

Research Progress in The Application of Bioactive Peptides in Cosmeceutical Products

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Abstract: Consumer demand for natural source cosmeceutical products with protective and therapeutic functions has prompted the cosmeceutical industry to look for alternative active ingredients. Natural bioactive peptides have single or multifunctional biological properties, such as anti-aging, anti-inflammatory, antibacterial, antioxidant activities, etc., which can promote the improvement of skin health by providing specific physiological functions, and are ideal raw materials for the development of medicinal cosmetics. In this paper, the preparation and characterization methods of bioactive peptides were reviewed, and the bioactivities of peptides related to their potential cosmeceutical applications were introduced in detail, and the challenges and opportunities of developing bioactive peptide-based cosmeceutical formulations were expounded, so as to provide references for the application and commercial development of natural bioactive peptides in the field of medicinal cosmetics.

1. Introduction

Cosmeceutical products, referred to as cosmeceuticals, also known as functional cosmetics, are a class of products between drugs and cosmetics, with both the therapeutic efficacy of drugs and the skin-care function of cosmetics [1]. The term cosmeceutical, originally proposed by Raymond E. Reed in 1960, is specifically described as a substance that meets the following points: (1) external application; (2) produces useful and desired results; (3) has desirable aesthetic properties; (4) complies with chemical, physical, and medical standards [2]. Cosmeceuticals combine the aesthetic properties of cosmetics with the efficacy of dermatologic drugs, aiming to achieve a dual purpose: to provide the desired aesthetic effect and to treat the dermatologic condition. Modern cosmeceuticals must achieve two specific benefits: immediate response (as in cosmetics) and prolonged effect of use (as in drugs) [1]. However, over time, both in terms of actual efficacy and cosmetic effect, the term has been expanded to include any cosmetic product claiming to have medicinal properties [3]. The medicinal cosmetics industry has grown tremendously over the past few decades, due in large part to increasing consumer demand for safer and more effective products, as well as advances in technology that have made the development of medicinal products more feasible [4].

In recent years, the addition of organic, sustainable and natural ingredients has become one of the popular directions in the development of cosmeceuticals due to the growing concern about maintaining healthy skin [5]. Within this trend, natural bioactive peptides are attracting attention for their multiple functions and potential applications [6]. Bioactive peptides are short-chain protein fragments composed of amino acids, which can be biologically active in the human body through different mechanisms and thus fulfill their regulatory functions [7]. For example, some bioactive peptides can achieve their specific effects by regulating cell physiological processes, interacting with the external environment, and promoting tissue repair [8]. Compared with chemically synthesized active ingredients, natural bioactive

peptides have many advantages and are considered a more reliable and safer alternative active ingredient [9]. In addition, the natural bioactive peptide market is expanding as consumers increasingly demand health and beauty products [4]. In the U.S. alone, more than 25 different peptides are regularly featured in a variety of skin care products [2]. Many more peptides are currently under development around the world, which will not only increase the number of peptide ingredients, but also expand the diversity of their applications [10].

This paper provides an overview of the preparation and characterization of natural bioactive peptides, and reviews the bioactivities of bioactive peptides in cosmeceuticals, such as anti-aging, antibacterial, anti-inflammatory, antioxidant and multifunctional bioactivities for improving skin health. It provides valuable theoretical references for the application and commercial development of natural bioactive peptides in the field of cosmeceuticals, and further promote the development of beauty and health-related fields.

2. Preparation and Characterization of Bioactive Peptides

2.1. Preparation of bioactive peptides

Current methods for preparing bioactive peptides mainly include enzymatic hydrolysis, microbial fermentation and chemical hydrolysis [11].

2.1.1. Enzymatic hydrolysis method

Enzymatic hydrolysis of protein is the most commonly used method to produce active peptides. Enzyme type, enzymolysis time, temperature, pH, and enzyme dosage affect the enzymolysis results and efficiency, and thus affect the preparation of bioactive peptides [12]. Enzymatic hydrolysis method has the advantages of strong controllability, strong reproducibility, mild conditions, high safety and no side reactions, and has become the preferred process for the production of bioactive peptides in food and pharmaceutical industry in recent years [13]. However, pH adjustment before enzymatic hydrolysis will introduce inorganic salt ions, which increases the difficulty of subsequent separation and

purification of active peptides [14].

