Food Science of Animal Products

Review Article

Advances in research on the effects of lactic acid bacteria on the physicochemical, flavor, and nutritional characteristics of fermented egg products

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Received: September 1, 2024; Revised: December 6, 2024; Accepted: December 18, 2024

Abstract: Eggs, as a high-nutritional food, face challenges such as limited flavor diversity and high cholesterol content in their processed products. Lactic acid bacteria (LAB) fermentation has been introduced as a method to address these issues by reducing pH, producing organic acids, and facilitating enzymatic hydrolysis, thereby enhancing the flavor and nutritional quality of egg products. This review explores the effects of lactic acid fermentation on the physicochemical, flavor, and nutritional characteristics of egg products. Research indicates that LAB can effectively improve the flavor of egg products, reduce pH, and enhance protein solubility and gel characteristics. During fermentation, LAB regulate the production of flavor compounds through enzymatic systems while promoting protein breakdown to generate bioactive peptides, which confer health benefits such as immune enhancement and lipid reduction. Furthermore, extending fermentation time significantly influences the pH and flavor compounds of egg products. This review provides a theoretical foundation for the further development of fermented egg products and is of considerable importance in meeting consumer demand for healthier foods.

Keywords: fermented egg products; lactic acid bacteria; physicochemical properties; flavor improvement; nutritional enhancement

Citation: Z. Q. Shang, D. R. Yu, J. J. Zhang, et al., Advances in research on the effects of lactic acid bacteria on the physicochemical, flavor, and nutritional characteristics of fermented egg products, Food Sci. Anim. Prod. 3 (2025) 9240112. https://doi.org/10.26599/FSAP.2025.9240112.

1 Introduction

Eggs are recognized as one of the most nutritionally complete natural foods available to humans, offering a well-balanced profile of high-quality proteins, lipids, carbohydrates, minerals, and vitamins[1]. Current technologies for egg product processing can be broadly categorized into two main types: whole egg processing and non-whole egg processing. Whole egg processing aims to maintain the freshness and nutritional value of eggs, such as fresh eggs processed into loose eggs, salted eggs, and so on. Conversely, non-whole egg processing involves the transformation of eggs into various other food forms, such as egg powder and ice eggs. The marketplace features a range of egg products including salted egg whites, defatted egg yolk protein, salted eggs, loose eggs, eggshell powder, yolk powder, protein powder, egg rolls, and egg sausages[2–4]. Although current production technologies for these products are quite advanced, they often suffer from a lack of variety in flavor, which struggles to cater to the diverse preferences of consumers.

The undesirable egg odor present in ordinary egg liquid seriously affects the flavor quality of the product and becomes one of the important factors restricting the development of egg products, for which there is a lack of effective solutions[5]. Furthermore, the high cholesterol content in eggs poses health risks, such as atherosclerosis, when consumed excessively[6]. With the continuous development of modern fermentation technology, its application in the processing of eggs and egg products has become increasingly widespread. Researchers have combined fermentation technology with egg product production to effectively enhance the quality and functional properties of egg products[4]. At present, many types of fermented egg products (FEP) have emerged in the market, including fermented egg yogurt[1], fermented egg drink[7], fermented egg powder[2], fermented egg white[8], fermented egg yolk[9], and mold FEP[10] (Figure 1). Among these, fermented egg yogurt stands out as a prominent example. This product is crafted using recovered milk and whole egg liquid as primary ingredients, which are then fermented by lactic acid bacteria (LAB), resulting in a product with enhanced flavors and nutritional benefits. The product has the characteristics of uniform color, delicate tissue, sweet and sour, and harmonious aroma, showing the innovative potential of egg product processing[1]. According to the flavor, egg yogurt can be divided into original egg yogurt, fruit-flavored egg yogurt, nut-flavored egg yogurt, and other flavors of egg yogurt. According to the functionality, it can be divided into several categories, such as skimmed egg yogurt, low sugar egg yogurt, high protein egg yogurt, probiotic egg yogurt, high calcium, and other nutrient-fortified egg yogurt. According to the state of the product, it is also categorized into solid egg yogurt and liquid egg yogurt. The fermented egg lactic drink, characterized by its unique flavor and acidity, is produced through the fermentation of whole egg and

Figure 1 Types of major FEP in the market.

skimmed milk powder or milk with LAB. This category encompasses a variety of products, including fermented egg yolk lactic drinks, fermented egg white lactic drinks, fermented whole egg liquid lactic drinks, and a composite of soymilk and egg fermented drink[11]. FEP represents a class of products renowned for their balanced nutritional profile, extended shelf life, and ease of transport. This is achieved through a series of processes that commence with the preparation of egg liquid, followed by pH adjustment to the optimal level conducive to enzymatic hydrolysis. Subsequent steps involve the inoculation of flavor protease and lipase for a comprehensive enzymatic hydrolysis reaction, culminating in spray drying and other processing techniques. This yields a spectrum of products, including fermented whole egg powder, fermented eggshell powder, fermented egg yolk powder, and fermented protein powder[2]. In contrast to fermented egg powder, mold FEP represents a distinct category. These products utilize cooked or dried eggs as raw materials to produce white embryos, which undergo mold fermentation to yield gross mold embryos. Subsequent fermentation with red and noodle curd introduces further flavor complexity, resulting in a novel food product with unique taste and nutritional attributes[12]. At present, except for egg curd, research on mold FEP remains relatively sparse. In addition, other FEPs include fermented egg biscuits, fermented whole-egg cakes, fermented egg cheese, fermented egg bread, and so on.

FEP represents a novel combination of the nutritional attributes of eggs and fermented foods, simultaneously offering improved digestibility, absorption rates, and potential health benefits[13]. Research has demonstrated that the fermentation process significantly alters the fatty acid composition of eggs, resulting in a decrease in total fatty acid content and a notable decline in polyunsaturated and monounsaturated fatty acid levels[14]. Moreover, microbial fermentation can effectively reduce cholesterol content in eggs, thereby mitigating the risk of high cholesterol intake from egg products. For example, a study by Fujiwara et al.[10] found that cholesterol levels in egg yolks were significantly reduced by feeding 3% dried natto to chickens for 12 weeks. In addition to its health benefits, yeast-fermented desugarized egg products possess a unique aroma, which is achieved by eliminating egg odor and imparting a yeast-fermented flavor[15]. The production process for FEP is generally standardized, although it varies slightly depending on the type of product. It mainly includes raw material sterilization, mixing, cooling inoculation, fermentation, post-fermentation, blending, homogenization, sterilization, refrigeration, and so on[1]. During lactic acid fermentation, proteins are hydrolyzed into low molecular proteins, peptides, and amino acids. The exposure of hydrophobic amino acids, resulting from peptide chain breakage, can produce a bitter taste when interacting with bitter receptors on the tongue, thus affecting the sensory characteristics of the product. This limitation has hindered the widespread adoption of egg-lactic fermentation products[16]. At present, domestic research on egg-milk fermented beverages mainly focuses on optimizing the ratio of egg liquid, sugar raw materials, stabilizer ratios, and screening for suitable microbial strains, such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Nevertheless, the existing products are still difficult to meet the market demand[17]. Egg beverages, compared to bovine milk counterparts, lag in production and consumption, attributable to factors such as their high caloric content and pronounced egg flavor, which fail to align with the consumer inclination towards low-calorie, mild-tasting alternatives[5]. Furthermore, the production of egg-lacto-fermented yogurt is challenged by the thermal sensitivity of egg protein, which can lead to coagulation, flakes, and rough texture during heating[18]. In summary, the development of FEP necessitates overcoming the challenges posed by raw material characteristics, production processes, and product sensory characteristics, to meet the growing health and nutritional needs of consumers.

