review information

Life Research thanks Kasamba Ilunga Eric and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

SAA-HCM, Enhanced Mixed Supercritical Fluid Assisted Atomization; SAC, S-allyl-L-cysteine; SAMC, S-allylmercapto-cysteine; ROS, reactive oxygen species.

Citation

Yang MH, Wang YY, Dong CM. Structure, function, action mechanism and application prospect of black garlic peptide. *Life Res.* 2024;7(4):18. doi: 10.53388/LR20240018.

Executive editor: Chen-Hui Dong.

Received: 29 July 2024; Accepted: 11 September 2024;

Available online: 13 September 2024.

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Abstract

Black garlic is a processed product derived from fresh garlic, retaining the original nutritional components of garlic while enhancing many of its biological activities. Black garlic peptides are a class of bioactive peptides extracted from fermented black garlic, which have gained considerable attention due to their unique health benefits. This article comprehensively discusses the extraction technologies, structural composition, biological functions, and application potential of black garlic peptides in agriculture, medicine, and the food industry. Extraction methods for black garlic peptides include ammonium sulfate precipitation, alkali-soluble acid-precipitation, enhanced hybrid supercritical fluid-assisted atomization (SAA-HCM), and others, which can efficiently separate bioactive peptides from black garlic. Structural and compositional analysis reveals the characteristics of black garlic peptides as small molecule peptides and the mechanisms of action of these peptides within the body. Black garlic peptides demonstrate health benefits such as antioxidation, anti-inflammatory effects, lipid-lowering properties, and immunomodulatory effects, providing scientific support for their applications in the medical field. In terms of application potential, black garlic peptides can be used as natural plant growth regulators and disease control agents in agriculture; in the medical field, their health benefits make them an important resource for developing new health supplements and drugs; in the food industry, black garlic peptides can serve as functional food additives to enhance the nutritional value and health benefits of foods. As a multifunctional bioactive substance, the optimization of extraction methods, in-depth studies of structure and function, and the development of applications in various fields will bring new opportunities for human health and industrial development. Future research should further explore the detailed mechanisms of action of black garlic peptides and their specific effects in different application domains to promote their wider use.

Keywords: black garlic; black garlic peptide; physiologic function; anti-inflammatory

Overview

Black garlic, also known as fermented black garlic, is a new type of garlic product. It is produced by fermenting fresh garlic under strictly controlled temperature and humidity conditions through pre-treatment, maturation, and drying processes over a long period of time [1, 2]. Black garlic has a black appearance, a soft texture, and a unique taste, making it a popular health food among people [3].

Black garlic contains a rich array of nutrients, including proteins, polysaccharides, vitamins, and minerals [4]. Among these components, black garlic peptides have attracted significant attention in academic circles. Black garlic peptides are multifunctional peptides that were first discovered and studied in the 1990s. However, there are various methods for extracting black garlic peptides, such as ammonium sulfate precipitation, alkali-soluble acid-precipitation, ultrasonic-assisted extraction, water extraction, alcohol extraction, and enhanced hybrid supercritical fluid-assisted atomization (SAA-HCM). Given current operational and cost limitations, ammonium sulfate precipitation and alkali-soluble acid-precipitation are commonly used to extract protein active substances, and ultrasonic assistance can be used to improve overall extraction efficiency. Water or organic solvent extraction is based on a simple principle but is only effective for water-soluble proteins. The SAA-HCM method offers higher extraction efficiency and retains the high activity of protein active substances while reducing solvent residues, but it requires specialized equipment, is more complex to operate, and is less safe, with higher costs. Japanese scientists, while studying the antioxidant properties of black garlic, found that black garlic extracts had excellent antioxidant activity [5]. Following this discovery, researchers began focusing on the active components in black garlic and eventually isolated a peptide with antioxidant properties, known as black garlic peptide. Since then, many research teams have conducted in-depth studies on the biological activities, mechanisms of action, and application prospects of black garlic peptides. Based on current research, black garlic peptides exhibit a variety of biological activities, such as antioxidation, anti-inflammation, antiviral effects, cardiovascular benefits, and immune modulation, making them a highly promising ingredient for health foods and pharmaceuticals [6, 7].

Researchers have utilized various analytical techniques, such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR), to conduct in-depth analyses of the structure-activity relationships of black garlic peptides [8–11]. The results indicate that black garlic peptides are composed of multiple amino acids, and their sequence and structure significantly impact their biological activity. Furthermore, researchers have explored the mechanisms of action of black garlic peptides, and the findings suggest that the antioxidant properties of black garlic peptides may be related to the scavenging of free radicals, inhibition of oxidase activity, and enhancement of antioxidant enzyme activity [12, 13].

With the deepening of research, the application field of black garlic peptide is also expanding. At present, black garlic peptide has been applied in food, health care products, cosmetics, agriculture and other fields, and has achieved certain results. For example, in the food development of black garlic peptide, researchers have successfully developed a variety of products such as black garlic drinks [14], black garlic candy, and black garlic cookies [15], which have been welcomed by consumers. In agriculture, black garlic peptide is also used as a biopesticide, which has good insecticidal and bacteriostatic effects [16].

Despite the progress made in the study of black garlic peptides, there remain several challenges that require further investigation. These include the optimization of preparation methods, the stability of the peptides, and their bioavailability. Additionally, a deeper understanding of the mechanisms of action of black garlic peptides is needed to broaden their potential applications. With ongoing research, black garlic peptides hold promise for playing a larger role in fields such as food, dietary supplements, and agriculture,

contributing significantly to human health and societal development.

