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Ajiao Peptides: Transformation and Innovation from Traditional Tonics to Modern Precision Bioactive Ingredients

As a traditional tonic with nearly three millennia of heritage, donkey-hide gelatin's efficacy in “harmonizing blood, nourishing yin, dispelling wind, and moistening dryness” has long been documented in the Compendium of Materia Medica. However, the primary component of traditional donkey-hide gelatin—macromolecular collagen—faces modern application challenges such as limited absorption efficiency and unclear functional targeting. With the rapid advancement of biotechnology, donkey-hide gelatin peptides—small peptide fragments derived from the degradation of macromolecular proteins through specific techniques—are emerging as the key to solving this challenge. Not only do they significantly enhance bioavailability, but they also reveal the specific bioactive codes inherent in donkey-hide gelatin. This breakthrough is propelling the industry's transition from traditional boiled preparations to modern bioactive raw materials, opening up vast prospects in functional foods, pharmaceuticals, and cosmetics. This paper systematically reviews

the preparation techniques, compositional characteristics, biological functions, and diverse applications of donkey-hide gelatin peptides, while exploring the potential role of peptide synthesis technology in their future development.

I.Preparation Techniques for Ejiao Peptides: From Bioenzymatic Hydrolysis to Chemical Synthesis

The preparation of donkey-hide gelatin peptides is the first step toward achieving high-value transformation. Currently, mainstream technologies can be broadly categorized into two types: enzymatic hydrolysis and chemical synthesis. Each approach has distinct principles, yields specific products, and is suited for different application scenarios.

1.1Bioenzymatic Hydrolysis: The Mainstream Pathway for Industrialization

Biological enzymatic hydrolysis is currently the most core and mature technology for large-scale production of donkey-hide gelatin peptides. Its fundamental principle involves simulating the human digestive process by utilizing proteases to selectively cleave peptide bonds in proteins under mild conditions, thereby yielding peptide segments within the target molecular weight range.

The typical industrial preparation process has achieved a high degree of integration and automation. As disclosed in Patent CN117003825A, the process uses donkey-hide gelatin powder as raw material. After sequential

extraction with cyclohexane and dichloromethane to remove impurities, it undergoes pepsin hydrolysis followed by further enzymatic digestion with pancreatin. Finally, ultrafiltration and gel chromatography purification yield high-purity characteristic peptides from donkey-hide gelatin. This "bionic enzymatic hydrolysis" strategy effectively releases peptide segments with specific biological activities. Complementing this, highly efficient and energy-saving production equipment, as described in Patent CN219792994U, integrates dissolution, enzymatic hydrolysis, and filtration processes into a single unit. Utilizing temperature and pressure sensors alongside automated control systems, it significantly enhances production efficiency and product stability. The "Bionic Enzymatic Hydrolysis Engineering Research Center" established by Fupai Ejiao focuses precisely on such technologies, dedicated to developing Ejiao peptide products with specific physicochemical properties or biological activities.

1.2 Chemical Synthesis Method: Precision-Engineered Cutting-Edge Tools

Unlike biological methods based on the degradation of natural raw materials, chemical synthesis—particularly solid-phase peptide synthesis (SPPS)—represents cutting-edge technology for precisely constructing peptide chains from scratch. This method does not rely on donkey hide gelatin as raw material. Instead, it starts with amino acids. On automated peptide synthesizers, following a pre-designed amino acid sequence, the

target peptide chain is synthesized step by step on a resin carrier through repeated cycles of deprotection, coupling, and washing.

Modern peptide synthesizers are the core equipment enabling this process. As equipped by industry-leading service providers, these instruments—ranging from laboratory-scale to large-scale production models—can synthesize peptides from dipeptides to those exceeding 99 amino acid residues. These systems support mainstream chemical strategies such as Fmoc (fluorenylmethoxycarbonyl) and t-Boc (tert-butoxycarbonyl), and enable complex site-specific modifications including N-terminal acetylation, C-terminal amidation, phosphorylation, cyclization, and introduction of fluorescent labels. This facilitates the production of high-purity, customized bioactive peptides.