LAB are the primary microorganisms responsible for fermentation in various egg products, and their application in egg processing has garnered growing attention in recent years. As a group of Gram-positive, non-spore-forming bacteria, LAB is capable of fermenting carbohydrates to produce lactic acid, thereby conferring unique characteristics to fermented products. Furthermore, LAB is recognized as a probiotic, with numerous benefits for human health, including enhancement of immunity, improvement of bodily functions, and retardation of aging[19]. In a fermentation process, LAB produces substantial amounts of lactic acid, as well as various amino acids and enzymes, which contribute to the distinctive acidity and flavor of the product[20]. Studies have shown that LAB fermentation can significantly improve the nutritional value and sensory quality of egg products. For instance, Yang et al.[1] discovered that incorporating liquid whole eggs into LAB fermentation noticeably increased the protein content of yogurt and improved its aroma and flavor. Similarly, Mangalisu et al.[21] demonstrated that fermentation of eggs with *Lactobacillus plantarum* could effectively enhance the antioxidant content of egg products, with optimal results achieved by fermenting eggs for 96 h at an incubation temperature of 37 °C. In addition, Marette et al.[22] established that LAB can assimilate cholesterol, exhibit potential in reducing the risk of cardiovascular disease, and display significant degradation at varying cholesterol concentrations. In summary, LAB fermentation can enhance the nutritional value of egg products, improve their flavor and texture, and endow them with specific functional properties, such as antioxidant and cholesterol-lowering. Notably, a systematic investigation of the effect of LAB fermentation on egg products is still lacking, and further, in-depth studies are needed to screen for optimal LAB strains, optimize fermentation conditions, and elucidate the underlying mechanisms of action to fully realize the potential applications of LAB in egg processing.

This comprehensive review synthesizes the existing literature on the impact of LAB fermentation on the processing, flavor, and nutritional properties of egg products, with a particular emphasis on elucidating the underlying mechanisms by which LAB enhances the quality of these products. Furthermore, this paper provides an in-depth analysis of the current technical hurdles faced by the FEP industry, while also prospectively exploring its future development trajectory. It is anticipated that the curation and discussion presented herein will enrich readers' understanding of FEP, stimulate further research and innovation in this domain, and ultimately catalyze the transformation and upgrading of the egg products industry.

2 Current research status

In recent years, there has been an increasing interest in studying FEP processing. In order to study the current state of research in this field, we conducted a study in the Web of Science database of papers on "lactic acid bacteria" and "egg" published between 2014 and 2024. VOSviewer software facilitated visualization and analysis of author contributions. Duan Xiang, Jia Jie, Liu Xuebo, Yang Yanjin, Wang Jing, and Tian Liangjie emerged as the most prolific authors. Notably, publications co-authored by Chen Wei, Bednarczyk Marek, Siwek Marek, Slawincka Anna, Duan Xiang, Jia Jie, and Liu Xuebo garnered significant citations, each exceeding 136 citations, underscoring their substantial impact within the field. Furthermore, network analysis highlighted Yang Yanjun as a central collaborating figure, fostering a robust research network (total link strength of 32) (Figure 2A). The temporal distribution of publications reveals a recent surge in research activity, particularly after 2020, indicating FEP as a nascent yet rapidly expanding area of inquiry. Keyword co-occurrence analysis unveils two prominent clusters: "lactic acid bacteria" (frequent mention, 63) and "growth-performance" (frequent mention, 22) (Figure 2B). This suggests a prevailing emphasis on the application of LAB for enhancing FEP properties, highlighting its potential as a promising avenue for future investigation.

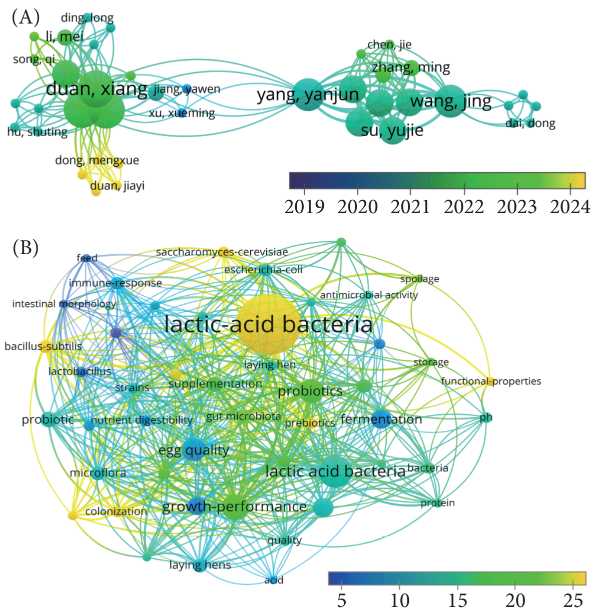


Figure 2 Current research status on effects of LAB on FEP. (A) Author cooperation map and co-cooperating authors-based clusters (2014–2024). (B) Visual representation of keyword co-occurrence networks within the field of effects of LAB on FEP based on the VOSviewer.