Extraction method of black garlic peptide

Black garlic peptide is a kind of small molecular active peptide extracted from black garlic. During the process of extracting black garlic peptide, multiple physical and chemical methods are usually adopted to ensure the maximum extraction of the effective components. Among numerous extraction technologies (Table 1), the water extraction method is the most common and traditional one. It uses water as the solvent and dissolves and separates the active components in black garlic through steps such as heating and stirring. Besides the water extraction method, there is also the alcohol extraction method. This method uses ethanol or other alcohols as the solvent and can effectively extract the fat-soluble components in black garlic. The ammonium sulfate precipitation method and the alkali dissolution-acid precipitation method utilize the solubility differences of proteins and polypeptides at different pH values and separate black garlic peptide through precipitation and redissolution. With the advancement of technology, some more efficient extraction technologies have also been developed. For example, the ultrasonic-assisted extraction method can improve the extraction efficiency and purity of the extract through the mechanical vibration and cavitation effect generated by ultrasound. The strengthened mixed supercritical fluid-assisted atomization method (SAA-HCM) is a more advanced extraction technology. It combines the characteristics of supercritical fluids with atomization technology and can achieve efficient extraction at lower temperatures and pressures while retaining more bioactive components. In actual production, an appropriate extraction method can be selected according to the actual situation. The following is an introduction to several extraction methods of black garlic peptide.

Ammonium sulfate precipitation method

The principle of ammonium sulfate precipitation is based on the differential solubility of proteins at varying concentrations of ammonium sulfate. At low concentrations, ammonium sulfate can keep proteins in solution; at higher concentrations, it disrupts the water layer around protein molecules, exposing hydrophobic regions, which leads to aggregation and precipitation of the proteins. This makes ammonium sulfate precipitation an effective method for protein extraction. The method is simple to perform and cost-effective, making it suitable for the extraction of a wide variety of proteins. Specifically, solid ammonium sulfate is added to a protein solution and stirred until uniformly distributed. As the concentration of ammonium sulfate increases, the solubility of proteins decreases. When the concentration of ammonium sulfate in the protein solution reaches saturation, the solubility of proteins is minimized, causing them to precipitate out of the solution. The saturation level of ammonium sulfate can be controlled by adjusting its mass fraction (such as 20%, 40%, 60%, 80%, etc) [17].

Ju Xiuyun and her team used ammonium sulfate precipitation to extract and study soluble proteins from black garlic [18]. They started by chopping and grinding the black garlic samples, then added an appropriate amount of 10 mmol/L phosphate buffer (pH 7.5) and ground the mixture thoroughly. Next, they added twice the volume of phosphate buffer solution and soaked the mixture at 4 °C for over 10 hours. They then centrifuged the soaking solution at 8000 r/min for 10 minutes at 4 °C and collected the supernatant. Subsequently, they added ammonium sulfate to the supernatant to reach 80% saturation and allowed it to sit overnight at room temperature. Finally, they centrifuged the mixture at 12000 r/min for 30 minutes and washed the precipitate with 10 mmol/L phosphate buffer (pH 7.5) to obtain a crude extract of soluble proteins, which they stored at –20 °C. Tao Fengting et al. used ammonium sulfate precipitation at different saturation levels to extract proteins from *Porphyra yezoensis* in their study on the extraction and structural analysis of separated proteins from this seaweed [19]. Additionally, Ismail MM et al. used ammonium sulfate precipitation, anion-exchange chromatography,

and size-exclusion chromatography to extract and purify phycocyanin (C-PC) from the cyanobacterium *Arthrospira platensis* and allophycocyanin (APC) from the red alga *Corallina officinalis* [20].

Proteins extracted using ammonium sulfate precipitation may retain some residual salt, requiring further purification. Nonetheless, ammonium sulfate precipitation remains an effective and commonly used method for protein extraction, suitable for many laboratory-scale protein preparations.

Alkaline solution and acid precipitation method

The alkali-soluble acid-precipitation method is a common technique for protein extraction, primarily used to isolate proteins from biological samples. Proteins are biopolymers composed of amino acids linked by peptide bonds and have amphoteric properties. When the pH of the solution equals the isoelectric point of the protein, the solubility of the protein reaches its minimum, making it prone to precipitation. Thus, we can change the pH of the solution to alter the solubility of proteins at different pH values, enabling their separation and extraction. Specifically, the biological sample is first mixed with an alkaline solution, causing the proteins to denature and dissolve in the solution. Then, the pH of the solution is altered by adding either alkaline or acidic reagents to meet the solubility requirements of the proteins. Typically, proteins have the lowest solubility under acidic conditions and exist mainly as precipitates; therefore, the addition of acid can precipitate the proteins. Finally, the protein precipitate is collected through centrifugation or filtration and subjected to washing and drying for further processing.

Zhou Zhen et al. used the alkali extraction and acid precipitation method to obtain protein active substances from garlic, purified these substances using different methods, and optimized the process to achieve the best extraction conditions [21]. The optimal conditions were: first, use an alkaline solution for extraction with a pH value of 7.2 for 30 minutes, then adjust the pH value to 4.2 to precipitate the desired protein active substances in an acidic solution. Gao X et al. also used the alkali extraction and acid precipitation method to extract garlic protein and investigate its antioxidant and antihypertensive effects and mechanisms [22]. The detailed steps were: accurately weigh the garlic residue, then add deionized water (weight ratio of 1 : 10), and adjust the pH of the mixture to 9.5 using 0.1 M sodium hydroxide solution. Next, treat the mixture with an ultrasonic extractor for 60 minutes. Afterwards, adjust the pH back to 4.5 using 0.1 M hydrochloric acid solution. Centrifuge the obtained supernatant at 4000 rpm for 15 minutes and collect the supernatant. Finally, wash the precipitate three times with deionized water and then freeze-dry it to obtain garlic protein.

