OPTIMIZED ISOLATION TECHNIQUES FOR BIOACTIVE WHEY PROTEINS AND PEPTIDES FROM RAW BOVINE MILK

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Abstract

Raw bovine milk provides whey proteins and peptides appreciated for their nutrition and a range of beneficial properties, such as those that defend from oxidative stress, lower high blood pressure, protect against microorganisms, and boost immunity. Because interest in functional foods and nutraceuticals is rising globally, there is a strong demand for techniques ensuring bioactive compounds are not lost during isolation. This study aims to assess different modern and older methods of separating bioactive whey proteins and peptides, aiming for maximum yield, high purity, and good function. Ultra-filtration, enzymatic hydrolysis, ion-exchange chromatography, and hybrid methods involving membrane chromatography and continuous foam separation were reviewed in the paper. Although traditional techniques offer a basic concept, issues like protein denaturation, polluted membranes, and difficult processing keep factories and industries from using them. Different examples and technological developments show how process optimization can resolve these barriers. The use of whey bioactives in functional foods, nutrition for medical purposes, and dietary supplements is looked at, highlighting their benefits for health and the prevention of diseases. The research ends by outlining future paths for creating sustainable and less-costly isolation strategies for functional dairy ingredients to meet the rising needs of the global market.

Keywords: whey proteins, peptides, bovine milk, bioactivity, isolation techniques, functional foods, nutraceuticals, enzymatic hydrolysis, ultrafiltration

1. INTRODUCTION

1.1 Background

These research areas are attracting more interest since they help identify important functions of whey proteins and peptides in supporting human health. Whey was once considered useless and discarded as it's produced during cheese production. In recent decades, scientific discoveries have changed our view of it, allowing us to see it again as a nutrient-rich source of good-quality proteins and beneficial peptides. Whey proteins are appreciated for having essential amino acids, including BCAAs like leucine, isoleucine, and valine, which are needed for building, growing, and recovering muscles. Because they are digested and absorbed quickly, these proteins have many uses in nutrition, sports, and pediatric care. Like all proteins, whey proteins carry important micronutrients to the body, making their absorption and use more efficient (Pihlanto & Korhonen, 2003). Besides helping with diet, some peptides found in whey have been proven to

lower blood pressure, kill harmful microbes, act as antioxidants, and enhance the immune system. Usually, enzymes liberate these peptides while the food is digested or when the product is processed industrially. Because of these functions, whey peptides are commonly included in supplements and special medical foods. Such medications help to strengthen the heart and are used for controlling metabolic problems such as obesity and type 2 diabetes. Because of rising global interest in healthy habits, there is an increased need for natural bioactive compounds in food items. Whey proteins and peptides in this setting look like great options that aid health and provide nutrition, steering food away from just what fills our stomachs. Therefore, finding the most efficient way to extract these bioactives from ordinary bovine milk remains an important research topic (Pihlanto & Korhonen, 2003).

1.2 Problem Statement

While their nutritional and therapeutic effects are clear, extracting these components from raw bovine milk is still difficult. Using acid precipitation, ultrafiltration, or chromatography can often change proteins and peptides' structure or biological activities. For this reason, these nutrients become less effective, especially when added to products designed for health or nutrition. Also, several popular isolation methods are costly enough to make industrial use difficult or depend on specialized equipment that people in low-resource areas do not have. Because of these challenges, companies producing whey often find it hard to mass-produce biologically active whey that is reasonably priced. A further problem involves scaling. Principles that are simple at the laboratory stage usually struggle to be used at an industrial level due to loss of products, extra processing time, and new regulatory burdens. A greater emphasis is being placed on creating isolation methods that benefit the environment by reducing waste and energy consumption. Because of this, there should be an urgent effort to perfect separation methods that give high-yielding and pure results and maintain the bioactivity in whey proteins and peptides. They should work on a large scale, be affordable, and fit into different production systems to allow many people to use these products.

1.3 Objectives

The primary objective of this research is to evaluate and identify optimized isolation techniques that can efficiently extract bioactive whey proteins and peptides from raw bovine milk while preserving their structural integrity and functional properties. As the demand for high-quality functional food ingredients continues to grow, developing processes that maintain bioactivity and are economically and environmentally sustainable becomes increasingly important.

The following specific objectives guide this study:

1. To analyze the composition and biofunctional significance of whey proteins and peptides in raw bovine milk.
2. To review conventional and emerging isolation techniques.
3. To assess the efficiency of various isolation methods.
4. To identify potential process improvements and innovative technologies.