3 Effect of LAB on the physicochemical properties of FEP

3.1 Effect of LAB on the pH of FEP

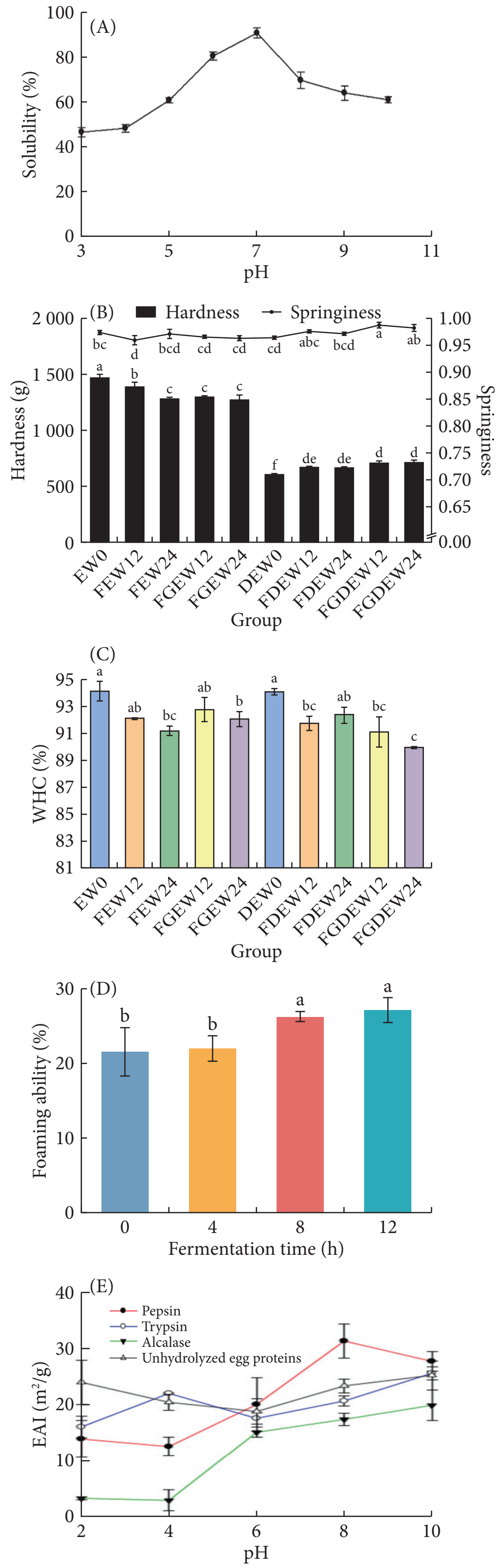
Whole egg liquid typically exhibits a neutral to alkaline pH, approximately 7.5, whereas the optimal growth pH range for LAB is 6.0–6.5, indicative of a mildly acidic environment[23]. Consequently, the pH of the whole egg solution must be modulated to align with optimal fermentation conditions before the initiation of LAB fermentation. For instance, citric acid is frequently employed as a pH buffer in the formulation of egg-milk fermentation products, facilitating the adjustment of the egg-milk mixture pH to 6.8 for subsequent fermentation processes[1,24]. LAB fermentation inherently involves acidification as these microorganisms metabolize glucose, producing organic acids such as lactic acid, resulting in a decrease in pH. Variations in the fermentation capabilities of distinct LAB strains significantly influence the pH of the final product, a phenomenon closely tied to the metabolic efficiency and substrate adaptability of these strains[23]. For instance, Yang et al.[1] demonstrated that co-inoculation of *L. bulgaricus* and *Streptococcus pyogenes* into liquid whole egg yogurt resulted in a near-linear pH reduction to 4.6 upon fermentation completion. Gan et al.[25] observed that homozygous or heterozygous LAB strains of fermented soybean yogurt exhibited the most significant pH drop within the first 3 h of fermentation, with pH ranging from 4.6 to 4.7 at 8 h. Similarly, Azizah et al.[26] found that *L. bulgaricus* inoculation of egg white powder significantly lowered its pH ($P < 0.01$), with a higher percentage of *L. bulgaricus* inoculation correlating with a lower pH of 6.63–7.33.

Numerous studies have established a clear correlation between fermentation time and the pH of FEP. Nahariah et al.[8] demonstrated that both milk addition and fermentation duration significantly influenced the pH of fermented egg whites, with an interaction observed between these factors. The pH of egg whites notably decreased from 6.80 to 5.49 following 24 h of fermentation, whereas the pH differences observed after 36 and 48 h were statistically insignificant. The inclusion of milk contributed to a further reduction in product pH, attributable to the metabolism of sugars (e.g., glucose, galactose) present in both egg whites and milk, catalyzed by LAB to produce lactic acid. Mourad et al.[7] similarly confirmed that after 24 h of inoculation with *Bifidobacteria*, the pH of the egg white drink significantly decreased compared to the initial value of 6.24. Yang et al.[1] also reported a significant pH drop in egg-milk mixture substrates inoculated with *Lactobacillus*. This trend exhibited a near-linear decrease, with a slight initial reduction during the first 2 h, followed by rapid decline from 2 to 4 h, and finally, gradual stabilization until the end of fermentation. These findings collectively indicate that both fermentation time and additives exert a significant effect on the pH of FEP. This understanding provides valuable insights for optimizing fermentation processes to achieve desired pH levels.

3.2 Effect of LAB on the processing characteristics of FEP

3.2.1 Effect of LAB on solubility of FEP

As a complete protein, egg protein provides all the essential amino acids required by the human body, with an amino acid composition closely resembling human needs, and exhibiting high bioavailability[27]. However, heat treatment can lead to protein denaturation, coagulation, and reduced solubility. LAB fermentation can affect protein solubility in egg product processing through multiple mechanisms. Firstly, lactic acid and other organic acids produced by LAB metabolism reduce pH, which in turn affects protein solubility. Secondly, protease synthesized by LAB can gradually hydrolyze high molecular weight proteins into more soluble small molecule peptides or amino acids, improving protein solubility. Jain et al.[28] demonstrated that protein concentration significantly increased with time during fermentation, peaking at 36 h and then stabilizing due to the inhibitory effect of LAB metabolites on microbial growth. Notably, the solubility of proteins is typically lowest at the isoelectric point (pH 4.5), resulting from the weakening of electrostatic repulsion and interaction with ionized water. The study also found that pH had a significant impact on protein solubility, with solubility at higher pH (6, 7, and 8) significantly higher than that at lower pH (3, 4, and 5) (Figure 3A). Ortiz et al.[29] proposed that protease-induced hydrolysis disrupts the secondary structure of the protein, increases the ionizable amino and carboxylic groups, and enhances the interaction with water molecules, ultimately increasing the solubility of the hydrolysate.