Ultrasonic assisted extraction method

Ultrasound, as a form of electromagnetic wave with extremely short wavelengths, enables rapid extraction of plant bioactive components and is characterized by its high efficiency, energy savings, and environmental friendliness [23]. Ultrasonic-assisted extraction is accomplished through three primary mechanisms: cavitation, thermal effects, and mechanical effects. This technology has been widely applied in the preparation of functional components such as polysaccharides, proteins, phenolic compounds, pigments, and alkaloids, effectively increasing product yields and improving their functional properties, demonstrating promising application prospects. Through its vibrational effect, ultrasound enhances the flow and mixing of solvents, expanding the contact area with cellular materials and accelerating the release and dissolution processes. This enhanced mass transfer process facilitates more effective extraction of active ingredients from cells. For example, through high-intensity ultrasonic treatment, the extraction efficiency of whey protein concentrates is improved, and the stability of complexes formed with pectin is enhanced [24].

Schulnies et al. showed that high-intensity ultrasound can reduce the viscosity of casein micelle concentrates and increase the solubility of their powders [25]. Madadlou et al. demonstrated that ultrasonic treatment can disrupt the casein micelle structure, affecting the

gelation of protein solutions and enhancing the strength of gels, which is beneficial for the production of yogurt and acid gels [26]. Zhang Xuan et al used ultrasound-assisted ethanol extraction to extract garlic oil from garlic, while Feng Yanwei et al. used ultrasonic disruption combined with phosphate buffer to extract superoxide dismutase (SOD) from garlic [27, 28]. They determined through single-factor experiments that the optimal disruption power was 300 W and the disruption time was 5 minutes, noting that ultrasonic treatment might cause partial enzyme inactivation.

Water extraction method

Water extraction is a basic method for protein extraction that uses water as a solvent to physically or chemically extract proteins from samples, primarily targeting water-soluble proteins. The specific procedure involves chopping black garlic and adding an appropriate amount of water, allowing the mixture to steep at room temperature for 24 hours. Afterward, the mixture is filtered, and the filtrate is concentrated under reduced pressure to obtain the black garlic peptide extract [29].

Ma Hongxin et al. extracted quinoa proteins using a water extraction method [30]. Quinoa powder was mixed with 15 volumes of 0.15 M NaCl solution and 0.02 M phosphate buffer (pH 7.4), then extracted at 40 °C for 3 hours. The mixture was centrifuged at 6000 r/min for 30 minutes to remove sediment, and this process was repeated three times. The supernatant was collected, adjusted to pH 4.5 with 1 M HCl, and left to stand for 30 minutes before being centrifuged again. The resulting precipitate was washed with deionized water, adjusted to pH 7, and freeze-dried to obtain water-soluble protein. This method could be adapted for black garlic peptide extraction, though further research on specific conditions is required due to limited studies in this area.

Alcohol extraction

Alcohol extraction is a commonly used method for protein extraction that utilizes alcohols as solvents to physically or chemically extract proteins from samples. This method is primarily used for extracting alcohol-soluble proteins and is characterized by high extraction efficiency and simplicity of operation, making it suitable for various types of samples. Pan Zhili et al. used alcohol extraction to isolate alcohol-soluble proteins from wheat [31]. Cao Wei et al. conducted in-depth research on the purity and extraction yield of wheat alcohol-soluble proteins, examining the influence of factors such as ethanol concentration, liquid-to-solid ratio, extraction temperature, and extraction time [32]. They also studied the physicochemical properties of alcohol-soluble proteins, including water-holding capacity, oil-holding capacity, emulsifying capacity, and emulsion stability. Through these studies, they determined the optimal conditions for alcohol extraction.

Enhanced Mixed Supercritical Fluid Assisted Atomization (SAA-HCM)

Enhanced Mixed Supercritical Fluid Assisted Atomization (SAA-HCM) is a method used for the extraction and purification of biomacromolecules, combining supercritical fluid technology with atomization technology. The basic principle of the SAA-HCM method is to extract the biomolecules from the sample by using the solubility of supercritical fluids for biomolecules, and then the extract is atomized into tiny droplets by nebulization technique to improve the mass transfer efficiency and reaction rate of biomolecules [33]. The protein extraction process using SAA-HCM can be summarized as follows: supercritical fluid dissolution, atomization, supercritical fluid circulation, protein collection, evaporation and concentration, and finally purification and identification. Jiang Shanshan et al. employed the enhanced hybrid supercritical fluid-assisted atomization technique (SAA-HCM) to prepare ultrafine particles of zein (a corn protein) [34]. Shen Yubin et al. used the SAA-HCM method to study the preparation of drug-loaded microparticles in aqueous systems [35].

Table 1 The advantages and disadvantages of various protein extraction methods

| Extraction method | Advantage | Shortcoming | Reference |
|---|---|--|-----------|
| Ammonium sulfate precipitation method | The operation is simple and convenient, the cost is low, it can maintain the structural stability and activity of proteins, and is suitable for the extraction of various proteins. | Low purity, corrosive to appliances, salt residue, and long desalination time. | [36, 37] |
| Alkali extraction and acid precipitation | The protein extraction rate is high, the operation is simple, the cost is low, it is suitable for large-scale protein extraction, and the method is well-developed. | Protein purity is relatively low, need to be centrifuged and washed several times and other operations, easy to cause protein loss and denaturation. | [38, 39] |
| Ultrasonic-assisted extraction | High extraction efficiency and solvent saving. | Complex operation requires certain equipment, and the thermal effect may have certain influence on the biological activity of protein. | [40, 41] |
| Water extraction | Low cost, high safety, stable protein structure. | Low extraction efficiency, complex purification process and long extraction time. | [42, 43] |
| Alcohol extraction | High extraction efficiency and easy purification. | Poor safety, high cost and high risk of protein denaturation. | [44] |
| Enhanced Mixed Fluid Assisted Supercritical Atomization (SAA-HCM) | High extraction efficiency, residue of protein active solvent. | High equipment cost, complex operation and low safety. | [45, 46] |