2.1.2. Microbial fermentation method

In this method, microbial strains (such as *lactic acid bacteria*, *yeast*, *cyanobacteria*, etc.) are used to degrade proteins by metabolites under suitable fermentation conditions, so as to generate peptides with biological activity [15]. Compared with enzyme method, microbial fermentation method has a relatively low cost to produce bioactive peptides, and has been widely used in food, health care products, drugs and other fields [16]. At the same time, by selecting different strains and adjusting fermentation conditions, a variety of different bioactive peptides can be obtained, which has great development potential [15]. However, the low yield of bioactive peptides produced by fermentation and the lack of specificity in peptide formation have severely hindered their industrial use [15, 17].

2.1.3. Chemical hydrolysis method

The chemical hydrolysis of protein is the method of cracking protein peptide bonds with acid or base reagents to produce peptides and free amino acids [14]. This method is used for the preparation of bioactive peptides because of its

simple operation and low cost [11]. In the United States, most flavoring peptides used in processed meats, cookies, and soups are produced by acid-hydrolyzing plant proteins [18]. However, chemical hydrolysis method has many limitations, such as difficult to control the hydrolysis process, easy to lead to chemical composition changes and other problems [19]. Hydrolysis using strong chemicals and solvents under extreme temperature and pH conditions can also lead to instability of product composition and reduced nutritional quality [6]. In addition, the neutralization step after acid hydrolysis requires the introduction of salts, while alkaline hydrolysis produces some undesirable amino acid derivatives. Therefore, chemical hydrolysis is rarely used for the industrial production of bioactive peptides [17].

The separation and purification of target peptides is particularly important in both research and industrial production of active peptides [11]. Due to the differences in the nature of the target peptide, its raw materials, preparation methods, etc., it is required to choose a suitable separation and purification method [20]. The commonly used separation and purification techniques for bioactive peptides are shown in Table 1.

Table 1. Commonly used separation and purification techniques for bioactive peptides

Technique/Method	Separation Characteristics	Source	Assessed bioactivity	References
Salt fractionation	Based on reduced peptide solubility	green pea protein		Chang et al. [21]
Ultrafiltration	Based on molecular weight size and shape	camel milk protein	Anti-diabetic and ACE inhibitory activities	Khakhariya et al. [8]
Nanofiltration	Based on the combined action of steric hindrance and charge effect	Dairy solution		Zhang et al. [11]
Gel filtration chromatography	Based on the size and character differences of polypeptide molecules	mulberry leaf enzymatic hydrolysate	anti-tyrosinase, anti- α -glucosidase and antioxidant activity	Cao et al. [13]
High performance liquid chromatography	Based on the different partition coefficients between the stationary phase and the mobile phase	Seaweed enzymatic hydrolysate	Antitumor activity	Shen et al. [16]

Table 2. Commonly used qualitative and quantitative identification techniques for bioactive peptides

Identification method	Characteristics	Suitable object	References
Terminal sequencing method	Advantages: Simple operation, high efficiency. Disadvantages: Not suitable for N-terminal closed ring polypeptides, often used in combination with other methods.	It can be applied to analyze peptides containing modification groups (such as phosphorylation, methylation, etc.).	Hong et al. [24]
Nuclear magnetic resonance	Advantages: It can provide atomic-level structural information, including bond length, Angle, etc. Disadvantages: Structural analysis of macromolecular peptide chains is difficult, requiring high concentration of samples and longtime measurements.	Suitable for smaller peptide molecules.	Roversi et al. [23]
Mass spectrometry	Advantages: It can provide precise molecular weight information of peptide molecules to help determine their molecular composition. Disadvantages: It is usually not possible to provide atomic-level structural information, and it may be difficult to parse complex peptide structures.	It can be used to detect peptide modification, dehydration, cleavage and so on.	Benavente et al. [22]

2.2. Structural characterization of bioactive peptides

In the study of active peptides, liquid chromatography-mass spectrometry (LC-MS/MS) is mostly used for the structural identification of active peptides, i.e., liquid chromatography is used to separate the peptides first, and then tandem mass spectrometry system is used for the structural characterization, which has now become a standard method for the identification of peptide sequences [22]. In LC-MS/MS analysis, the peptide sample is first ionized to form charged ions, and then enters the mass spectrometer for analysis [16]. The peptide mass spectrometry data obtained by mass spectrometry can be compared with the peptide sequence in the mass spectrometry database to analyze the primary structure of the peptide [22]. Numerous studies have shown that mass spectrometry, especially LC-MS/MS, is one of the most commonly used methods for structural characterization of purified peptides. This technique is highly accurate and widely adaptable to rapidly characterize the primary structure of peptides [23]. The qualitative and quantitative identification techniques of bioactive peptides have been reported in the literature as shown in Table 2.