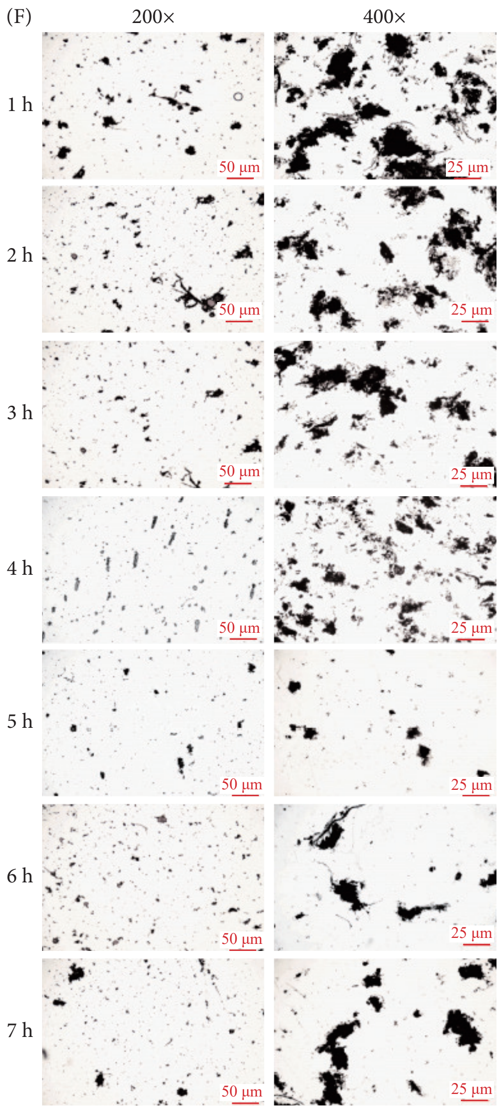


Figure 3 Effect of LAB on the physicochemical characteristics of FEP. (A) Solubility of proteins under various pH. (B) Textural properties and (C) water-holding capacity (WHC) of FEP. (D) Foaming ability of egg white powder fermented for different durations. (E) Emulsifying activity index (EAI) of enzymatic protein hydrolysates under various pH. (F) Optical micrograph of fermented egg-milk beverage (FEMB) under various cooling times. EW, egg white; FEW, fermented egg white; FGEW, fermented glucose egg white; DEW, diluted egg white; FDEW, fermented diluted egg white; FGDEW, fermented glucose diluted egg white. Numerals represent fermentation duration in hours. Different lowercase letters mean significant differences between the groups ($P < 0.05$).

3.2.2 Effect of LAB on the textural properties of FEP

The FEP by LAB exhibits a profound impact on their textural properties. Primarily, the metabolic by-products, such as lactic acid and bacteriocins, generated during LAB fermentation endow egg products with distinctive taste profiles and flavors, while concurrently refining their texture to a more delicate and smoother consistency. Additionally, proteolytic enzymes from LAB can modify the molecular conformation of proteins, thereby enhancing the gelling attributes of egg products and facilitating gel formation[30].

LAB fermentation yields bioactive substances, primarily organic acids (e.g., acetic acid, lactic acid) and bacteriocins, which exert significant bacteriostatic activity and contribute to enhancing texture in egg products. The production of organic acids, for instance, induces a pH reduction, which subsequently impacts the hardness of egg product gels. Furthermore, extracellular polysaccharides produced during LAB metabolism improve viscosity during fermentation and increase structural compactness[31]. Lü et al.[32] showed that the viscosity of fermented custard drinks increased with cooling time, a phenomenon that stems from the growth of LAB activity and acidity. Wang et al.[30] found that fermenting liquid whole egg with *L. plantarum* 90 enhanced gel properties, improving water retention and resulting in a smoother, more delicate surface with increased elasticity. Lü et al.[33] indicated that egg white protein molecules established a stable gel network via uniform aggregation and cross-linking throughout the fermentation process, thereby optimizing the textural properties of egg products by refining the rheological attributes such as hardness and springiness (Figure 3B). Jia et al.[34] observed in their investigation of egg white-fermented cake that the hardness, viscosity, and chewiness of the cake were significantly enhanced compared to the unfermented counterpart, with the fermentation process yielding a more compact pore space and a harder structural composition of the cake. However, the utilization of fermented egg whites in cake preparation may lead to excessive moistness and compromised quality due to reduced elasticity and viscosity.

3.2.3 Effect of LAB on gelation of FEP

Gelation is a ubiquitous phenomenon in the food industry, with thermal gelation being a primary method of protein gelation. Upon heat treatment, egg protein experiences denaturation, unfolding its structure to form a highly ordered three-dimensional network through intermolecular forces, including hydrophobic interactions, disulfide bonds, and hydrogen bonds[35]. However, heat treatment can result in a decrease in the thermal stability of proteins, thereby affecting the performance of the gel during subsequent processing or storage[36].

Food fermentation, a straightforward, risk-free, and eco-friendly technology, has a profound impact on enhancing the sensory properties of food, extending shelf life, increasing nutritional value, and reducing toxic and anti-nutritional factors[37]. For example, a study by Lü et al.[33] revealed the effects of LAB such as *Bifidobacterium* and *L. plantarum* on the protein structure and gel properties of egg white. The results demonstrated that *L. plantarum* fermentation significantly improved the texture, WHC, and elasticity of whole egg gels, forming a denser and less porous network structure. This study also found that undiluted fermented egg white showed decreased gel hardness (from 1 472.41 to 1 274.37 g) and WHC. However, diluted egg white with glucose supplementation significantly increased hardness by 16.64% (12 h) and 18.02% (24 h), while WHC still declined in both diluted and undiluted groups. The WHC reduction correlated with decreased protein concentration and altered water distribution patterns in gels. Dilution and glucose addition improved texture but reduced WHC in fermented egg white (Figures 3B and C). In the experimental study undertaken by Jiang et al.[38], the hydrophobicity index (H_0 value) of proteins experienced an elevation from 28.7 to 52.0 following 6 h of fermentation. This observation suggests that moderate fermentation facilitates the unfolding of protein molecules, and the modification or disruption of the protein molecular structure during the fermentation process disrupts the hydrophilic-hydrophobic equilibrium of the proteins, ultimately affecting their gelatinization properties. Similarly, Jia et al.[2] observed a significant decrease in the negative charge of protein dispersions after 8 and 12 h of fermentation. This enhanced electrostatic repulsion between protein particles was attributed to the gradual release of peptide chains with fermentation time, exposing charged amino acid residues on the protein surface. This increased electrostatic repulsion contributes to the stability of the overall colloidal system.