Chemical structure of black garlic peptide

Black garlic peptides are a series of small molecule peptides produced by enzymatic reaction of allicin (S-allylcysteine). Allicin (Hypoglycemic and hypolipidemic effect of S-allyl-cysteine sulfoxide) is an organosulfur compound with molecular formula: C₆H₁₁NO₃S and molecular weight of 177.22 g/mol as shown in Figure 1 [47]. Allicin is a non-protein sulphur-containing amino acid contained in fresh garlic (6–14 mg/g fresh weight) and is found in higher amounts in black garlic, where it is the main component of black garlic peptides. Allicin is soluble in water but insoluble in organic solvents such as anhydrous ethanol, acetone, chloroform, benzene and acetic acid, and is a colourless crystal or powdery substance at room temperature with a melting point between 165 °C and 166 °C [48]. It contains a sulphur atom in the chemical structure, which gives allicin its unique chemical properties. In the molecular structure of allicin, the sulphur atom is attached to a carbon atom to form a thiol group (-SH), which is the key to allicin's strong odour and antimicrobial properties. The structure of allicin also contains an amino group

(-NH₂) and a carboxyl group (-COOH), which are functional groups that enable allicin to be converted by enzymatic reaction to allicin (Figure 1), the main active ingredient in garlic, with antibacterial, antioxidant and antitumour properties. In addition, sulphur-containing compounds such as S-allyl-L-cysteine (SAC) and S-allylmercapto-cysteine (SAMC) in black garlic peptides also have antioxidant and anti-inflammatory activities [49].

Apart from allicin, the specific amino acid composition of black garlic peptides may vary depending on the extraction and purification process, but usually contains alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, threonine, serine, glutamic acid, aspartic acid, lysine, histidine, arginine, proline, glycine, tryptophan, and cysteine [50]. The free amino acid content is shown in Table 2.

These amino acids occur in different combinations and sequences in the black garlic peptide, giving the black garlic peptide its unique biological activity. Since the amino acid sequence and structure of black garlic peptides have important effects on their biological activity, it is important to study the precise composition and structure of these peptides to understand their function.

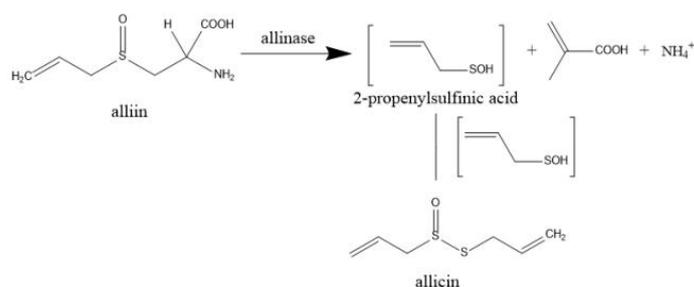


Figure 1 Process of alliin conservin

Table 2 Contents of various amino acids in black garlic

| Amino acid | Content (mg/100 g) |
|-----------------|--------------------|
| Asp | 72.1 |
| Glu | 51.5 |
| Asn | 225.4 |
| Ser | 156.8 |
| Gly | 72.1 |
| Gln | 42.5 |
| His | 46.7 |
| Thr + Pro + Ala | 292.1 |
| Arg | 9647 |
| Tyr | 69.7 |
| Val | 98.6 |
| Cys-Cys | 21.4 |
| Met | 78.8 |
| Cys + Leu + Ile | 49.8 |
| Phe | 229.7 |
| Trp | 68.9 |
| Lys | 385.9 |
| Total | 2926.7 |

Biological function of black garlic peptide

Antioxidant effect

Black garlic peptide is a bioactive compound extracted from fermented black garlic that exhibits significant antioxidant properties [51, 52]. This antioxidant property is closely related to water-soluble organosulfur compounds such as alliin, allicin, S-allyl-L-cysteine (SAC) and S-allylmercapto-cysteine (SAMC), which are present in the black garlic peptides. In fresh garlic, SAC and SAMC are initially present as γ -glutamyl-S-allylcysteine, and after heat treatment, these compounds are converted to water-soluble organosulfur compounds such as SAC and SAMC by the action of endogenous γ -glutamyl transpeptidase. These transformed compounds are effective in scavenging free radicals in the body and protecting cells from reactive oxygen species (ROS)-induced oxidative damage [53]. In the human body, alliin and allicin can be converted to SAC and SAMC, while SAC and SAMC in black garlic show high stability during digestion.

In studies comparing the antioxidant properties of fresh garlic and black garlic in type 2 diabetes animal models, mice fed with black garlic exhibited higher superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities compared to those fed with fresh garlic, indicating that black garlic has superior antioxidant properties [54]. P.J. G et al. investigated the changes in antioxidant compounds and antioxidant activity of black garlic before and after processing, finding that after treatment at 70 °C and 90%

humidity, the content of flavonoids, polyphenols, and sulfur compounds significantly increased, along with enhanced antioxidant activity [55]. Black garlic has a high DPPH radical scavenging ability, with a scavenging capacity of 74.48% for DPPH radicals and over twice the scavenging ability for ABTS radicals compared to fresh garlic. Sun Yue'e et al. found that during the Maillard reaction, the reductive power of black garlic increased tenfold within 28 days, and the stronger the reductive power, the greater the antioxidant capacity [56, 57].

Mitochondria-mediated apoptosis is closely related to the expression of Bcl-2 family genes [58]. Bcl-2 and Bax genes represent proteins that inhibit cell proliferation and promote cell death, respectively. The balance between these two genes is typically measured by their expression ratio. Additionally, members of the Caspase family, such as Caspase-3, -8, and -9, play crucial roles in the process of apoptosis. Caspase-3 is directly involved in the apoptotic process, while Caspase-8 and Caspase-9 are also key proteins closely associated with apoptosis. When cells are treated with alliin, the expression of the Bax gene decreases, whereas the expression of the Bcl-2 gene increases. At the same time, the expression levels of Caspase-3, Caspase-8, and Caspase-9 also show a decreasing trend. Alliin may alleviate oxidative stress-induced cellular damage by modulating the expression of these apoptosis-related proteins.