3. Bioactivities of Peptides Related to Cosmeceutical Applications

3.1. Anti-aging active peptides

3.1.1. Collagenase inhibitory peptide

Collagen is the most abundant protein in the human body. As the main structural component of the dermis, collagen provides strength and support to human skin and is responsible for restoring elasticity and flexibility [25]. In general, there are at least 16 types of collagen, and about 90% of the collagen in the human body is composed of types I, II, and III, which contain repeated Gly-Pro-X sequences and fold into a characteristic triple helix structure [26]. In normal skin, collagen is repeatedly produced and degraded to maintain skin's elasticity and firmness. However, due to the excessive production of matrix metalloproteinases and collagenase, collagen protein is continuously reduced [27]. Sun et al. [20] reported that three related peptides GYTGL, LGATGL, and VLGL isolated from the skin gelatin hydrolysate of tilapia upregulated collagen I by inhibiting the expression of collagenase. Liu et al. [28] showed that the highly absorbent peptides GLPY and GPGGVGAL derived from elastin can protect fibroblasts from UV damage by inhibiting calcium influx and improving collagen and elastin loss. These results suggest that natural active peptides play a crucial role in reducing collagen loss.

3.1.2. Hyaluronidase inhibitory peptide

Hyaluronic acid, also known as hyaluronic acid, occurs naturally in human tissues [1]. Because hyaluronic acid has excellent moisturizing properties and hydrogel properties, it can absorb and retain a large number of water molecules, making it play an important role in maintaining skin rejuvenation [29]. Under normal circumstances, human skin produces hyaluronic acid to adsorb and retain water around skin cells, increasing the water content of the skin and improving the softness and elasticity of the skin [12]. Hyaluronidase is an enzyme protein that breaks down hyaluronic acid. When hyaluronidase is produced in excess, hyaluronic acid degrades and loses its original moisturizing

and filling function [30]. Although various cosmetic industries have provided topical application of hyaluronic acid products according to consumer needs, exogenous application of hyaluronic acid often causes inflammatory reactions [7]. Montalvo et al. [19] reported that different peptide levels obtained from spirulina (i.e. Pepsin-PHP, Subtilis proteinase-PHA and the mixture of two enzymes-PHS) showed anti-hyaluronidase activity, among which PHS peptide had the highest activity (38.8%, IC_{50} = 0.92 mg/mL). Norzagaray-Valenzuela et al. [31] also found that peptides obtained from three microalgae (*Dunaliella tertiolecta*, *Tetraselmis suecica*, and *Nannochloropsis* sp.) inhibited hyaluronidase in a significantly microalgal species-dependent manner. In summary, the search for natural hyaluronic acid inhibitors is essential to protect the skin.

3.1.3. Tyrosinase inhibitory peptide

Melanin is a biological pigment found in areas such as skin, hair, and eyes that determines the color of skin, hair, and eyes in humans and animals [26]. The formation of melanin involves the participation of several biochemical reactions and enzymes, among which tyrosinase is an enzyme that catalyzes the conversion of tyrosine into intermediates such as dopamine and dopaquinone [32]. Dopa and dopaquinone are key molecules in the production of melanin. In the cosmetic field, melanin production is usually inhibited by regulating tyrosinase activity [33]. Tyrosinase is an enzyme that contains copper at its active site and is used to catalyze oxidation reactions [4]. Therefore, blocking the active site of the enzyme or chelating copper ions can inhibit tyrosinase activity. Nakchum and Kim [29] prepared five kinds of low-molecular weight squid skin collagen hydrolysates by ultrafiltration method. Among them, peptide effluents with molecular weight of 3-10 kD showed a certain copper chelation ability and showed a peptide concentration dependence. When the concentration was 1 mg/mL, the inhibitory effect on tyrosinase activity reached 39.65%. In addition, Karkouch et al. [4] isolated and purified seven peptides from the protein hydrolysate of broad bean seed and determined their common biological activities. The results showed that these peptides have significant antioxidant effects and inhibit anti-tyrosinase activity, and can be used as a source of natural bioactive peptides for cosmetic and pharmaceutical applications.