3.2.4 Effect of LAB on the foaming properties of FEP

Foaming properties are influenced by several factors, including the properties of the protein, environmental conditions, and the specific conditions of the foam formation process. Foam formation process conditions significantly influence its performance. For instance, at pH 4, foaming capacity increased substantially during the initial 5 h of fermentation as proteins unfolded, exposing hydrophobic groups. However, beyond 5 h, foaming capacity declined, attributed to an excess of hydrophobic groups hindering protein migration to the air-liquid interface. Conversely, at pH 7, both the foaming ability and foam stability remained relatively constant throughout the fermentation period, attributable to the relatively stable surface properties at this pH level[39]. Furthermore, the foaming characteristics were also influenced by the microbial species employed in the fermentation process. Jain et al.[28] demonstrated that protein fermentation hydrolysis products exhibit superior foam stability by effectively reducing interfacial tension at the air-water phase interface. With increasing fermentation concentration, the foaming ability of the fermentation products was significantly enhanced. The high concentration of proteins promotes intermolecular interactions, leading to the formation of a multilayer membrane structure that substantially reduces interfacial tension, thereby significantly improving foaming ability. Moreover, the capacity to form stable foams is closely associated with the elevated content of hydrophobic amino acids in the fermentation hydrolysate. These amino acids strengthen interfacial binding forces, endowing the product with excellent foaming properties. Jiang et al.[38] used LAB to ferment egg whites, and the results showed that the foaming capacity of egg whites increased significantly from 72.3% to 93.0%, indicating that LAB fermentation can effectively improve the foaming capacity of egg whites. Jia et al.[2] demonstrated that egg white powder fermented for 8 and 12 h exhibited significantly enhanced foaming ability compared to unfermented natural egg white powder (21.48%), achieving 26.30% and 27.22%, respectively. Following 4 h of fermentation, the foam stability of the egg white powder solution was significantly augmented from 40.97% to 48.84%, suggesting that the protein molecules adsorbed at the air-water interface exhibited enhanced strength post-fermentation, and the bubbles were more resilient after the fermentation treatment (Figure 3D).

3.2.5 Effect of LAB on the emulsifying properties of FEP

Emulsification is a key property in food processing, involving the adsorption capacity of proteins at the oil-water interface, which plays a decisive role in the formation of stable emulsions. After the protein enters the oil-water interface, its structure undergoes denaturation and rearrangement, and the hydrophobic amino acid locates to the oil phase and the hydrophilic amino acid locates to the water phase, thus reducing the interfacial tension and enhancing the emulsification property[40]. Jain et al.[28] revealed that the fermented protein hydrolysate produced smaller peptides due to the higher degree of hydrolysis, which was easier to migrate to the oil-water interface and showed excellent emulsified performance. This finding is further corroborated by the research of Chalamaiah et al.[41], which demonstrated that the EAI of 30% hydrolyzed pepsin hydrolysates was notably higher than that of trypsin and alcalase hydrolysates at pH values of 6, 8, and 10. This suggests that the 30% hydrolyzed pepsin hydrolysates may contain peptides with enhanced amphiphilic characteristics, which are likely to promote the migration of these peptides to the oil-water interface (Figure 3E). Xiao et al.[4] demonstrated that LAB fermentation significantly alters egg yolk protein structure, enhancing emulsifying properties. Gundogan et al.[42] similarly observed that protein aggregate formation, a consequence of fermentation, can alter egg white protein emulsifying capacity, increasing it from 57.00% to 73.92%. Emkani et al.[39] further noted that fermentation modifies protein properties (structure, molecular size, conformation, flexibility, solubility, and surface hydrophobicity) and environmental conditions (pH, mineral content), potentially enhancing protein emulsifiability. However, low emulsification in fermented samples may be attributed to protein aggregation and interactions with by-products, potentially hindering hydrophobic interactions between proteins and oil molecules, thereby reducing protein amphiphilicity. Additionally, emulsification stability is contingent upon factors such as pH, the soluble concentration of proteins, hydrophobicity, and structural flexibility.

3.3 Effect of LAB on the structure of FEP

The structure of proteins has an important influence on their functional properties. Therefore, it is crucial to study the effect of LAB fermentation on the structure of egg protein. For example, Emkani et al.[39] showed that lactic acid fermentation alters the structure of proteins due to the presence of enzymes and organic acids. Firstly, microbial proteases can break peptide bonds and produce new peptides and even free amino acids. LAB has a complex protease system that consists of extracellular proteases that degrade polypeptides into short oligopeptides and free amino acids. Secondly, organic acids such as lactic acid and acetic acid may disrupt the secondary and tertiary structure of proteins, and studies of the peaks in the thermograms by differential scanning calorimetry have also shown that fermentation causes intermolecular bonds to break within the proteins, which denatures the proteins with fewer heats. As another example, García-Cano et al.[43] in their study observed several protein hydrolases in different cell fractions using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and the molecular weights of the enzymes detected by the enzyme spectra ranged from 40 to 100 kDa, suggesting that LAB produces a wide range of proteases that are capable of further altering the structure of proteins. Jia et al.[2] employed Fourier transform infrared spectroscopy to analyze the secondary structural alterations of egg white proteins during fermentation. Their findings revealed a reduction in β-sheet and α-helix content, indicating a decrease in the ordered structure of the proteins. Furthermore, the fermented product exhibited an increase in β-turn content, suggesting a more disordered structure. This transition from order to disorder implies an increase in unfolded proteins within the fermentation product, which facilitates conformational changes at the air-water interface and enhances foaming capacity. Simultaneously, the fermented egg white particles adopted a concave spherical morphology. As fermentation time extended, the particles became smaller and more evenly distributed with an increased number of concave pores. This transformation is attributed to the hydrolysis of egg white proteins during the fermentation process. The smaller size and porous structure of the fermented particles augment the specific surface area, thereby enhancing the adsorption between polar groups and water, leading to improved foaming properties[2]. Wang et al.[30] also observed a transformation of β-sheet and β-turn structures into α-helix and disordered structures. This shift suggests an increase in the flexibility of the protein structure, as β-sheet exhibits a stable conformation while α-helix possesses a more flexible structure. With prolonged fermentation, the β-turn and α-helix content in the fermented protein gels exhibited an initial increase followed by a decrease, while β-sheet content decreased initially and then increased. The disordered structure showed a general decrease, indicating a trend towards a more ordered and stabilized secondary structure in the late stages of fermentation compared to the pre-fermentation state. Lü et al.[32] employed microscopy to examine protein aggregation and further confirm the structural changes in FEMB under various cooling times. Microscopic images revealed that protein micelles were larger at the beginning of cooling. The action of LAB resulted in the breakdown of proteins into polypeptides, disrupting the micellar structure and reducing aggregation. After more than 5 h of cooling, protein aggregation occurred, and the micellar structure gradually densified in the presence of a decreased pH, ultimately leading to an increase in viscosity (Figure 3F).

