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Phosphoproteomics revealed the sites and functions of whey proteins exclusive to human and bovine mature milk

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ABSTRACT

The phosphorylation modification of whey protein, an important nutrient of milk, has a key role in functional regulation. However, differences in the phosphorylation modifications of exclusive whey proteins in human and bovine milk are not known. In this study, phosphoproteomics analysis of exclusive whey proteins was performed in human mature milk (HM) and bovine mature milk (BM). 52 whey phosphoproteins and 128 phosphorylation sites were identified in HM, with Osteopontin phosphorylation sites being the most abundant. 59 whey phosphoproteins and 151 phosphorylation sites were identified in BM, with Osteopontin and Alpha-S2-casein phosphorylation sites having the highest number. The results indicate that phosphoproteins mainly play roles in immune defense, coagulation regulation and transmembrane signaling synergy in HM, and are mainly associated with immune regulation in BM. The most strongly interacting phosphoproteins in HM and BM were Serum albumin and Heat shock protein HSP 90-alpha (HSP 90-alpha), respectively. These results suggest functional differences between HM and BM whey phosphoproteins and enhance our understanding of the exclusive phosphoprotein sites. This study provides a theoretical basis and technological targets at the molecular level for the development of functional dairy products and infant formulas.

1. Introduction

Milk is a source of high-quality protein, which consists of two main groups: casein and whey proteins (F. Zhu, 2024). Whey protein is an important functional component of mammalian milk, which not only provides essential amino acids and nutritional support for newborns (Tsakali et al., 2023), but also participates in immune regulation, intestinal development, and metabolic regulation through a variety of biologically active components (e.g., immunoglobulins, lactoferrin, growth factors, etc.) (Dullius et al., 2018; Olvera-Rosales et al., 2023). Whey proteins are widely used in infant formulas, baking, sports nutrition and other products (J. C. Li & Zhu, 2024). The percentage of whey protein in human milk is the largest, up to 60–80 % (X. Li et al., 2025). As the ideal food for infants (Wang et al., 2020), human milk is irreplaceable in terms of composition, function and post-translational modification of its components for infant growth and development, immune maturation and metabolism (Andres et al., 2023; J. Zhu & Dingess, 2019). Although human milk is the ideal food for infants, some mothers may have insufficient breastmilk, in which case infant formula can temporarily replace breastmilk. Bovine milk is the main basic raw material of infant formula milk powder. Because the composition ratio of its macronutrients is relatively close to that of human milk and can be further adjusted through standardization to simulate the nutritional composition of human milk (Yu et al., 2025). All infant formulas are modeled after human milk. Therefore, understanding the differences between HM and BM whey proteins can provide a theoretical basis for optimizing infant formulas that are closer to breast milk.

Post-translational modifications occur in most proteins and are the main cause of changes in protein structure, function and properties (Javitt & Merbl, 2023). Post-translational modifications regulate processes such as metabolism, protein localization and turnover, signal transduction (Dutta & Jain, 2023). As one of the central forms of post-translational modifications, phosphorylation modifications are widely found in proteins (Duan et al., 2020). It profoundly affects the

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nutritional properties and biological functions of proteins by regulating their conformation, stability and interactions (Muneer et al., 2025; Rout & Verma, 2021). Protein phosphorylation occurs mainly on serine (Ser), threonine (Thr) and tyrosine (Tyr) residues (X. B. Chen et al., 2022). Mature milk provides a sustainable supply of nutrients and adaptive growth support, especially bovine milk, which is a core component of the dairy industry. Phosphoproteomic analysis focuses on analyzing the biological functions, differences in nutritional properties, and potential applications of protein phosphorylation modifications. Therefore, it is necessary to perform phosphoproteomic analysis of whey proteins in HM and BM.

Although modern infant formula milk is close to breast milk in terms of the composition of macronutrients such as protein and fat, there is still a significant gap in bioactive ingredients (Lönnerdal, 2016). Studies have shown that casein phosphorylation promotes the dissolution and absorption of minerals (calcium, iron) (Y. M. Zhang et al., 2024). Lactoferrin, an important component of whey proteins, has been shown to affect cellular metabolism by modulating the phosphorylation of proteins such as AKT (Moreno-Navarrete et al., 2009). Bovine milk is currently the main protein source for formula, but its phosphorylation modifications differ from breast milk. It has been shown that human milk milk fat globule membrane (MFGM) phosphoproteins are mainly involved in developmental regulation and immune-related functions, whereas bovine milk MFGM phosphoproteins are more significantly involved in signaling response functions (X. Y. Liu, Bai, et al., 2025). However, the analysis of cross-species phosphorylation differences of whey proteins still needs further systematic studies.