Additionally, alliin may exert its antioxidant effects through the activation of the Nrf2 signaling pathway and by regulating apoptosis.

Nrf2 is a critical antioxidant molecule within cells that typically binds to Keap1, maintaining its stability in the cytoplasm. Under stress conditions, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it activates antioxidant response elements (ARE), thereby initiating the expression of a series of antioxidant genes. These genes include HO-1 and NQO-1, which are important proteins for combating oxidative stress. HO-1 catalyzes the degradation of heme, producing biliverdin, bilirubin, and iron, thus providing antioxidant protection [59]. NQO-1 combats oxidative stress by reducing the production of reactive oxygen species (ROS) and alleviating oxidative damage to DNA. When cells are subjected to oxidative stress or damage, the expression of HO-1 and NQO-1 is upregulated to help defend against ROS generation and oxidative damage to DNA. In summary, alliin can exhibit antioxidant activity, with the mechanism likely involving the Nrf2 signaling pathway (Figure 2), providing a scientific basis for further investigation into the antioxidant mechanisms of alliin.

Anti-inflammatory effect

Biological tissues, when stimulated by damage factors such as trauma or infection, undergo a fundamental pathological process primarily characterized by a defensive response known as inflammation. The local manifestations of inflammation include redness, swelling, warmth, pain, and functional impairment, accompanied by systemic responses such as fever and changes in peripheral white blood cell counts. Inflammation is an adaptive defense mechanism that has evolved to maintain the body's internal stability. Under normal circumstances, this response is beneficial, helping to protect the body from further harm and initiating the repair process. However, if the inflammatory response becomes uncontrolled and persists for too long, it can become harmful, potentially leading to serious health issues such as septic shock or multi-organ dysfunction. Inflammation is also a key factor in many physiological and pathological processes, including the development of cancer and certain degenerative diseases. Studies have shown that black garlic peptides possess anti-inflammatory properties, capable of inhibiting inflammatory responses in various tissues [60].

Min L and colleagues studied the effects of alliin, a compound found in garlic, on the inflammatory response in RAW264.7 cells [61]. Through quantitative analysis and fluorescence staining techniques, they found that alliin significantly reduced the production of nitric oxide (NO) and reactive oxygen species (ROS) in a dose-dependent manner. Additionally, using polymerase chain reaction (PCR) and Western blotting, they observed that alliin could significantly decrease the mRNA and protein expression of inflammation-related factors in the cells. Further studies have shown that alliin can inhibit myeloperoxidase activity in the lungs, reduce the expression of TNF- α and IL-1 β in bronchoalveolar lavage fluid, and alleviate acute lung injury induced by lipopolysaccharide (LPS), thereby exerting anti-inflammatory effects. Black garlic peptides were also found to attenuate the phosphorylation of JNK and p38 MAPK in macrophages in a dose-dependent manner, which in turn suppressed the expression of inflammatory mediators and pro-inflammatory cytokines. This anti-inflammatory effect was demonstrated in human umbilical vein endothelial cells treated with TNF- α and in a mouse model of phorbol ester-induced dermatitis [62]. Research further revealed that black garlic peptides regulate the NF- κ B signaling pathway, reducing the release of inflammatory cytokines such as TNF- α and IL-6, thus alleviating inflammation (Figure 2). Compared to fresh garlic, black garlic has a more pronounced protective effect on the gastrointestinal mucosa; it does not damage the mucosa but rather reduces esophageal inflammation and relieves gastric spasms [63]. These findings suggest that black garlic peptides have potential applications in anti-inflammatory and gastroprotective roles, particularly in the treatment of inflammatory diseases [64].

During the initial stages of tumorigenesis, there is a delicate balance between the inflammation caused by emerging tumor cells and the host's anti-tumor immune response. When the host's intrinsic anti-tumor activity is weaker than the inflammation and

immunosuppressive activity caused by tumor cells, immune-evasive tumor cells can grow rapidly. Conversely, when the host's intrinsic anti-tumor activity is stronger than the inflammation and immunosuppression caused by tumor cells, the tumor cells are cleared. Long-term consumption of organic sulfur compounds from black garlic can help suppress the inflammation caused by tumor cells (left) and/or enhance the anti-tumor immune response (right), disrupting the balance within the host and shifting from an inflammatory response to an anti-tumor response, gradually eliminating the tumor cells. Notably, cytokines IL-10 and TGF- β have dual roles in the process of tumorigenesis, determined by the specific microenvironment at the time of tumor initiation.

Anti-tumor effect

In current garlic research, results indicate that alliin and sulfur compounds have certain anticancer effects [65]. When garlic cell membranes are damaged, alliin interacts with allinase to produce alliin. During fermentation, alliin in black garlic undergoes transformation, leading to an increase in other types of sulfur compounds. Among these, water-soluble sulfur compounds include SAC (S-allyl cysteine) and SAMC (S-allyl mercaptocysteine), while fat-soluble sulfur compounds include diallyl sulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide. These compounds, especially SAC and SAMC, play significant roles in antioxidant and anticancer activities (Figure 2). The processing of black garlic also facilitates the conversion of garlic proteins into amino acids, which can help enhance the human immune system, reduce fatigue, and fight cancer. Additionally, trace elements such as selenium and germanium in black garlic have a positive impact on anticancer effects [66].

