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From Ancient Oilseed to Modern Nutrient Source: The Journey of Refining Flaxseed Peptide Production Techniques

Flaxseed, this ancient crop dating back thousands of years, has long been prized primarily for its rich content of alpha-linolenic acid (an omega-3 fatty acid) and dietary fiber. However, beyond its oils and fiber, the high-quality protein resource—accounting for approximately 20% of the seed's mass—has long remained underutilized. The peptides generated through the degradation of these proteins not only retain the essential amino acids of flaxseed protein but may also unleash unique bioactivities such as antioxidant effects, blood pressure reduction, and immune enhancement. Efficiently converting flaxseed protein into high-activity peptides is key to enhancing its value. This review systematically traces the optimization journey of the production process from flaxseed to high-activity peptides, revealing the scientific rationale behind each refinement step.

I. Starting Point and Challenges: Why Process Optimization is Needed?

Processing flaxseed directly presents two inherent challenges that make process optimization essential:

1. “Impurity” interference: Flaxseed contains antinutrients or undesirable components such as phytic acid, cyanogenic glycosides (e.g., linamarin), and lignin. These substances not only potentially compromise the safety and flavor of the final product but also interfere with protein extraction and enzymatic hydrolysis efficiency during processing.

2. Protein “Difficult Release”: Flaxseed protein is enclosed within a dense cell wall structure and exists in complex with polysaccharides, phenolic compounds, and other substances. This results in low extraction rates and insufficient purity, directly impacting the subsequent yield and activity of peptides. Therefore, an optimized preparation process must prioritize achieving high extraction rates of flaxseed protein, high conversion rates to peptides, and maximizing the biological activity of the final product—all while ensuring safety.

II. The Three-Step Process Optimization

The preparation of modern flaxseed peptides is not a one-step process but rather a meticulously interlinked procedure comprising three major stages: pretreatment, protein extraction and enzymatic hydrolysis, and separation and purification.

First Step: Raw Material Preprocessing — Separating the Wheat from the Chaff, Clearing the Path

The goal of preprocessing is to create optimal conditions for subsequent steps.

Detoxification and Dehulling: Traditional wet-heat processing (steaming and roasting) effectively degrades cyanogenic glycosides, but excessive heating can cause protein denaturation. Modern optimized techniques favor low-temperature baking combined with solvent (e.g., ethanol) washing. This approach removes most antinutrients and oils while maximizing the preservation of natural protein activity.

Cell Wall Disruption and Activation: Physical grinding serves as the foundation, but optimization focuses on adopting ultrafine grinding technology. This process pulverizes raw materials to micron or even nanometer levels, significantly increasing specific surface area and disrupting cell walls. This facilitates greater contact between internal proteins and extraction solvents. Research indicates that compared to conventional grinding, ultrafine grinding can enhance the final extraction rate of flaxseed protein by over 15%.

Second Step: Protein Extraction and Enzymatic Digestion — Core Transformation, Precision Cleavage

This is the core of the preparation process, determining the yield and activity profile of the peptide.

1. Protein Extraction Optimization:

Alkaline solubilization and acid precipitation remains the traditional mainstream method: leveraging the property of flaxseed protein to dissolve under alkaline conditions and precipitate at its isoelectric point (around pH

4.5). Optimization hinges on precise control of pH, temperature, solid-to-liquid ratio, and duration. Research confirms that extraction at pH 9.5 and 50°C for 1-2 hours typically yields high protein extraction rates (>80%).

Hydrolytic enzymatic extraction represents an emerging green alternative: Within an aqueous system, proteases perform limited hydrolysis to disrupt protein-polysaccharide complexes, thereby releasing lipids and proteins. This method operates under mild conditions but incurs higher costs and requires more complex process control.

2. Enzymatic Hydrolysis Process Optimization (Key Steps):

Enzymatic hydrolysis precisely cleaves macromolecular proteins into small-molecule bioactive peptides. Optimization focuses on “enzymes, parameters, and modes.”

Enzyme Selection and Combination: Different proteases yield peptides with distinct sequences and activities due to their unique cleavage sites (enzymatic specificity).

Alkaline Proteases: Feature broad cleavage sites and high hydrolysis efficiency, commonly used to achieve high degree of hydrolysis and high yield.

Flavor Proteases: Capable of cleaving hydrophobic amino acids at peptide chain termini, effectively enhancing product flavor and reducing bitter peptides.