We provide valuable information to the dairy industry in order to "humanized" infant formulas and close the gap between formula and breast milk in terms of immunity. In this paper, we applied label-free quantitative techniques for phosphoproteomic analysis of whey proteins in HM and BM. We have applied bioinformatics to analyze the identified whey phosphoproteins, phosphorylation sites and biological functions. Understanding the phosphorylation of whey proteins between species may provide new insights in the optimization and development of infant milk powders. Our study is the first to show exclusive phosphoproteins and phosphorylation sites in HM and BM. The results provide information to better mimic human milk and to promote the transformation of milk powder from "nutritional replacement" to "biofunctional replication".

In general, our results not only reveal the functions and species differences of whey proteins, but also provide a scientific basis for dairy processing optimization and nutritional fortification. In this paper, phosphoproteomics analysis of whey proteins in HM and BM was performed, but the functional study of whey proteins needs to be further researched, and we hope that scholars will conduct in-depth research on the sites of whey phosphorylated proteins in the future.

2. Materials and methods

2.1. Collection of two types of milk samples

The human milk samples were donated by 45 healthy mothers aged between 25 and 30 years. Bovine milk samples were collected from 45 Holstein cows (aged 3.5–4 years old and weighing 550–650 kg) at Huishan Farm, Shenyang, Liaoning, China. These cows ate the same food and lived in the same environment. All human donors have been screened, including (1) no chronic diseases; (2) Do not smoke; (3) No antibiotics have been used within one month. The cows are selected from the same production batch and are milked at exactly the same time. The 45 samples were divided into 3 groups of 15 each mixed to produce 3 representative samples. The study was approved by the Ethics Committee of Shenyang Agricultural University (approval number [24060502]) and the Medical Ethics Committee (approval number [Y (2024)232]).

2.2. Extraction and digestion of two whey proteins

HM and BM samples were removed from the refrigerator (−80 °C) and thawed to room temperature (25 °C). These samples were processed by centrifugation (12000×*g*, 4 °C, 15min) aiming at removing milk fat. Centrifugation again (15000×*g*, 4 °C, 1h) yielded whey protein, the concentration of which was measured by the Caumas Brilliant Blue method. Two samples were taken and 10 mmol/L DTT (37 °C, 2.5h) was added to each sample and brought down to room temperature (25 °C). Subsequently, 50 mmol/L IAA was added and protected from light for 30 min. Then 5-fold volume of water was added separately to dilute the concentration of UA lysate to 1.5 M. Trypsin was added in the ratio of 1:50 (37 °C digestion for 18h). Finally, it was desalted using a Sep-Pak C18 column and lyophilized.

2.3. Peptide enrichment

The peptides were put into the buffer for re-solution. The solution was shaken for 40 min after addition of TiO_2 beads, followed by centrifugation (12000×*g*, 4 °C, 15min). The beads were collected and washed three times with buffers I and II. Finally, the phosphorylated peptides were collected by elution with elution buffer.

2.4. LC-MS/MS

Data were obtained by Thermo Q-Exactive mass spectrometer and Easy nano-Liquid Chromatography system. Each sample was redissolved in 0.1 % formic acid solution. The mobile phase consisted of 0.1 formic acid acetonitrile aqueous solution. The concentrations of acetonitrile in mobile phases A and B were 2 % and 84 %, respectively. The C18 column (Thermo EASY column SC200 150 μm*100 mm) was equilibrated with mobile phase A, while mobile phase B performed a gradient elution. The linear gradient of liquid B ranged from 0 % to 55 % at 0 min–115 min, from 55 % to 100 % at 115 min–120 min, and was maintained at 100 % at 120 min-125 min. The enzymatic products were separated by capillary HPLC and mass spectrometry analysis was performed by a Q-Exactive mass spectrometer (Thermo Finnigan). The mass spectrometer takes 125 min to acquire data. It mainly detects positive ions and has a scanning range of 300–1800 m/z for precursor ions. MS^1 and MS^2 have resolutions of 70,000 and 17,500 at M/Z 200, respectively.

2.5. Data analysis

LCMS/MS data are imported into Maxquant software for library search (UniProt). Main parameters: MS/MS tolerance ppm: 20; Missed cleavage: 2; Enzyme: Trypsin; Main search ppm: 6; Variable modification: Oxidation (M), Acetyl (Protein N-term), Phospho (STY); Peptide and Protein FDR: 0.01; Fixed modification: Carbamidomethyl (C). MaxQuant was used to calculate PTM scores, localization probabilities, and quantization values.

2.6. Bioinformatics and results analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of whey proteins were performed using the DAVID online database in conjunction with the WeiShengXin online platform. The STRING database was combined with Cytoscape software for protein-protein interaction (PPI) analysis. MeMe and Weblogo were used for motif analysis. All results analysis was done using Office and Prism.