Recent epidemiological studies have shown that garlic consumption may reduce the risk of some cancers, including biliary tract, gastric, colorectal, and breast cancers [67]. The intake of allium vegetables, such as onions, garlic, and leeks, is negatively associated with the risk of gallbladder cancer, suggesting that consuming anti-inflammatory foods can lower the risk of biliary tract cancer. An Italian case-control study found that frequent consumption of allium vegetables like onions and garlic can decrease the risk of gastric cancer. A study in South Korea discovered that regular garlic intake can also lower the risk of gastric cancer. Research has shown that aged black garlic extract can delay the progression of breast cancer by regulating hormone balance in mice [68]. Meta-analyses have found that both high and low garlic intakes are associated with reduced gastric cancer risk, with higher intakes showing particularly significant effects. This suggests a link between garlic intake and a reduction in gastric cancer risk. Animal experiments have demonstrated that black garlic can inhibit the growth rate of gastric, liver, and colon cancers in mice. Studies have found that black garlic extract can significantly slow the growth of tumors in mice carrying them [69]. Further experiments have shown that the levels of superoxide dismutase, glutathione peroxidase, and interleukin-2 are increased in mice, indicating that the anticancer effects of black garlic are related to its antioxidant and immunomodulatory properties. After transplanting H22 liver cancer cells into Kunming mice, a water extract of black garlic significantly inhibited the growth of liver cancer tumors in the mice, with an inhibition efficiency exceeding 40%. Additionally, when colon cancer Ht-29 mice were treated with a combination of black garlic extract and γ irradiation, the growth of transplanted tumors was inhibited, and the duration of tumor growth was extended, suggesting an improvement in the sensitivity to radiation therapy.

Studies have shown that a combination of black garlic extract and γ radiation exhibits growth-inhibitory effects. Research on the impact of black garlic extract on colorectal cancer xenografts in mice has found that black garlic extract can enhance radioprotective effects [70]. In studies on the antitumor properties of black garlic, it was observed that, compared to the high-fat model group, the high-dose black garlic group showed significantly elevated activities of catalase and glutathione peroxidase, indicating strong glucose metabolism and antioxidant capacities [71]. When a black garlic peptide sample at a concentration of 1 mg/mL was diluted to a 1:10 ratio, it displayed a

strong inhibitory effect on the liver cancer cell line HepG2, suggesting that the soluble proteins in black garlic possess antitumor properties. While there is extensive research on the total extracts of black garlic, further work is needed to isolate and screen its effective components and to conduct detailed studies on its antitumor mechanisms related to cell apoptosis. These areas require more in-depth exploration in the future.

Immune regulatory effects

The smooth functioning of the immune system is closely linked to our overall health. It serves as a vital defense mechanism against invading substances and pathogens. Within this system, macrophages play a critical role, not only engulfing and clearing foreign microorganisms but also recognizing and destroying tumor cells, thus protecting our bodies from infection and disease [72].

Alliin in black garlic peptides has demonstrated regulatory effects on the immune system in multiple animal experimental models (Figure 3). This regulation might include enhancing the functionality of immune cells, improving their ability to recognize and eliminate pathogens, or modulating the immune response to prevent excessive reactions that can lead to tissue damage. Through these mechanisms, alliin helps maintain balance in the immune system, ensuring it can effectively protect us from diseases while avoiding unnecessary immune responses. This balance is crucial for preventing infections, cancer, and other diseases caused by immune system dysregulation. Therefore, alliin, as a potential immunomodulator, provides a possible strategy to enhance the function of the immune system, thus improving overall health [73].

Research has found that alliin effectively inhibits the expression of the receptor CD36 on macrophage surfaces induced by homocysteine, suggesting its potential to regulate immune responses. Further studies, through feeding alliin to mice, observed an increase in the number of hemolytic plaques formed by lymphocytes, indicating enhanced lymphocyte activity. Additionally, alliin enhanced phagocytic function in mice and intensified their type IV hypersensitivity reactions, further confirming its role in augmenting cellular immune function [74]. In a rabbit model of swelling induced by *Staphylococcus aureus*, researchers further explored the impact of alliin on immune function. They found that alliin not only promotes the maturation and release of immune cells but also enhances the body's immune response, thereby improving its immune defense capabilities. Furthermore, black garlic peptides stimulate B cells to produce antibodies, enhancing humoral immune responses, which aids in fighting pathogens and foreign substances [75]. Given that oxidative stress and inflammatory responses can suppress immune function, the antioxidant and anti-inflammatory properties of black garlic peptides also contribute to immune regulation.

These findings collectively suggest that black garlic peptides have potential positive effects in modulating the immune system, which could be significant for the treatment of certain immunological disorders.

Anti-cardiovascular effects

Cardiovascular diseases are among the most prevalent and deadly conditions worldwide, with complex pathogenic mechanisms involving numerous factors [76]. Studies have shown that regular consumption of garlic can reduce risk factors for arteriosclerosis, such as hypertension and total cholesterol levels [77]. This effect may be related to alliin, allinase, and allicin present in black garlic.

Currently, research on the roles of alliin, allinase, and allicin in myocardial ischemia and myocardial infarction remains limited. Sangeetha et al. explored the protective effects of alliin against myocardial ischemia in a rat model of isoproterenol-induced myocardial damage. In this study, the rats were treated with varying doses of alliin (0.04 and 0.08 mg·g⁻¹) [78]. Through histopathological assessment and serum biochemical marker detection, including CK, CK-MB, TC, TG, free fatty acids, as well as the activities of ICDH, SDH, α-KG-DH, NADH dehydrogenase, cytochrome c oxidase, Na⁺, K⁺-ATPase, and Ca²⁺, Mg²⁺-ATPase in myocardial tissue, they found

that alliin effectively alleviated isoproterenol-induced myocardial damage by enhancing mitochondrial function and antioxidant capacity in cardiac muscle cells [79].