3.4 Effect of LAB on the antioxidant properties of FEP

Anti-oxidation is a functional property capable of delaying, preventing, or eliminating oxidative damage to target molecules[44]. Some active peptides and amino acids formed during protein hydrolysis have significant antioxidant activity. Recent studies have demonstrated the antioxidant activity of active peptides and amino acids generated during protein hydrolysis. For instance, Jain et al.[28] found that amino acids derived from fermentation hydrolysis contribute to the antioxidant and angiotensin-converting enzyme (ACE) inhibitory activities of proteins and peptides. Furthermore, the presence of these amino acids indicated a high free radical scavenging and ACE inhibitory capacity in fermented protein hydrolysates. Presently, Nimalaratne et al.[44] have elucidated that egg white hydrolyzed by “protease P” yields two potent antioxidant peptides, both of which feature leucine residues at the N-terminal, enhancing their antioxidant capabilities. Additionally, egg yolk protein hydrolysate exhibits superoxide and hydroxyl radical scavenging activities, suggesting its potential as a natural antioxidant. Lü et al.[32] observed an increase in the antioxidant capacity of FEMB samples with cooling time, which attributed to the breakdown of proteins by LAB to form active components with antioxidant activity. This finding aligns with previous research that has verified the ability of LAB to produce antioxidant peptides. The results of Jain et al.[28] showed that the fermented protein hydrolysate had excellent free radical scavenging and reducing ability, especially at concentrations of 3, 4, and 5 mg/mL. In addition, the study also evaluated the efficacy of the fermentation products in inhibiting lipid peroxidation, and the results showed that their inhibitory effect on lipid peroxidation was significantly enhanced. The antioxidant properties of fermentation products are closely related to their amino acid composition and molecular size. For example, the presence of tyrosine and histidine residues contributes to improved antioxidant properties. The study also confirmed the presence of electron-donating peptides, which act as major antioxidants by inhibiting free radical chain reactions[28]. These findings indicate that protein hydrolysis products have potential antioxidant and bioactive applications, which are important for the in-depth study of their mechanisms and the development of novel antioxidants.

4 Effect of LAB on the metabolism and flavor of FEP

4.1 LAB influences the production of flavor substances in egg products through enzymatic systems

Egg products exhibit certain limitations in flavor, and LAB, a beneficial microorganism with significant application potential, substantially alters the flavor characteristics of egg products through the modulation of protein metabolism. The regulatory role of LAB elucidates the pivotal mechanisms of protein catabolism and transformation, underscoring the crucial role of microorganisms in enhancing food quality. The biomodification induced by LAB leads to the conversion of proteins and other components in egg yolks, thereby improving their taste characteristics and markedly influencing the quality of egg products.

During egg fermentation, the cooperative action of enzymolysis and LAB facilitates the shift of phenylalanine to floral and almond aroma compounds. Enzymatic digestion not only accelerates the fermentation process but also releases key flavor precursors. LAB significantly enhances the flavor of FEP through the combined action of extracellular enzymes, cytoplasmic metabolism, and amino acid degradation, which form, transform, or break down aroma components[45]. Smid et al.[46] demonstrated that the extracellular and cell envelope binding protease (PrtP) of *Lactis lactatus* can hydrolyze casein to produce over 100 distinct oligopeptides. These oligopeptides are subsequently transported into the cell via dipeptides and oligopeptide transporters, and free amino acids are released by intracellular peptidase to support the growth and maintenance of LAB. In protein-rich environments such as custard, sufficient protein hydrolysis activity ensures that the supply of amino acids in the environment far surpasses the biosynthetic requirements of the fermenting LAB. Additionally, the amino acid composition of the protein source typically does not align with the amino acid composition of the fermenting microbial biomass, leading to another fate for the released amino acids, their conversion through catabolic reactions to form a range of volatile aromatic compounds. Jia et al.[47] discovered that during egg yolk fermentation, LAB secrete specific enzymes that hydrolyze carbohydrates, fats, and proteins into fatty acids, glucose, amino acids, and other small molecule metabolites. These small molecules serve as flavor precursors and engage in various chemical reactions to produce a multitude of volatile compounds that directly influence the final flavor of the food.

4.1.1 LAB mediates lipid metabolism to regulate flavor production in egg products

Free fatty acids, both saturated and unsaturated, released during lipid hydrolysis contribute significantly to the aroma profile of fermented foods[37]. Cheng et al.[48] demonstrated that short-chain fatty acids, particularly those derived from saturated fatty acids, play a crucial role in developing strong product flavors. Unsaturated fatty acids, in the presence of free radicals, undergo oxidation to form hydroperoxides, which rapidly decompose to produce hexanal or unsaturated aldehydes. Furthermore, unsaturated fatty acids generate 4- or 5-hydroxy acids that readily cyclize to form γ- or

δ-lactones or odd-carbon methyl ketones via β-keto acid decarboxylation.

4.1.2 LAB mediates protein metabolism to regulate flavor generation in egg products

LAB influences the flavor of egg products through a series of biochemical processes, including protein catabolism, synthesis, and transformation. During egg fermentation, proteases and other hydrolytic enzymes produced by LAB specifically hydrolyze proteins into small molecule metabolites such as amino acids[47].

During the baking process, these small molecules act as flavor precursor substances and participate in the Maillard reaction, producing volatile compounds such as aldehydes and furans. Consequently, they directly influence the final flavor of baked goods. Studies have shown that LAB fermentation during the processing of egg-containing cookie products significantly reduces baking losses and increases the diffusion factor of the cookies, resulting in improved texture[49]. For example, Jia et al.[47] fermented and hydrolyzed egg yolk proteins with *Lactobacillus rhamnosus*, hindering gluten network formation and reducing dough elasticity, leading to a softer cookie texture. Additionally, they employed headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) to identify the volatile components in the cookies and discovered that fermented egg yolk altered the flavor profile of the cookies, with the fermented egg yolk treated with *L. rhamnosus* exhibiting the most pronounced effect. In the processing of egg white flour, LAB fermentation generates organic acids such as lactic acid, leading to the precipitation of proteins and the formation of aggregates. Enzymatic degradation by flavourzyme further disrupts the three-dimensional structure of proteins. For example, Chen et al.[45] employed LAB fermentation followed by enzymatic degradation with flavourzyme to disrupt the hydrophobic cavities that bind to off-flavor compounds, thereby releasing and removing these compounds through fermentation.

The combination of enzymatic digestion and fermentation significantly enhances the flavor of egg powder. Proteolysis by flavor enzymes generates amino acids and peptides with favorable flavor characteristics, while simultaneously promoting the growth and metabolism of LAB. The metabolites produced by LAB further optimize the flavor profile of the egg powder[50]. Studies have demonstrated that the enzymatic-with-fermentation combined treatment excels in reducing the undesirable flavors of egg powder, effectively diminishing the presence of four major undesirable flavor compounds: geranylacetone, 1-octen-3-ol, octanal, and nonanal. Additionally, the content of free amino acids in the treated egg powder was substantially increased, leading to a marked improvement in its flavor[51]. These findings indicate that the enzymatic combined fermentation approach effectively enhances the flavor of egg powder by modulating amino acid metabolism.