3. Results

3.1. Identification of whey phosphoproteins in HM and BM

In this study, we used a label-free quantitative technique for phosphoproteomic analysis of whey proteins in HM and BM. In this analysis, we chose two and more results from three replicate experiments to analyze the phosphorylation sites. 52 whey phosphoproteins and 128 phosphorylation sites in HM and 59 whey phosphoproteins and 151 phosphorylation sites in BM were identified (Table S1). There were 283 and 336 phosphorylation-modified peptides in HM and BM, respectively (Fig. 1A). Among the identified proteins (Table 1), Osteopontin (P10451) was the most abundant in HM with 24 phosphorylation sites which were S215, S219, S228, S254, S258, S263, S267, S270, S243, S275, S280, S191, S195, S24, S26, S27, S76, S62, S63, T227, T237, T185, T190, and Y225. Osteopontin (P31096) was also the most abundant in BM with 23 phosphorylation sites which were S211, S216, S220, S193, S194, S197, S223, S228, S188, S167, S171, S23, S24, S26, S27, S76, S60, S62, S63, T191, T161, T166 and T22. This was followed by Alpha-S2-casein (BM) with 17 phosphorylation sites. These sites were S258, S23, S24, S25, S28, S31, S71, S72, S73, S76, S46, S52, T145, T153, T18, S150 and S68 on Alpha-S2-casein. The mass error of all whey phosphorylation-modified peptides was less than 6 ppm (Fig. 1B), indicating the reliability of the data. The distribution of whey phosphorylation sites in HM and BM is shown in Fig. 1C, and one site occupies the largest proportion in both. The number of three sites is higher than two sites in HM, while the number of four sites is greater than three sites in BM. Ser, Thr, and Tyr are phosphorylation sites, and Ser is the most predominant in HM and BM, 85.2 % and 86.1 %, respectively. Tyr was the smallest percentage with 3.9 % and 2 % respectively (Fig. 1D and E). Fig. 2A and B are visualizations using Weblogo, centered on the phosphorylation site and flanked by 15 amino acids on each side. Fig. 2C and D shows the probability of phosphoprotein sites appearing in HM and BM. The major phosphorylation motifs are analyzed in Fig. 2E and F. Fig. 2G and H are the global motif analyses for human and bovine milk.

Table 1
The number of top ten phosphoproteins and phosphorylation sites in HM and BM.

UniProt accession	Description	Gene	Number of phosphorylation sites	HM/ BM
P10451	Osteopontin	SPP1	24	HM
P05814	Beta-casein	CSN2	7	HM
C4B6Q2	Osteopontin	opn	4	HM
A0A161I202	Lactoferrin	LTF	4	HM
A6NMQ1	DNA polymerase	POLA1	4	HM
Q6WN34	Chordin-like protein 2	CHRDL2	4	HM
Q3LGB0	Osteopontin	SPP1	4	HM
Q86TD3	Secreted phosphoprotein 1	SPP1	3	HM
P01833	Polymeric immunoglobulin receptor	PIGR	3	HM
P01042	Kininogen-1	KNG1	3	HM
P31096	Osteopontin	SPP1	23	BM
P02663	Alpha-S2-casein	CSN1S2	17	BM
P02662	Alpha-S1-casein	CSN1S1	9	BM
P80195	Glycosylation-dependent cell adhesion molecule 1	GLYCAM1	5	BM
P02666	Beta-casein	CSN2	4	BM
O18836	Growth/ differentiation factor	MSTN	3	BM
P00711	Alpha-lactalbumin	LALBA	3	BM
P02672	Fibrinogen alpha chain	FGA	3	BM
P12763	Alpha-2-HS-glycoprotein	AHSG	3	BM
P18892	Butyrophilin subfamily 1	BTN1A1	3	BM