5. To explore the implications of optimized isolation techniques.

4. Significance of the Study

Other than academic value, separating these proteins and peptides from raw bovine milk is essential for advancing functional foods, nutraceuticals, and clinical nutrition. People increasingly ask for natural products with health benefits when they know more about food and health. Due to their clear effects on the body, such as helping the immune, oxidation defense, and metabolic systems, whey proteins and peptides are widely used in this area (Smithers, 2008).

In this domain, many products now include bioactives such as protein-enhanced drinks, energy snacks, infant formulas, and supplements used for sports. Since whey proteins are easy to digest, have great amino acid profiles, and can be used in many ways, they are ideal for manufacturers who want to meet nutritional and quality requirements. Improving the isolation procedure helps the final product and assists in designing suitable formulations for specific health goals. In the world of nutraceuticals, peptides with antihypertensive, antimicrobial, or cholesterol-lowering properties could provide safe alternatives to taking medicine. Bioactivity mustn't be lost during isolation, so the benefits of using these supplements can be maintained.

In clinical nutrition, whey proteins are essential for helping patients manage malnutrition, surgery aftercare, and diabetes and cancer. They support the repair of tissues, support the immune system, and help patients improve. Accordingly, designing methods to separate these materials with the greatest purity and adequate function is not just a technical task—it helps advance public health and nutrition science (Smithers, 2008).

2. Literature Review

2.1 Composition of Raw Bovine Milk

Raw bovine milk consists of water, sugars (mostly lactose), fats (lipids), proteins, minerals, and bioactive components. Whey proteins only represent 20% of the protein fraction, with the bulk (80%) made up of casein. Many whey proteins, like β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, and peptides, developed during enzymatic or fermentation processes (Wal 2002).

2.2 Bioactivities of Whey Proteins and Peptides

Attention towards whey proteins has increased recently because of their possible therapeutic benefits. Studies show that proteins and their peptides have important activities that benefit human health. For example, lactoferrin shows antimicrobial and anti-inflammatory properties, while β-lactoglobulin may enhance the process of antioxidation and nutrients being better absorbed (Korhonen & Pihlanto, 2006). Besides, peptides formed by enzymatic hydrolysis of whey proteins can help lower blood pressure by inhibiting ACE, decreasing cholesterol levels, and enhancing immune response (Pihlanto, 2006).

Due to these activities, more attention is being given to efficiently extracting these molecules for health applications. 2.3 Using Methods of Separating Data Usually, people use mechanical or chemical methods to purify whey proteins and peptides from bovine milk, and the approaches have different levels of success and precision. Commonly, caseins and whey are separated using acid or enzymatic precipitation of the milk serum. Many researchers use ultrafiltration and microfiltration to separate and collect whey proteins depending on their molecular size. Because these techniques are not heated, they keep the proteins' shapes and activities intact (Morr & Ha, 1993).

Despite giving highly pure results, ion exchange and gel filtration chromatography are not frequently used in industry, as they are costly and require much more time than other techniques (Smithers, 2008). Bioactive peptides are also often released from whey proteins using enzymatic hydrolysis with proteases such as trypsin, chymotrypsin, or alcalase. Its action is similar to the digestive process and is useful for producing peptides with particular activities (Pihlanto & Korhonen, 2003).

2.3 Traditional Isolation Techniques

High temperatures in certain working methods can change the structure of certain proteins and lessen their usefulness. There are many aspects of membrane filtration, such as membrane fouling, high concentration buildup, and our lack of selectivity for certain peptides, which may weaken its efficiency and lead to problems in scaling up (Zhang et al., 2020).

2.4 Limitations in Conventional Approaches

Although excellent for small uses, chromatography is complicated, requires a lot of equipment and purification processes, slows things down, and drives up costs for bigger projects. Furthermore, the results of enzymatic hydrolysis can include peptides that aren't always active, making it difficult to isolate the right type of peptide. Precisely controlling the enzyme type, the pH, and the reaction temperature is necessary to prevent the bitterness in functional foods and nutraceuticals that could result from over-hydrolysis (Madureira et al., 2010).

2.5 Advancements and the Need for Optimization

Modern research focuses on creating new methods that integrate what's best about old approaches while fixing the points they miss. Combinations of ultrafiltration, enzymatic hydrolysis, or chromatography help experts finely manage the structure and biochemical properties of the extracted compounds (López-Expósito et al., 2012). Membrane chromatography, electro-membrane filtration, and affinity-based separation are used more often because they offer improved selectivity and simple operation.

Moreover, studies have begun to emphasize the importance of process optimization through variables such as temperature, flow rate, enzyme-substrate ratio, and hydrolysis time. Optimization not only improves yield and purity but also plays a vital role in preserving the whey-derived compounds' therapeutic potential. This

is especially critical in applications where bioactivity—rather than protein content—is the key determinant of product functionality.