AMPK is a protein kinase that regulates energy, glucose and lipid metabolism, as well as mitochondrial function. Alliin, by activating AMPK and modulating lipid metabolism, helps alleviate hyperlipidemia [80]. PPAR are a group of nuclear receptors activated by fatty acids, including PPAR-α, PPAR-β/δ, and PPAR-γ. PPAR-α reduces triglyceride levels, whereas PPAR-γ influences adipocyte differentiation and lipid metabolism. Alliin inhibits the expression of PPAR-γ. SREBP are transcription factors regulated by glucose and insulin that control lipid synthesis, with SREBP-1c promoting fatty acid and triglyceride synthesis. A high-fat diet increases SREBP-1c, which is inhibited by alliin [81]. CD36, also known as a scavenger receptor, binds to fatty acids and activates the ERK pathway, promoting the secretion of inflammatory factors. Alliin, by decreasing CD36 expression, prevents or treats lipid metabolic disorders (Figure 4). FASN and ACC are key enzymes involved in hepatic fatty acid synthesis. Alliin improves the metabolism in hyperlipidemic mice by inhibiting these enzymes, with higher concentrations leading to stronger inhibition. Additionally, alliin inhibits the protein expression of inflammatory factors IL-6, IL-1β, TNF-α, and iNOS [82].

Application prospects and challenges of black garlic peptide

In the food and beverage industry

Black garlic peptides, due to their antioxidant, anti-inflammatory, and antimicrobial properties, are extensively studied for the development of novel health foods and dietary supplements [83]. They can help improve cardiovascular health, enhance immunity, and potentially play a positive role in preventing certain chronic diseases. Black garlic peptides can be added to yogurts, juices, and energy bars to provide antioxidant and immune-enhancing benefits. As a natural food additive, black garlic peptides can enhance the taste, color, and shelf life of foods while offering additional health benefits [84, 85]. Black garlic peptides can serve as nutritional supplements, supplying essential amino acids and trace elements, enhancing immunity, and promoting overall health. Due to their potent antioxidant capabilities, black garlic peptides can be utilized in developing anti-aging health supplements, helping to slow down the aging process and improve skin health. They can also be added to health drinks, such as sports and energy beverages, to provide extra health benefits, such as increased endurance and recovery [86]. Furthermore, black garlic peptides can be used to develop functional beverages, such as immune-boosting and cardiovascular health drinks, to meet consumers' demands for healthier lifestyles.

In the pharmaceutical industry

As a potential antibiotic substitute, nigra peptide has great potential in the field of medicine. Its antibacterial activity and inhibitory effect on drug-resistant strains provide a new idea for the treatment of bacterial infection [87]. Especially in the context of the increasingly serious problem of antibiotic resistance, its antibacterial activity is particularly important. Black garlic peptide has potential application value in the treatment of inflammatory diseases such as arthritis and inflammatory bowel disease [88]. By inhibiting inflammatory reaction, black garlic peptide can help relieve symptoms and improve patients' lives. Black garlic peptide is the choice of antioxidant and free radical scavenger. These antioxidants have certain preventive and therapeutic effects on diseases related to oxidative stress, such as cardiovascular diseases and neurodegenerative diseases [89]. It can also enhance the immune response of the body and improve the body's resistance to pathogens, which is of great significance for improving the efficacy of vaccines and developing drugs to strengthen immunity. Although black garlic peptides have broad application prospects in the medical field, there are still some challenges to overcome in practical applications. The safety and efficacy of black garlic peptides in humans need to be validated through clinical trials to ensure their safety as a drug ingredient.