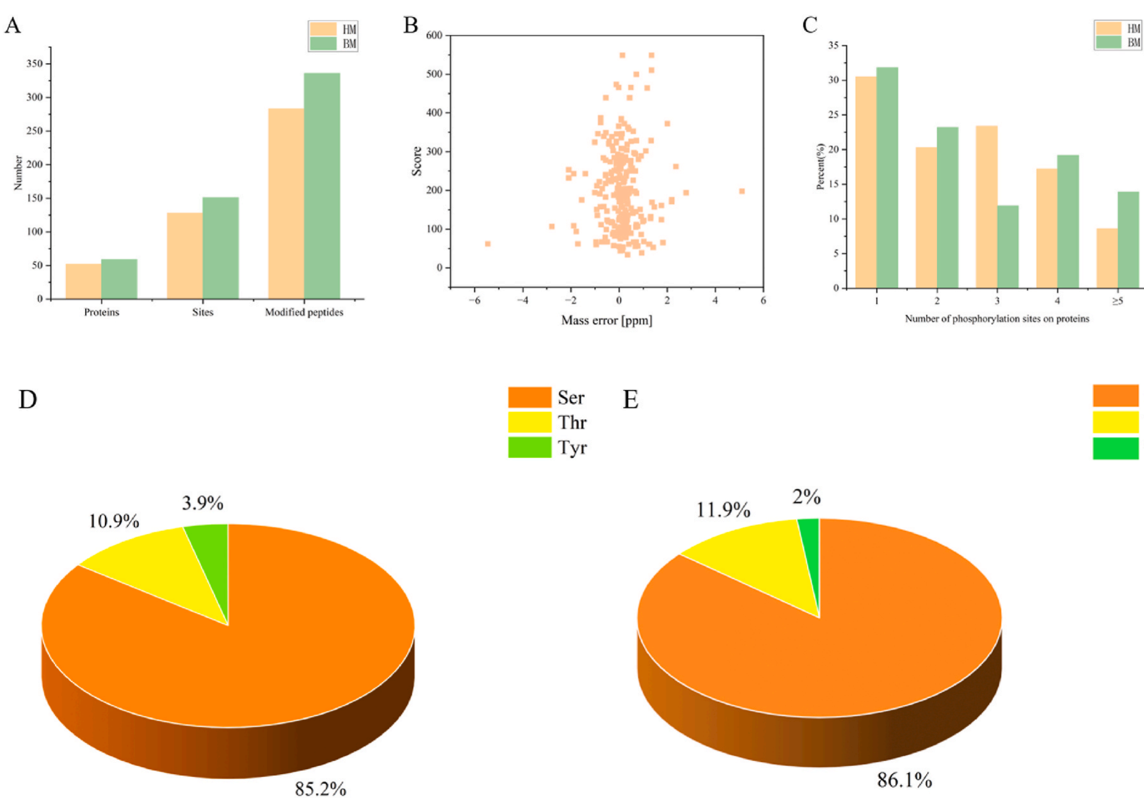

Fig. 1. Analysis of whey phosphoproteomics. (A) The number of proteins, sites and modified peptides. (B) Mass error analysis of phosphorylation-modified peptides. (C) The number of phosphorylation sites on phosphoproteins. The percentage of Ser, Thr and Tyr phosphorylation sites on whey phosphoproteins in HM (D) and BM (E).