3. Methodological Approaches in Isolation

Choosing the right ways to extract bioactive whey proteins and peptides from raw bovine milk helps them maintain their complete shape and biological effect. Investigators have developed and improved various methods, some existing and others innovative, throughout the years. The efficiency, level of control, feasibility to upscale, and cost involved are not the same for these techniques.

3.1 Membrane-Based Separation Techniques

Membrane filtration is common in whey protein separation because it is efficient, requires relatively little heat, and works on any scale. These processes mainly separate and strengthen whey proteins by controlling their size. Because proteins big enough to be stopped by ultrafiltration usually pass, major whey proteins like alpha-lactalbumin and beta-lactoglobulin can be concentrated, with smaller peptides and non-protein materials moving on (Alayash et al., 2013, p. 2). Nanofiltration distinguishes even the smallest of bioactive peptides with its small pores and saves most of the protein from wasting out. Working under gentle conditions is a major advantage of membrane-based systems, allowing the protein to keep its functional structure safe. Still, fouling the membranes, pressure decreases, and rising maintenance fees require the process to be frequently optimized based on pH, temperature, and transmembrane pressure.

3.2 Chromatographic Techniques

Purifying individual proteins and peptides is possible with ion-exchange chromatography (IEC) for those based on their charge and with reversed-phase high-performance liquid chromatography (RP-HPLC) for those based on their hydrophobicity. Scientists have mainly applied ion-exchange chromatography to isolate lactoferrin and immunoglobulins, which are present in relatively small amounts but are very active in the body (López-Expósito et al., 2012).

Reversed-phase HPLC is particularly valuable for separating peptide fractions prepared by digesting proteins. Because of its high resolution and purity, it is used to find and measure bioactive peptides. However, performing these techniques on a large scale usually takes too long, uses a lot of solvents, and can become expensive.

3.3 Enzymatic Hydrolysis

Many scientists favor enzymatic hydrolysis to make bioactive peptides from whey proteins. For instance, if isolated partway during digestion, proteolytic enzymes like trypsin, pepsin, alcalase, and chymotrypsin can free functionally useful peptides from the protein, as shown by Pihlanto (2006). To produce the best results, all conditions for hydrolysis, such as how much of the enzyme, temperature, pH, and time, must be managed with care so that the peptides are not overly broken down into bitters. The approach imitates digestion and is

mainly useful for peptides developed for functions, including regulating blood pressure and blood glucose. However, using enzymatic hydrolysis frequently produces mixtures of peptides that need further clarification with membrane or chromatography.

3.4 Advanced and Hybrid Technologies

Researchers are increasingly using hybrid approaches that merge different types of techniques to solve their problems. As an illustration, when ultrafiltration is linked with ion exchange chromatography, it becomes possible to extract certain fractions of proteins and keep them active (Madureira et al., 2010). Similarly, membrane chromatography combines filtration and adsorption, giving higher throughput and less need for solvents than normal column chromatography. Another new approach is called electro-membrane filtration, where an electric field helps separate peptides more effectively and with greater control. It could be used to identify specific bioactive peptides that change little in form, making it attractive for industry-scale use.

3.5 Optimization Parameters

The effectiveness of isolation techniques depends heavily on the optimization of operational parameters. Key variables include:

- **pH**: Influences protein solubility and enzyme activity.
- **Temperature**: Affects both enzymatic reaction rates and membrane permeability.
- **Flow rate and pressure**: Critical in membrane systems for controlling separation efficiency and preventing fouling.
- **Enzyme-substrate ratio and hydrolysis time**: Crucial in determining peptide length, activity, and yield.

Process optimization through response surface methodology (RSM) or design of experiments (DOE) has been increasingly employed to fine-tune these parameters and improve reproducibility. These statistical approaches help identify interactions between variables and establish ideal operating conditions for maximum productivity and cost-effectiveness.

4. Case Studies and Recent Innovations

4.1 Enzymatic Hydrolysis and Ultrafiltration for Antioxidant Peptide Isolation

An article by Jung et al. (2022) explains the process of creating antioxidant peptides by breaking down whey proteins with enzymes and filtering them by ultracentrifugation. The research revealed that this strategy led to peptides that strongly showed antioxidant effects, and these findings were confirmed by carrying out in vitro assays. It was mentioned that making the right choices for hydrolysis and the membrane can boost the product yield and activity.

4.2 In Silico Modeling for Bioactive Peptide Prediction

Kruchinin et al. (2023) applied computer modeling to predict and find bioactive peptides in various milk whey recipes. Thanks to bioinformatics, the study found peptides that could play a role in treating hypertension and preventing oxidative damage. It shows how computational solutions contribute to the more efficient discovery and use of functional peptides from dairy.