Although chemical synthesis is not currently the primary method for producing bulk ejiao peptide raw materials due to cost considerations, it plays an irreplaceable role in the fundamental research of ejiao peptides: By synthesizing specific active peptide sequences identified in donkey-hide gelatin (such as characteristic peptide segments), researchers can precisely validate their structure-activity relationships, elucidate their mechanisms of action, and lay the groundwork for developing more effective peptide-based pharmaceuticals or cosmetic leads centered on the core structure of donkey-hide gelatin peptides.

II.Composition and Structural Characteristics of Ejiao Peptides

The composition of donkey-hide gelatin peptides inherits the protein essence of native donkey-hide gelatin, yet exhibits unique functional advantages due to its small-molecule characteristics.

Chemical Composition: Ejiao peptides are rich in essential amino acids for the human body and amino acids characteristic of collagen. Analysis indicates that Ejiao oligopeptides contain 8 essential amino acids and 2 semi-essential amino acids, with a relatively high proportion of hydrophobic amino acids accounting for approximately 46.23% of the total. A high proportion of hydrophobic amino acids is often closely associated with the biological activities of peptides, such as antioxidant properties. Additionally, collagen-specific amino acids like proline and hydroxyproline are also present in the peptide segments.

Molecular weight distribution: Low molecular weight is key to ensuring high absorption rates and activity. Research indicates that donkey-hide gelatin oligopeptides prepared via a composite enzymatic hydrolysis method exhibit a relative molecular mass predominantly concentrated below 900 Dalton (Da), accounting for up to 83.87% of the total. These oligopeptides typically consist of 2-10 amino acid residues, possessing a significantly smaller volume than proteins. This enables them to be more readily absorbed intact by intestinal mucosal cells or penetrate the skin barrier to exert their effects.

III.Biological Activity and Functional Mechanisms of Ejiao Peptides

Modern research has gradually unveiled the diverse biological functions of donkey-hide gelatin peptides, revealing effects that extend far beyond the traditional understanding of "blood tonification."

3.1 Antioxidant and Cellular Protection

This fundamental activity has been fully validated by experimental data. The scavenging capacity of donkey-hide gelatin oligopeptides against DPPH and ABTS radicals (IC_{50} values of 1.39 and 2.06 mg/mL, respectively) significantly outperformed that of unenzymatically processed raw donkey-hide gelatin. At the cellular level, pretreatment with 10 mg/mL ejiao oligopeptides increased hydrogen peroxide-induced fibroblast survival by 14.34% and elevated intracellular superoxide dismutase activity by 497.88%, effectively reducing reactive oxygen species levels. This potent antioxidant capacity provides the molecular basis for its anti-fatigue and anti-photoaging effects.

3.2 Promoting Mineral Absorption and Endocrine Regulation

A 2023 patent revealed new functions of the characteristic peptide in donkey-hide gelatin: this peptide segment possesses iron ion chelation capacity and excellent intestinal mucosal permeability, enabling it to serve as a carrier to promote iron absorption and utilization in the body, offering a novel approach to improving iron deficiency anemia. Additionally, the study found that this characteristic peptide regulates gene expression in rat

hypothalamic KNDy neurons, elevating serum estradiol and progesterone levels. This indicates its regulatory role in the endocrine system, providing scientific support for its application in preconception health management.

3.3 Immunomodulation

Donkey-hide gelatin peptides can regulate immune balance. Studies indicate they can reverse the imbalance of Th17/Treg immune cells in mouse models of airway inflammation. Small-molecule donkey-hide gelatin has also been shown to enhance immune function in mice. This immunomodulatory property holds potential for improving the body's defense capabilities and alleviating excessive inflammatory responses.