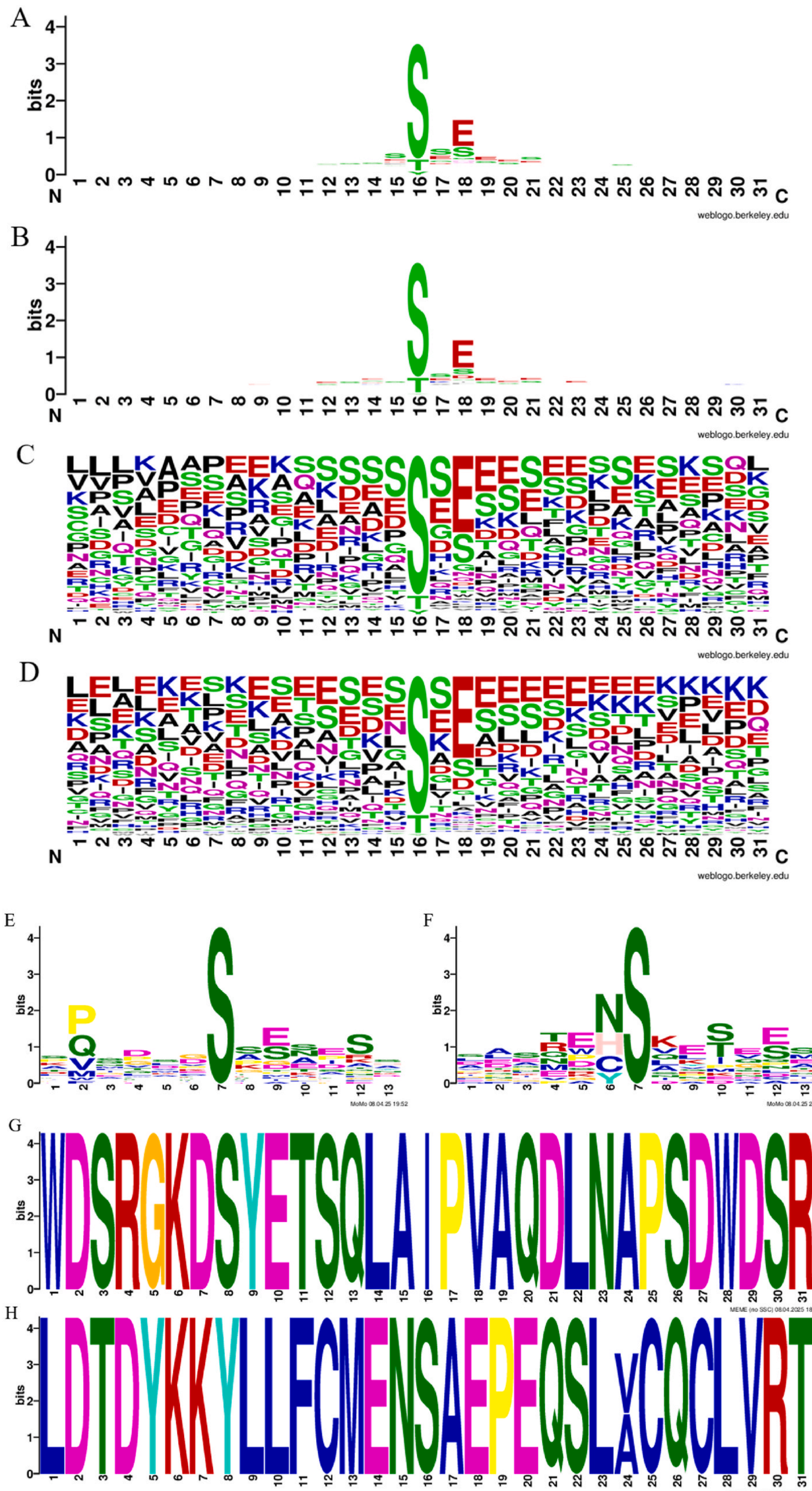

Fig. 2. Analysis of phosphorylation sites. The whey protein phosphorylation sequence motifs were found in HM (A) and BM (B) using WebLogo software. The probability of occurrence of each phosphorylation site in HM (C) and BM (D). Whey protein phosphorylation sequence motifs were identified in HM (E) and BM (F) by MoMo. Global sequence of whey phosphorylation in HM (G) and BM (H).

3.2. GO analysis of exclusive whey phosphoproteins in HM and BM

GO analysis was performed on whey Phosphoproteins, which were classified based on the DAVID online database in terms of biological processes, cellular components, and molecular functions (Fig. 3A and B). We focused on a comparative analysis of the gene functions of the exclusively phosphoproteins in HM and BM, respectively. In terms of biological processes, HM whey phosphoproteins are mainly involved in signal transduction, and BM is mainly engaged in response to dehydroepiandrosterone. In terms of cellular components, extracellular space is mainly involved in HM and extracellular region in BM. In terms of molecular function, it is mainly involved in identical protein binding in HM and calcium ion binding in BM. Chordal plots were visualized showing the number of phosphoproteins (Fig. 3C and D). There are 33 phosphorylation sites of six proteins involved in signal transduction in the HM, which are S297 on Complement C3 (P01024, C3), S96 and T95 on Tumor necrosis factor ligand superfamily member 10 (P50591, TNFSF10), S215, S219, S228, S254, S258, S263, S267, S270, S243, S275, S280, S191, S195, S24, S26, S27, S76, S62, S63, T227, T237, T185, T190 and Y225 on Osteopontin (P10451, SPP1), S80 on SPARC-like protein 1 (Q14515, SPARCL1), S332 and T327 on Kininogen-1 (P01042, KNG1), S627, S629 and S630 on Polymeric immunoglobulin receptor (P01833, PIGR). There are 35 phosphorylation sites of five proteins in the BM involved in response to 11-deoxycorticosterone and response to dehydroepiandrosterone. These phosphorylation sites are S258, S23, S24, S25, S28, S31, S71, S72, S73, S76, S46, S52, T145, T153, T18, S150 and S68 on Alpha-S2-casein (P02663,CSN1S2), S59 on kappa-casein (P02668,CSN3), S88, S89, and S95 on Alpha-lactalbumin (P00711, LALBA), S103, S56, S130, S79, S81, S82, S83, S90, S6 and Y106 on Alpha-S1-casein (P02662, CSN1S1), S30, S32, S33 and S34 on Beta-casein (P02666, CSN2). The number of phosphoproteins in HM that are mainly engaged in extracellular space and identical protein binding are 25 and 8, respectively. The number of phosphoproteins in the BM that are primarily involved in extracellular region and calcium ion binding are 15 and 7, respectively.

3.3. KEGG analysis of exclusive whey phosphoproteins in HM and BM

KEGG analysis was performed on whey phosphoproteins in HM and BM, which are Complement and coagulation cascades and Various types of N- glycan biosynthesis in HM, and Complement and coagulation cascades, Protein processing in endoplasmic reticulum and Galactose metabolism in BM (Fig. 4A and B). Significantly enriched in HM were Complement and coagulation cascades (phosphoprotein genes for C3, FGA, KNG1), and in BM were Complement and coagulation cascades (C3, FGA, CFI, KNG1) and Protein processing in endoplasmic reticulum