3.1.4. Elastase inhibitory peptides

Elastin is an important extracellular matrix protein that plays an important role in maintaining the elasticity and toughness of human tissues [28]. Found mainly in connective tissues such as the aorta, lungs, cartilage, elastic ligaments and skin, elastin is about 1,000 times more flexible than collagen, so elastin is key to maintaining tissue elasticity [26]. In addition to collagen, the production of elastin maintains the elasticity and firmness of the skin. The dynamic balance between synthesis and degradation of elastin is an important mechanism to maintain the elasticity and toughness of tissues [28]. However, with age and external factors, elastin can be overproduced, causing it to degrade faster than it can be synthesized. The reduction of elastin fibers in turn leads to a decline in the elastic and mechanical properties of the tissue[34]. Norzagaray-Valenzuela et al. [31] found that microalgal peptides had anti-elastase activity. When the concentration of peptides obtained from *Dunaliella tertiolecta* was 0.0026 μ g/ μ L, the inhibitory activity on elastase reached 32.5%. The IC_{50} values of peptides

extracted from *Tetraselmis suecica*, and *Nannochloropsis* sp were 0.038 $\mu\text{g}/\mu\text{L}$ and 0.025 $\mu\text{g}/\mu\text{L}$, respectively. Therefore, natural active peptides play an important role in protecting and promoting elastin synthesis and maintaining tissue health and function.

3.2. Antimicrobial peptides

As the outermost barrier of the human body, the skin needs to constantly resist the influence of microorganisms in the external environment [23]. However, with age, the antibacterial function of the skin gradually weakens. It has been reported that the occurrence of some skin diseases is closely related to microorganisms in the environment, such as *staphylococcus aureus*, which causes atopic dermatitis, and *propionibacterium acnes* in acne vulgaris [35]. The study found that peptides with antimicrobial activity could serve as a potential alternative to common acne antibiotic treatment [36]. Antimicrobial peptides are an important part of the natural immune defense of human and higher animals, and are the first line of defense of the immune system [23]. The unique structure of antimicrobial peptides makes it difficult for bacteria to develop drug resistance [35]. Antimicrobial peptides are expected to become a substitute for antibiotics and have broad application prospects in agriculture and biomedicine. Qi et al. [36] optimized the hydrolysis process of milk casein and prepared the active casein antimicrobial peptide mixture. The results showed that the peptide mixture showed high activity in inhibiting *Streptococcus mutans* and *Porphyromonas gingivalis*. Morphological analysis showed that incomplete or irregular collapse occurred on the surface of bacterial membrane after treatment with peptide mixture. In addition, Răileanu et al. [35] found that some antimicrobial peptides can also support wound healing by promoting cell proliferation and migration, and can be used to treat skin and soft tissue infections, to a certain extent, to solve the problem of unprecedented rise in bacterial antibiotic resistance. Therefore, finding bioactive peptides with antibacterial effects and using them as functional components of cosmeceuticals has broad application prospects.

3.3. Antioxidant peptides

UV-induced ROS promotes the activity of MMP-1 in human keratinocytes and dermal fibroblasts, leading to photoaging of the skin [37]. To combat this effect, researchers usually neutralize ROS with antioxidants and antioxidant bioactive substances to mitigate its damage to the skin and slow the skin photoaging process [27]. Recent studies have shown that bioactive peptides with antioxidant properties can be used to prevent oxidative stress caused by excessive ROS production in the skin [4]. Specifically, this antioxidant active peptide can reduce the activity of MMP-1 when resisting UV-induced ROS, thereby reducing the degradation of collagen and maintaining skin elasticity and health. Xiao et al. [14] used a combination of Alcalase, Flavorzyme and Protamex to hydrolyze corn protein powder and the hydrolysates were subjected to antioxidant activity analysis after further fractionation. The obtained peptide components displayed preferable scavenging capacities for ABTS radicals and DPPH radicals, with IC₅₀ values of 0.122 mg/ml and 0.180 mg/ml, respectively. Dong et al. [37] isolated antioxidant peptides from soybean can inhibit xanthine oxidase activity and have protective effects on oxidative stress damage caused by hyperuricemia. In addition, natural antioxidants are safer and more sustainable than chemically synthesized

antioxidants [29]. Therefore, the use of natural antioxidants is the trend of the future, not only to meet people's needs for healthy and sustainable products, but also to provide better protection for people's health and quality of life [27].