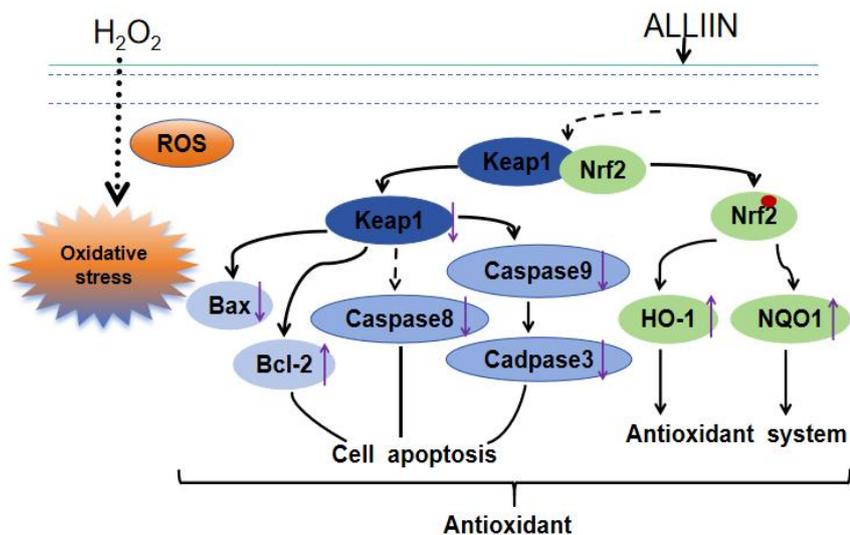


Figure 2 Antioxidant mechanism of black garlic peptide

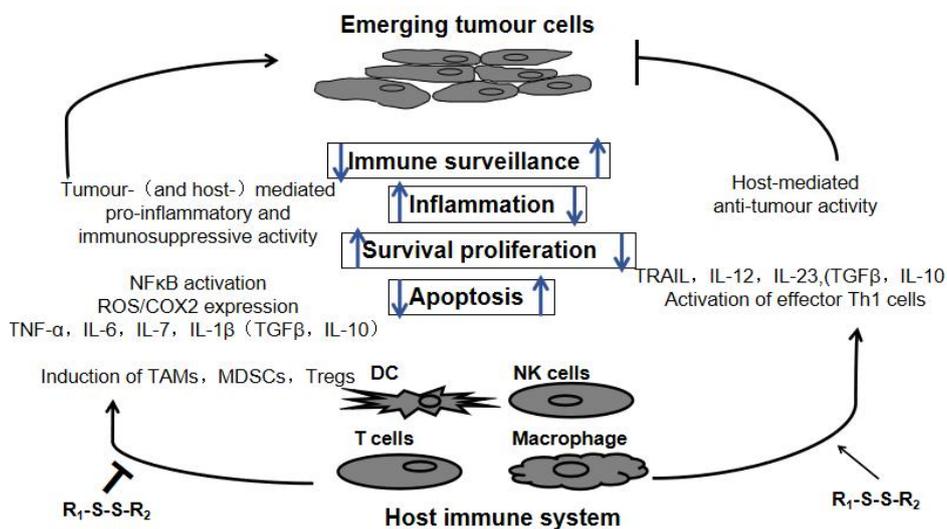


Figure 3 Anti-inflammatory and anti-tumor immune mechanism of black garlic peptide

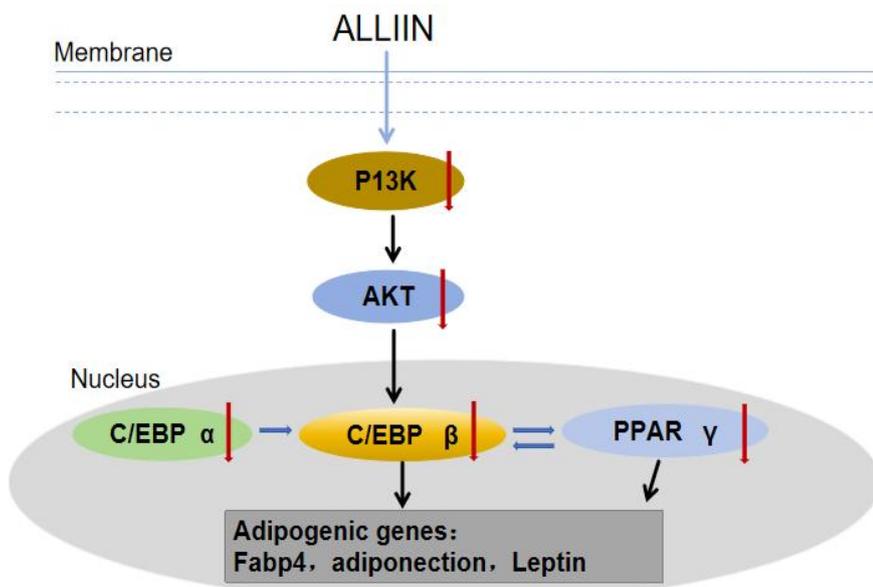


Figure 4 Anti-lipogenesis mechanism of black garlic peptide

Agricultural Industry

As a natural bioactive substance, black garlic peptides exhibit potential applications in plant protection, crop yield enhancement, and improving the quality of agricultural products. With antimicrobial and antifungal properties, black garlic peptides can help control plant diseases, significantly reducing the frequency of chemical pesticide usage and thereby lowering environmental pollution and chemical residues in agricultural products. Research indicates that black garlic peptides can promote crop growth by enhancing plant resilience. They assist plants in better withstanding adverse conditions such as drought, salinity, and low temperatures, thus increasing crop yields and quality [90]. Additionally, black garlic peptides not only promote crop growth but also enhance the quality of agricultural products. They can improve the efficiency of photosynthesis and facilitate the accumulation of nutrients, leading to enhanced flavor, nutritional value, and shelf life of fruits.

Outlook

Black garlic peptides, as natural products with diverse bioactivities, hold promising research and application prospects. Extracting and purifying these peptides from black garlic is the most critical step in the research process. Currently, common extraction methods used in laboratories include aqueous extraction and ultrasound-assisted extraction, but these techniques suffer from low efficiency, high impurity content, and complex, costly purification processes. Therefore, exploring new extraction and purification methods to increase yields and purity while reducing costs represents a significant challenge in the current research. With ongoing advancements in science and technology, particularly the application of nanotechnology, membrane separation, mass spectrometry, and nuclear magnetic resonance (NMR) analysis, the extraction efficiency and purity of black garlic peptides are expected to improve significantly, while production costs will be controlled. This will lay a solid foundation for the large-scale production and application of black garlic peptides. Regarding structural identification and bioactivity studies, as our understanding of the amino acid sequences and structural characteristics of black garlic peptides deepens, we can better elucidate the molecular mechanisms underlying their antioxidant, anti-inflammatory, and cholesterol-lowering activities. This provides a scientific basis for the application of black garlic peptides in disease prevention and treatment. Future research may uncover additional targets and signaling pathways, guiding the development of new drugs and dietary supplements. Moreover, clinical trials and safety assessments are critical steps in developing black garlic peptides as health products and pharmaceuticals. Through conducting more clinical studies, we can gain a comprehensive understanding of the long-term effects and safety of black garlic peptides, providing scientific support for their market entry and widespread use. In the application domain, black garlic peptides have significant potential as functional food ingredients and could become an essential component of health foods and dietary supplements, catering to the growing demand for healthy food options. To maintain the biological activity of the extracts and prevent deactivation during extraction and purification, gentle extraction methods can be employed to preserve active components. Simulating the complexity of foods, studying interactions among components, and replicating them in supplements can also be pursued. Clinical studies should be conducted to evaluate supplement efficacy and compare it with the original food source.

The development of black garlic peptides as nutritional supplements or drugs must be grounded in safety assessments. However, there is currently a lack of data on the long-term safety of consuming black garlic peptides. Therefore, more clinical studies will be needed in the future to further evaluate the long-term effects and safety of black garlic peptides. The prospects for the application of black garlic peptides in the pharmaceutical industry are extensive. As research into black garlic peptides continues to advance, they have the potential to become novel treatments for chronic diseases such as cardiovascular

disease and diabetes.

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