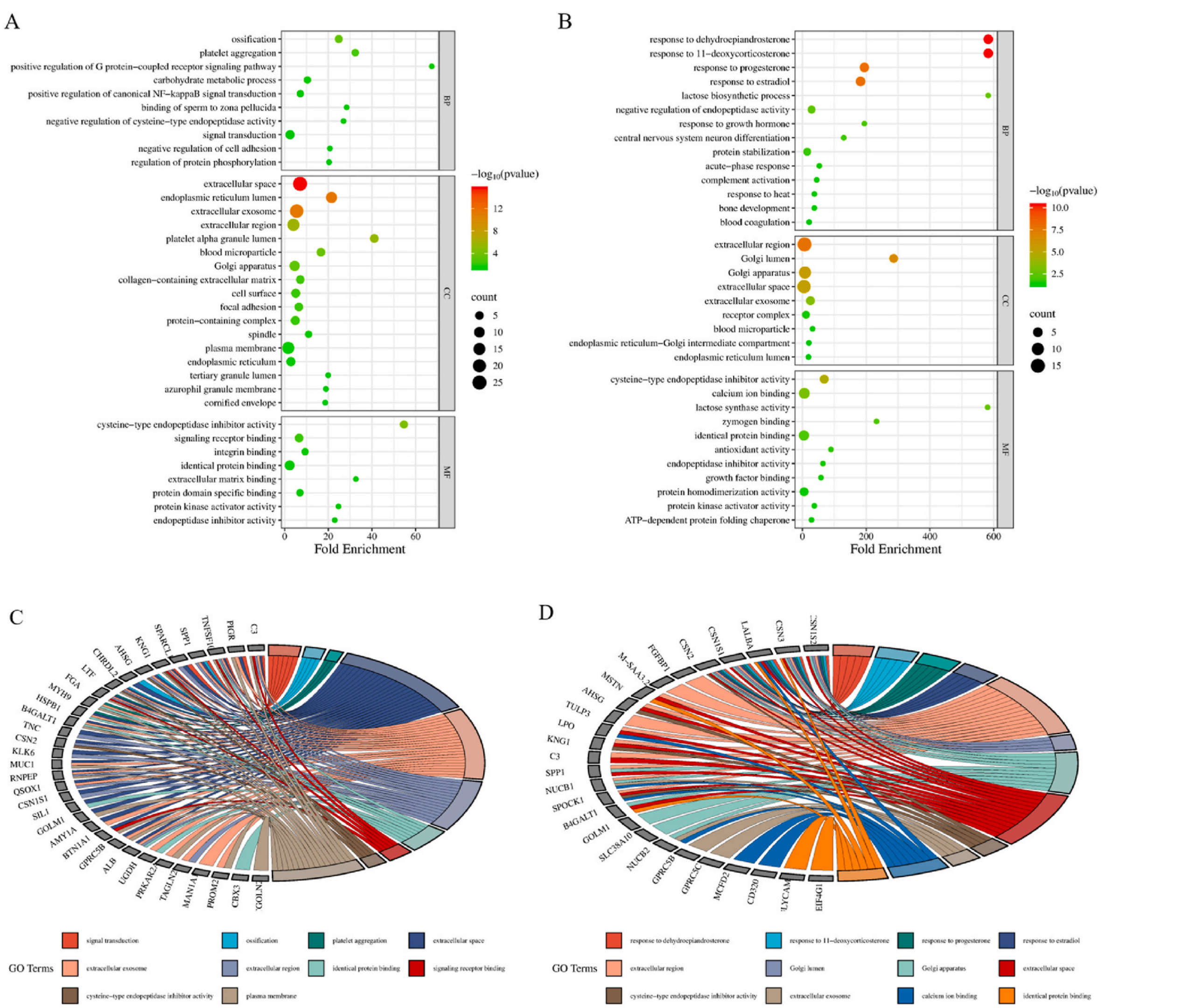

Fig. 3. GO analysis of the phosphoproteome. GO analysis of whey phosphoproteins in HM (A) and BM (B). Chord diagrams for HM (C) and BM (D) (gene names on the top, GO terms on the bottom).