Furthermore, the sulfhydryl metabolism of LAB exerts a significant influence on the structure and functionality of egg white proteins. The sulfhydryl metabolism pathway mediated by LAB species can effectively modulate egg white protein properties through the accumulation of sulfhydryl compounds, which subsequently engages in sulfhydryl/disulfide bond exchange reactions with egg white proteins. This process alters protein conformation and ultimately enhances protein flavor[52]. For instance, sourdough fermentation with *Lactobacillus sanfranciscensis* resulted in near-complete hydrolysis of ovalbumin (OVA), with hydrolysis significantly increasing post-treatment. Concurrently, a substantial increase in sulfhydryl group levels within the sourdough was observed, correlating with OVA degradation. These findings indicate that LAB sulfhydryl metabolism effectively modulates egg white protein properties. While the impact of LAB sulfhydryl metabolism may be influenced by specific conditions, it demonstrates considerable potential for regulating protein hydrolysis and improving food quality. To further enhance the effects of LAB sulfhydryl metabolism on egg white proteins, researchers often employ strategies such as adjusting fermentation conditions and integrating other modification methods to optimize nutritional value and sensory attributes[53].

4.1.3 LAB mediates carbohydrate metabolism to regulate flavor production in egg products

LAB exerts a substantial influence on the flavor profile of egg products by modulating carbohydrate metabolism during fermentation. The metabolic activity of LAB results in the conversion of carbohydrates into a diverse array of organic acids and other flavor compounds, thereby contributing to the unique flavor and textural characteristics of egg products. LAB metabolism facilitates the conversion of carbohydrates into lactic acid and other organic acids, directly impacting the flavor profile of egg products[54]. For instance, Jia et al.[55] employed GC-MS to analyze the volatile compounds in mayonnaise produced with varying durations (0, 3, 6, and 9 h) of lactic acid fermentation. The production of these organic acids primarily occurs through the glycolytic pathway of LAB. This pathway initiates with the phosphorylation of glucose to glucose-6-phosphate, followed by a series of transformations culminating in the formation of pyruvate. Consequently, the organic acids generated through LAB fermentation constitute the primary source of sour flavor in egg products.

4.2 Effect of fermentation time on the flavor of egg products

LAB fermentation yields organic acids, including lactic acid, and a diverse array of enzymes such as proteases. This process leads to a decrease in product pH and subsequent protein degradation, along with other compositional alterations. Extending the fermentation duration enhances the production of fermentation metabolites, consequently impacting the overall flavor profile of the product. Extended fermentation durations result in a progressive decrease in pH, which enhances intermolecular protein interactions, including hydrophobic bonding and electrostatic interactions. This facilitates protein aggregation and the formation of gel structures. Concurrently, an increase in both inter- and intra-molecular hydrogen bonding contributes to a denser network structure, resulting in improved product stability and moisture retention[56]. For instance, Yang et al.[1] observed a distinct transition in egg-lactic acid mixed fermentation substrates as fermentation time increased from 0 to 7 h and pH concomitantly decreased. The initial liquid substrate gradually evolved into a weak gel with enhanced stability, while the yogurt texture became increasingly viscous with prolonged fermentation. Furthermore, LAB-produced proteases hydrolyze proteins into peptides and amino acids, with the degree of hydrolysis increasing over time. This proteolysis induces structural modifications in proteins, potentially leading to the loss or gain of certain flavor compounds, thereby influencing the overall flavor profile of the product[1]. Milawati et al.[57] reported a decrease in the relative abundance of hexanoic acid in mayonnaise and an increase in formic acid, phenylglyoxal, 1-octen-3-one, and heptanal with extended fermentation. The sensory evaluation further revealed that mayonnaise fermented for 3 and 6 h exhibited a more desirable texture and aroma.

5 Effect of LAB on the nutritional characteristics of FEP

5.1 Effect of LAB on the nutritional components of FEP

The edible properties of FEP are intricately linked to their nutritional characteristics, particularly the alterations in their nutritional composition. Fermentation of proteins by LAB results in their decomposition and transformation, subsequently influencing the nutritional profile of the proteins and providing beneficial effects on health. For instance, Lü et al.[32] employed microscopy to investigate the microstructure of fermentation products and analyze protein aggregation behavior. The microscopic images revealed that varying cooling times significantly affected the structure of protein micelles. Initially, the micellar clusters in the samples were larger; however, as LAB acted on the proteins, they were progressively broken down into polypeptides, leading to the disintegration of the micelle structure and a notable reduction in the degree of aggregation.

Research has demonstrated that LAB fermentation can yield a range of bioactive peptides, including antihypertensive, antioxidant, antimicrobial, and antidiabetic peptides. Specific antihypertensive peptide fragments, such as VPP (Val-Pro-Pro) and IPP (Ile-Pro-Pro), have been identified in fermented dairy products and have exhibited blood pressure-lowering effects in clinical trials[58]. Antioxidant peptides contribute to reducing oxidative stress and preventing cellular damage by scavenging free radicals, chelating metal ions, and enhancing the body's intrinsic antioxidant defenses. These peptides often contain specific amino acid sequences, including aromatic amino acids like tyrosine and tryptophan, which possess significant antioxidant properties due to their high proton-donating capacity. Consequently, antioxidant peptides play a crucial role in maintaining cell membrane integrity, preventing DNA damage, and slowing the aging process[59]. Antimicrobial peptides (AMPs) are biologically active molecules with potent antimicrobial properties, capable of inhibiting or eliminating pathogenic microorganisms through various mechanisms, including disruption of microbial cell membrane integrity, interference with protein synthesis, and inhibition of key enzymatic activities. These peptides typically possess specific structural features, such as α-helices or β-sheets, which facilitate their interaction with microbial cell membranes. AMPs are utilized in food preservation to inhibit the growth of spoilage microorganisms and are emerging as novel antibiotic candidates in the medical field, particularly against drug-resistant bacteria[59]. Antidiabetic peptides primarily function by inhibiting the activity of dipeptidyl peptidase-IV, an enzyme responsible for the degradation of incretin hormones, such as glucagon-like peptide-1, which play a crucial role in regulating blood glucose levels. By extending the half-life of enteric insulin, these peptides enhance its insulin-promoting and blood glucose-lowering effects. The incorporation of these peptides into food products may offer significant benefits for individuals with diabetes or those at high risk, aiding in better management of blood glucose levels. Additionally, their inclusion in the diet could provide preventative benefits for healthy individuals by reducing the risk of developing diabetes[58].