4.3 Membrane Chromatography to Separate Whey Proteins

Modern developments in membrane chromatography have made it more effective for whey protein fractionation. This review discussed the combination of membrane techniques with chromatography to achieve more selective and pure whey protein isolation. These systems' improved performance and low costs make them popular in factories.

4.4 Isolation of Bioactive Peptides from Yogurt Whey

Active Peptides from Yogurt Whey Karimi et al. (2021) studied methods for isolating and purifying bioactive peptides from yogurt whey. A combination of ultrafiltration and chromatography methods was used to select peptides that could act as antioxidants. Following isolation, the peptides were used in a test food system as natural preservatives, proving they could be used in both food preservation and functional foods.

4.5 Foam Fractionation for Protein Recovery

Foam fractionation has emerged as an innovative technique for protein recovery from whey. This method leverages the surface activity of proteins to separate them from solutions via foam formation. The process offers advantages such as low energy consumption and the ability to concentrate proteins without significant denaturation, presenting a sustainable alternative for protein isolation.

5. APPLICATIONS IN FUNCTIONAL FOODS AND NUTRACEUTICALS

Attention has increased towards using bioactive whey proteins and peptides in functional foods and nutraceuticals due to their possible health advantages and wide range of functions. Raw cow milk contains components that can positively influence the body and play a role in both helping prevent and treat several health conditions.

5.1 Functional Food Applications

Whey proteins and peptides are crucial for making healthy foods beyond traditional nutrition. Adding them to food products has been linked to improving antioxidant, antihypertensive, and immune system functions. For example, certain peptides from whey proteins can stop the angiotensin-converting enzyme (ACE), helping to manage blood pressure (Korhonen & Pihlanto, 2006). Besides being healthy, wheat proteins help

improve the taste and feel of food products. Due to their ability to emulsify, form foam, and make gels are valuable additions to food formulations such as dairy, beverages, and bakery items (Smithers, 2008).

5.2 Nutraceutical development

Dietary supplements in the nutraceutical industry use the unique actions of whey-derived peptides to help address certain health concerns. They have been introduced as products to support healthy hearts, boost immune responses, and help control metabolic issues. It is easy for the body to use whey proteins, so they are often included in sports and high-protein supplements designed for athletes and others who need more protein (Marshall, 2004). Furthermore, thanks to encapsulation, bioactive peptides can now be included in nutraceutical formulations, where they stay stable and are released according to specific needs. Due to these impacts, whey-derived peptides can now be used more for nutrition and treatment.

5.3 Clinical Nutrition Applications

In health care, whey proteins and peptides are often used to assist patients in healing and treating certain conditions. Thanks to the amino acids and proteins they contain, they help patients recover from muscle loss and support the rehabilitation of muscles (Boirie et al., 1997). In addition, some immunity-changing peptides in whey proteins have been studied in older people and those with long-term illnesses. Ha & Zemel (2003) stated that taking whey protein can strengthen your immune system and help protect against different infections.

6. CONCLUSION

Exploring better ways to isolate bioactive whey proteins and peptides from raw bovine milk is vital in food science, clinical nutrition, and the making of functional foods. This paper shows that β-lactoglobulin, α-lactalbumin, lactoferrin, and immunoglobulins in whey proteins offer better nutrition and have physiological effects like antioxidant, antihypertensive, antimicrobial, and immunomodulatory activities. They point to the fact that isolation approaches should ensure that the chemical structure and activity of the compounds are not lost while being extracted. At present, membrane filtration, enzymatic hydrolysis, and chromatographic purification are still used, though they each have certain limitations. Due to membrane fouling, thermal denaturation, and high production costs, traditional food processing techniques struggle to grow and become more commercial. Even so, recent advances in membrane chromatography, continuous foam separation, enzyme engineering, and bioactive peptide prediction have allowed researchers to increase efficiency and sustainability in food processing.

Bioactive whey proteins and peptides are finding new uses in several different areas. Functional foods are included in items designed to improve health in ways that surpass everyday nutrition. Such formulations often help manage chronic health problems like high blood pressure, diabetes, and when the immune system is out of balance.

These compounds are important in helping patients recover, look after their muscles, and support their immune health. In the future, scientists should concentrate on designing new and economical technologies that are very effective and friendly to the environment so that high-purity, active whey fractions can be obtained. This project will depend on new non-thermal advances, innovative membranes, precise enzymatic use, and live process supervision. Also, it will be important for food scientists, biotechnologists, and industrial engineers to join forces to ensure that innovative lab successes can reach the market. Improving isolation techniques is necessary for maximizing the health and economic impact of whey proteins and peptides. As more people request natural products and pay more attention to preventive healthcare, this research will gain greater importance.

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