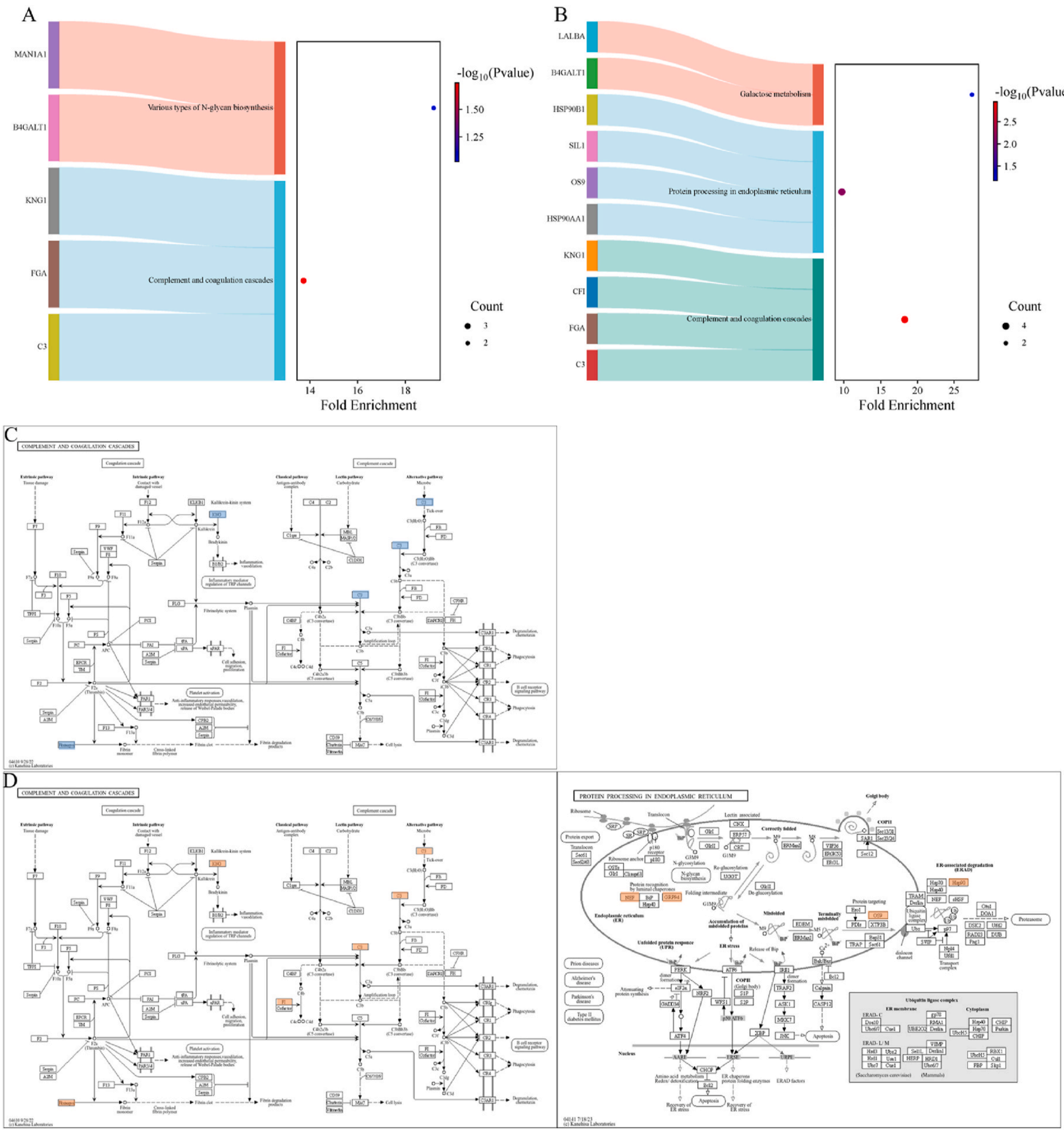

Fig. 4. KEGG analysis of the phosphoproteome. KEGG analysis of whey phosphoproteins in HM (A) and BM (B). Complement and coagulation cascades (C). Complement and coagulation cascades and Protein processing in endoplasmic reticulum (D). The blue and orange boxes in the pathway are the identified phosphoproteins.

(HSP90AA1, OS9, SIL1, HSP90B1) (Fig. 4C and D). Phosphoprotein sites in HM associated with Complement and coagulation cascades are S297 on Complement C3, S364 on Fibrinogen alpha chain, S332 on Kininogen-1 and T327. Phosphoprotein sites associated with Complement and coagulation cascades in BM are S967 and S1321 on Complement C3, S517, S518, and S578 on Fibrinogen alpha chain, S51 on Uncharacterized protein, and S331 on Kininogen-1. Meanwhile, the phosphoprotein sites related to Protein processing in endoplasmic reticulum are S263 on HSP 90-alpha, S148 and T55 on Nucleotide exchange factor SIL1, S532 on Protein OS-9, and S604 and S607 on Endoplasmin. Table S2 demonstrates the phosphoproteins and their sites, GO and KEGG.

3.4. PPI analysis of exclusive whey phosphoproteins in HM and BM

PPI network analysis of whey phosphoproteins in HM and BM was performed using the STRING database and combined with Cytoscape software, respectively (Fig. 5A and B). Fig. 5C, D shows the PPI analysis of the top ten whey phosphoproteins in HM and BM. There are 36 proteins and 183 interactions in HM, with the most strongly interacting proteins being Serum albumin (ALB) and Mucin-1 (MUC1). The two phosphoprotein sites are S82 on Serum albumin and S1229 on Mucin-1. There are 40 proteins and 226 interactions in BM, with the strongest interacting proteins being HSP 90-alpha (HSP90AA1) and Nucleobindin 2 (NUCB2). The phosphoprotein sites are S263 on HSP 90-alpha and S196 on Nucleobindin 2.