3.4. Anti-inflammatory peptides

Inflammation is the body's natural defense response to tissue damage, bacterial infection, chemicals, or other stimuli [38]. It has been reported that inflammation can promote aging and age-related diseases, mainly due to redox imbalances and immune system disorders during aging, inducing the continuous upregulation of pro-inflammatory mediators and activating many pro-inflammatory signaling pathways [8]. Peptides with anti-inflammatory activity are a class of bioactive peptide molecules that can inhibit inflammatory response and alleviate inflammatory symptoms [38]. These peptides can be obtained from a variety of sources, including natural sources such as animals, plants, and microorganisms, as well as synthetic peptides. Yang et al. [9] isolated and identified 115 peptides from yak bone collagen by ultrafiltration, reversed phase high performance liquid chromatography (RP-HPLC) and nano liquid chromatography tandem mass spectrometry (Nano LC-MS/MS). Twelve peptides were selected based on the predicted bioactivity, six of which could effectively inhibit inflammation by regulating the NF- κ B signaling pathway and NO production. Chen et al. [38] used ultrafiltration, chromatography and mass spectrometry to isolate and characterize novel anti-inflammatory peptides from perch, providing a chemical basis for active ingredients in the treatment of inflammation-related diseases. The anti-inflammatory mechanisms of these peptides include inhibiting the production of inflammatory cytokines, regulating immune response, alleviating oxidative stress, etc., and have potential roles in the treatment of inflammation-related diseases [8].

3.5. Multifunctional peptides for improving skin health

Multifunctional bioactive peptides have the ability to trigger, regulate or inhibit multiple physiological pathways simultaneously [27, 38]. Bioactive peptides with antioxidant, antibacterial, anti-inflammatory, and anti-aging activities have been reported to provide partial or total protection against skin aging [4, 35]. This versatility makes bioactive peptides have a wide range of applications in skin care products [35]. Wang et al. [15] used *Bacillus subtilis* to ferment rapeseed meal, and the natural active peptide obtained was verified by cell experiments to have high antioxidant and anti-aging properties. Castro-Jacome et al. [12] extracted the active peptides beneficial to skin function from sorghum crops by alkaline enzyme hydrolysis and ultrafiltration technology. In particular, the two peptide extracts with different molecular weights were able to reduce the levels of pro-inflammatory cytokines, such as interleukin 1- β (IL- β), interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α). In addition, these peptides can also inhibit the activity of collagenase, elastase and tyrosinase, and can be used as a natural skin care agent in cosmeceutical products [5]. In summary, these multifunctional bioactive peptides are often added to skin care products, such as creams, serums, masks, etc., which not only help improve skin health, but also have superior functional properties compared to single active peptides [2].

4. Challenges and Opportunities in The Development of Bioactive Peptide-based Cosmeceuticals

Bioactive peptides require a complex research and development process, including the search for suitable peptide bases, optimization of formulations, and validation of efficacy, as well as a significant investment of time, money and other resources [14]. Peptide-based active ingredients may also be affected by environmental conditions during preparation, transportation and storage, leading to a decrease in their stability [18]. Therefore, maintaining the stability of peptide-based formulations is a challenge that needs to be addressed with the help of suitable technologies [29]. In addition, the use of bioactive peptides may be associated with a number of uncertainties and safety concerns. This means that validation of the safety and efficacy of peptide-based formulations is crucial and rigorous clinical trials need to be conducted prior to their use [2]. Although there are still many challenges in developing active peptide-based cosmeceutical formulations, the increasing consumer demand for natural, green and therapeutically protective cosmeceutical products provides a market opportunity [35]. As science and technology continue to evolve, researchers can leverage new technologies and methods to find and optimize new formulations of bioactive peptides [11].

5. Conclusion

Natural bioactive peptides prepared by modern separation techniques usually have a wide range of biological activities, among which those relevant to medicinal cosmetics mainly include anti-aging, anti-inflammatory, antibacterial, and antioxidant. In recent years, the development of bioactive peptide-based medicinal cosmetic formulations has faced many challenges, but has also presented opportunities, as science and technology have advanced and consumer demand has increased. By overcoming these challenges, the development of safe and effective bioactive peptide cosmeceutical formulations can not only meet the needs of consumers for functional and health care cosmeceutical products, but also promote the innovation and development of the cosmetics industry.

6. Author Contribution Statement

All authors listed have significantly contributed to the development and the writing of this article.

7. Data Availability Statement

All data generated or analyzed during this study are included in this published article.

8. 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.

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