IV.Application Domain Expansion: From “Medicinal Foods” to “Cosmeceuticals”

The diversified applications of donkey-hide gelatin peptides are continually expanding beyond the boundaries of traditional donkey-hide gelatin.

• Functional Foods and Health Supplements: This represents the primary application area at present. Leveraging its functions in promoting iron absorption, providing antioxidant effects, combating fatigue, and regulating endocrine activity, donkey-hide gelatin peptides have been developed into specialized formula foods and dietary supplements targeting pre-pregnancy conditioning, anemia improvement, and immune enhancement. For instance, compound products containing active donkey-

hide gelatin peptides, Chinese herbal peptides, and isomaltulose have been formulated into various dosage forms such as oral liquids and capsules.

• Cosmetics and New Ingredients: This represents the most groundbreaking advancement in recent years. In 2025, Ejiao Peptide was successfully registered as a National Cosmetic New Ingredient (National Cosmetic New Ingredient Registration No. 20250155), marking its official entry into the beauty industry. The small-molecule structure of Ejiao Peptide enables effective penetration through the skin barrier, stimulating collagen and elastin regeneration in the dermis. This positions it as a representative "Chinese-origin ingredient" in the anti-aging skincare sector. Building on this, Fupai Ejiao has implemented a "cosmetics-food convergence" strategy, establishing a product ecosystem centered on "internal consumption and external nourishment."

• Pharmaceutical Research and Development: Although currently used directly as a drug in limited applications, the well-defined biological activity of donkey-hide gelatin peptides positions them as potential drug lead compounds. In the future, precise synthesis or modification of key active sequences using peptide synthesizers holds promise for developing next-generation peptide therapeutics targeting osteoporosis (by enhancing osteoblast activity), metabolic disorders, or specific inflammatory conditions.

V.Future Outlook and Challenges

Despite promising prospects, the development of the donkey-hide gelatin peptide industry still faces challenges, while also pointing to future directions for innovation.

Future Research Directions and Industry Trends:

1. In-Depth Analysis of Active Mechanisms: Future research should integrate techniques such as mass spectrometry, NMR, molecular docking, and cellular signaling pathway analysis to more precisely identify key active sequences within donkey-hide gelatin peptides and elucidate their microscopic mechanisms of interaction with receptors.

2. Standardization and Quality Control: Establishing a peptide fingerprint profile and quantitative detection method for donkey-hide gelatin based on characteristic peptide segments is crucial for achieving product quality standardization and market regulation.

3. Synthetic Biology and Green Manufacturing: Exploring the use of genetically engineered microorganisms to directly ferment and produce specific active peptide sequences of donkey-hide gelatin may become a new pathway for low-cost, sustainable large-scale production in the future.

4. Multi-technology Integration and Precision Application: Precision synthesis technologies, exemplified by peptide synthesizers, will complement natural peptide libraries prepared via enzymatic hydrolysis.

Chemical synthesis can be employed to produce reference standards and validate active core sequences, enabling rational drug design to develop donkey-hide gelatin peptide analogues or mimetics with enhanced activity and improved stability.

VI.Summary

The successful development and application of donkey-hide gelatin peptides exemplify the profound integration of traditional Chinese medicine wisdom with modern biotechnology. Through enzymatic hydrolysis, we have unlocked the active essence of small molecules within donkey-hide gelatin. Cutting-edge tools like peptide synthesizers provide us with the keys to delve into its mysteries, even redesigning and creating superior active molecules. From functional foods to novel cosmetic ingredients, the industrialization of donkey-hide gelatin peptides not only breathes new life into this millennia-old substance but also offers a valuable “peptide-based” model for the modernization and value enhancement of the entire traditional tonic industry. With ongoing advances in fundamental research and the innovative application of cross-disciplinary technologies, donkey-hide gelatin peptides are poised to play an increasingly pivotal role in the precision nutrition and health industries.