Combined Enzyme Systems: Currently the most effective

optimization strategy. For example, deep hydrolysis with alkaline proteases followed by “tail trimming” with flavor proteases significantly improves product palatability and flavor while maintaining high activity. Additionally, targeted use of trypsin (specifically cleaving lysine and arginine) may more readily yield peptides with specific antihypertensive (ACE inhibitory) activity.

Systematic parameter optimization: Experimental design methods like response surface analysis enable precise establishment of mathematical models linking multiple factors (hydrolysis temperature, pH, enzyme loading, substrate concentration, time) to hydrolysis degree, peptide yield, and target activity. This identifies globally optimal process parameter combinations.

Dynamic and directed hydrolysis: Traditional batch hydrolysis is evolving toward more advanced approaches. Pulsed ultrasonic-assisted hydrolysis serves as an efficient optimization method. Moderate ultrasonic cavitation continuously renews the enzyme-substrate interface, preventing product aggregation, thereby enhancing hydrolysis efficiency by 20–30% and potentially releasing new active fragments.

Section Three: Separation, Purification, and Drying — Pursuing Excellence, Locking in Activity

The crude enzymatic hydrolysate is complex in composition, containing unhydrolyzed proteins, large peptide fragments, small peptides,

amino acids, and salts, necessitating further purification.

Separation and Purification: Multi-stage membrane separation technology is commonly employed. First, microfiltration removes large particulate impurities, followed by ultrafiltration systems that fractionate based on molecular weight. For instance, sequential use of ultrafiltration membranes with 10 kDa, 5 kDa, and 3 kDa pore sizes yields peptide fractions across different molecular weight ranges. Extensive research indicates that the antioxidant and ACE-inhibitory activities of flaxseed are predominantly concentrated in small peptide fragments below 3 kDa. For high-end research or products, gel filtration chromatography or preparative high-performance liquid chromatography can be further employed to purify and identify individual active peptides.

Drying Technology: The drying method directly impacts the stability, solubility, and activity retention of peptide products. Spray drying is the preferred industrial method, with optimization centered on controlling inlet air temperature and atomization pressure to prevent inactivation of heat-sensitive peptides. For extremely high-value products, vacuum freeze-drying maximizes preservation of the peptide's natural conformation and activity, despite its high cost.

III. The Ultimate Goal of Process Optimization: Function-Oriented Customized Production

The pinnacle of modern process optimization lies in shifting from

“producing peptides” to “customizing peptides with specific functions.”

Through reverse thinking, we first define the target biological activity (e.g., potent antioxidant properties), then work backward:

1. Select proteases likely to generate sequences with that activity (e.g., proteases preferring to cleave hydrophobic amino acids are often associated with antioxidant peptides).

2. Design corresponding enzymatic digestion and purification processes to enrich the target molecular weight range.

3. Finally, validate the process route's effectiveness through activity testing and structural identification, forming a precise “function-process” closed-loop system.

IV. Challenges and Future Outlook

Despite significant advancements in the process, challenges remain: achieving continuous, automated production throughout the entire workflow to reduce costs; and gaining deeper insights into the structure-activity relationships among peptide sequences, structures, and activities to guide more precise enzymatic design.

In the future, flaxseed peptide preparation processes will integrate deeply with cutting-edge technologies:

Artificial Intelligence and Big Data: Machine learning will analyze vast amounts of enzymatic hydrolysis experimental data to predict optimal enzyme combinations and process parameters.

Synthetic Biology Technologies: Once the sequences of key active peptides are identified, future production may be achieved through direct fermentation using engineered bacterial strains, completely bypassing the complex processes of extraction from raw materials and enzymatic hydrolysis.

V.Conclusion

The journey from a whole flaxseed to a bottle of high-purity bioactive peptide powder is far more than a simple “grinding-hydrolysis” process. It represents a systematic engineering endeavor spanning pretreatment, extraction, precise enzymatic hydrolysis, and meticulous separation. The optimization of each step embodies a profound understanding of material properties, enzyme principles, and separation science. As the process continues to advance, flaxseed is evolving from a traditional oilseed crop into a sustainable, high-value treasure trove of bioactive peptides. This transformation is injecting new vitality into functional foods, specialized nutritional products, and even the biopharmaceutical sector. The path of optimizing this preparation process not only enhances the value of the final product but also serves as a vivid example of modern food technology fully utilizing natural resources to achieve green value-added outcomes.