Lü et al.[60] conducted LC-MS/MS analysis to investigate the protein composition of a novel fermented dairy product prepared using equal quantities of egg white and milk, supplemented with 1% (*m*/*m*) glucose and 4% (*m*/*m*) sucrose, and fermented at 39 °C for 12 h using the ABY-8 lactic acid strain from Chr Hansen. The analysis revealed that the predominant protein in the product was OVA, with sequence coverage ranging from 42% to 63%. Additionally, minor quantities of lysozyme, κ-casein, ovotransferrin, ovocleidin-like protein, and glycoproteins were detected. Peptide analysis indicated that the parent protein was primarily lecithin, confirming the proteolytic activity of LAB on the protein. Studies have shown that the polypeptides produced during the fermentation process can be further hydrolyzed into bioactive small molecule peptides in the human digestive system, which may have functions such as anti-inflammatory and immune-enhancing functions[60]. Moreover, metabolites such as lactic acid, hydrogen peroxide (H_2O_2), and bacteriocins produced by *L. plantarum* during fermentation exhibit antimicrobial properties, thereby further enhancing the functional attributes of the product[61]. H_2O_2, a reactive oxygen species, plays a critical dual role in biological systems. At low concentrations, it functions in cell signaling and physiological regulation, while at high concentrations, it can induce oxidative stress and cause cellular damage. H_2O_2 can generate highly reactive hydroxyl radicals through the Fenton and Haber-Weiss reactions, leading to oxidative damage to lipids, proteins, and DNA, and is closely associated with the development of various diseases[62]. LAB produces bacteriocins, a class of ribosomally synthesized AMPs that exhibit low cytotoxicity under laboratory conditions while demonstrating antagonistic properties and additional benefits. Unlike conventional antibiotics, these bacteriocins possess species-specific antimicrobial activity, selectively targeting pathogens without disrupting other microbial populations within the same ecosystem. The combination of bacteriocins with antibiotics presents novel therapeutic opportunities for foodborne pathogens[63].

For example, it was found that 17 LAB isolates exhibited the ability to produce a diverse range of amino acids extracellularly under varying pH conditions, albeit in a strain-specific manner. Most strains demonstrated the ability to produce glutamic acid, valine, methionine, lysine, threonine, and tryptophan, all of which are also present in egg products. Additionally, the study noted that these LAB isolates were unable to produce arginine, resulting in a decrease in arginine concentration[64].

5.2 Effect of LAB on the nutritional function of FEP

LAB can enhance immune system function by promoting the proliferation and differentiation of immune cells, thereby strengthening the immune response. For instance, *L. plantarum* not only produces lactic acid but also degrades nutrients in eggs and demonstrates significant bacteriocin production capacity[61]. Bacteriocins, which are peptide or protein compounds secreted by LAB, possess specific bactericidal effects against phylogenetically related harmful bacteria. This antimicrobial activity provides an effective means of inhibiting the growth of pathogenic bacteria, offering significant potential in the fields of food safety and biopreservation. Moreover, bacteriocins produced by *L. plantarum* not only inhibit the growth of related pathogens but may also enhance host immune function by modulating the balance of intestinal flora[61]. To evaluate the positive effects of FEMB on intestinal flora structure, Lü et al.[60] employed 16S rRNA gene sequencing to analyze changes in the gut microbiome. Their study demonstrated that LAB exerts a significant regulatory effect on intestinal flora through careful grouping and analysis. The results indicated that FEMB treatment with LAB significantly increased the microbial diversity and abundance of mouse cecal contents, promoting the health of the intestinal microbial community as reflected in an improved α-diversity index. Furthermore, FEMB treatment was able to restore structural changes in the gut flora induced by dextran sulfate sodium to resemble the composition of normal controls. Notably, at the portal level, FEMB treatment significantly altered the ratio of Firmicutes to Bacteroidetes, which is an important indicator of gut health.

Egg products fermented by LAB exhibit significant hypolipidemic effects. For instance, *in vivo*, experiments on mice demonstrated that serum and liver triglyceride concentrations, as well as serum free fatty acid levels, were significantly lower in the lactic acid-fermented egg white group compared to the casein group. However, no significant differences were observed among the unheated egg white group, the heated egg white group, and the casein group regarding these parameters[65]. The intake of lactic acid-fermented egg whites was associated with a marked reduction in fat absorption, leading to decreased visceral fat mass. The potential mechanisms underlying this reduction in visceral fat include: 1) enhanced fatty acid β-oxidation in the liver, 2) decreased fatty acid synthesis in the liver, and 3) reduced adipocyte size within visceral fat tissue[66]. Moreover, an increasing body of research has demonstrated that fermented milk peptides (FMP) possess significant antihypertensive effects. In studies involving spontaneously hypertensive rats (SHR), those fed FMP showed a marked reduction in systolic blood pressure compared to SHR-fed skimmed milk[67]. In human studies, daily consumption of 95 mL of fermented milk containing the tripeptides Val-Pro-Pro and Ile-Pro-Pro resulted in a reduction of systolic blood pressure by 14.1 mmHg[68]. Additionally, a study by Seppo et al.[69] found that hypertensive patients experienced a significant decrease in blood pressure after a daily intake of 150 mL of a kefir product containing the same tripeptides, fermented by *Lactobacillus helveticus*, over periods of 21 and 10 consecutive weeks, with no adverse effects reported. This antihypertensive effect is likely attributed to the substantial inhibition of ACE activity by FMP.

6 Conclusion

This paper investigates the effects of LAB fermentation on the physicochemical properties, flavor, and nutritional characteristics of egg products in detail. The results indicate that the pH of FEP decreases with prolonged fermentation time, with varying fermentation abilities among different LAB strains leading to distinct final pH values. The action of LAB enhances the foaming and emulsification stability of egg products, increases protein solubility, and improves their physicochemical characteristics. Additionally, LAB fermentation alters protein structures, which subsequently affects the functional properties of the egg products. Moreover, the antioxidant activity of LAB-FEP is significantly greater than that of unfermented egg products. LAB regulates the production of flavor compounds in egg products primarily through enzyme systems involved in lipid, protein, and carbohydrate metabolism. The overall flavor profile of egg products is notably influenced by the growth of fermentation products as fermentation time increases. Furthermore, LAB fermentation facilitates the decomposition and transformation of proteins, promoting the formation of smaller peptides, which enhances protein digestion and absorption[4]. FEP also offers potential health benefits, including immune support and hypolipidemic properties, due to their functional characteristics. However, the field of LAB fermentation of egg products remains underexplored. Future research should focus on further investigating the fermentation characteristics of various LAB strains and optimizing fermentation conditions to achieve optimal product quality and functional properties. Additionally, there is a need to develop novel FEP, such as low-fat, low-cholesterol, and high-protein options, to satisfy contemporary consumer demands for health and nutrition.

Conflict of interest

The authors of this study have declared no conflict of interest.

Acknowledgment

This work was financially supported by the College Students Innovation Training Program of Shanxi Agricultural University (S202410113033) and the Shanxi Provincial General Program for Basic Research Project (202303021211093).

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