4. Discussion

We have performed phosphoproteomic analysis of whey proteins in HM and BM using label-free quantitative techniques. The phosphorylation sites were identified and analyzed from three aspects of GO, KEGG

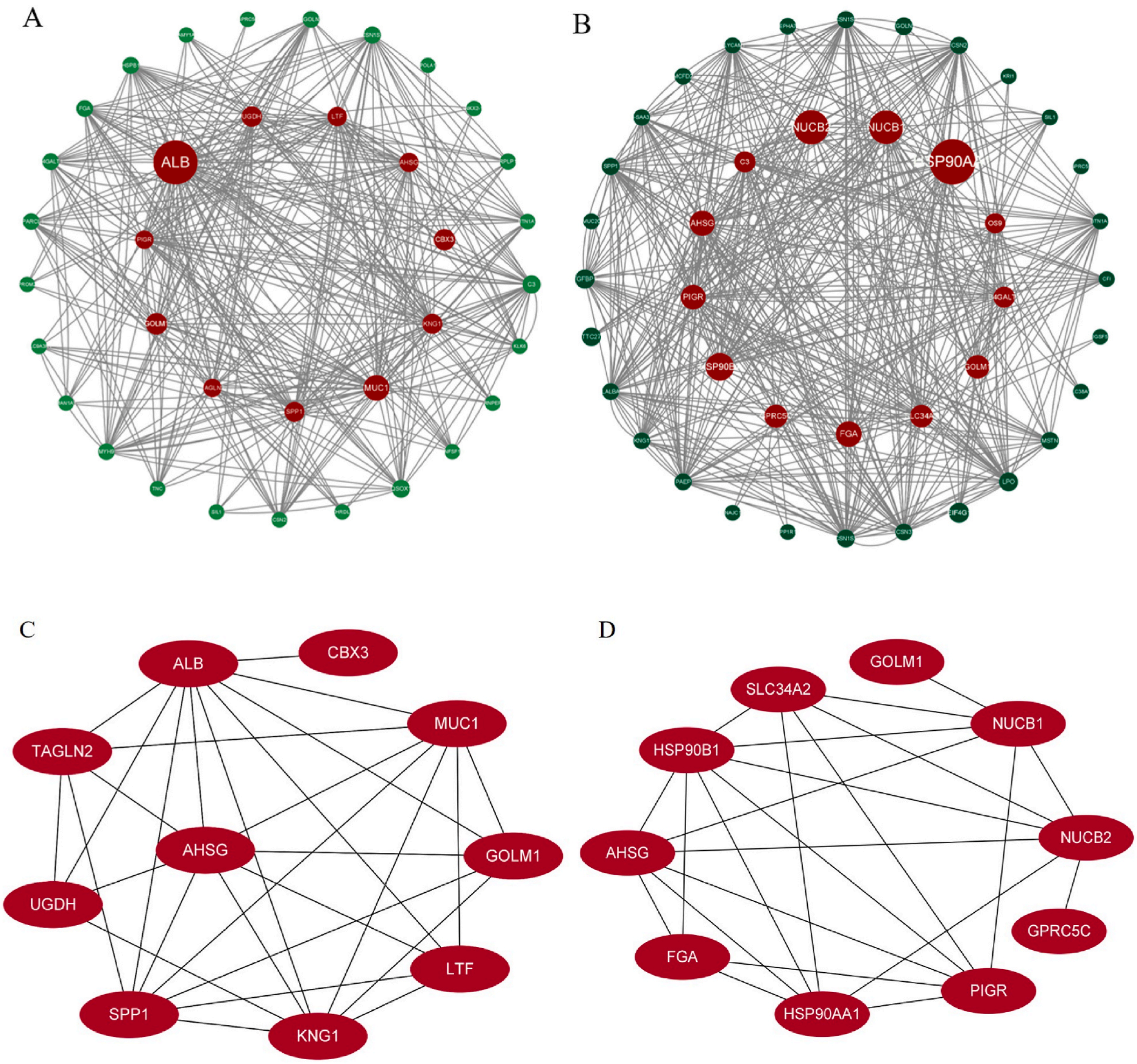

Fig. 5. PPI analysis of the phosphoproteome. PPI analysis of whey phosphoproteins in HM (A) and BM (B). The larger the red circle, the stronger the protein interaction. PPI analysis of the top ten whey phosphate proteins in HM (C) and BM (D).

and PPI by bioinformatics analysis. Phosphorylation modification is an important part of protein research and has not been reported up to now regarding exclusive whey phosphoproteins in HM and BM. Our research results are based on a strictly screened internal database. In this study, 52 whey phosphoproteins, 128 phosphorylation sites and 283 phosphorylation-modified peptides were identified in HM. There are 59 whey phosphoproteins, 151 phosphorylation sites and 336 phosphorylation-modified peptides in HM. The research results provide further insight into the role of whey proteins among different species as well as new ideas for future dairy powder design.

Osteopontin (P10451) was found to be the most abundant of the 52 phosphoproteins identified in HM in our study, with 24 phosphorylation sites. Osteopontin (P31096) was also found to be the most abundant of the 59 phosphoproteins in BM, with 23 phosphorylation sites. Osteopontin is a multifunctional phosphorylated glycoprotein that is highly phosphorylated on Ser and Thr residues (Hu et al., 2021). Osteopontin is present in different tissues, milk and urine. It is widely engaged in a variety of biological processes, such as cell adhesion, migration, immune regulation, signaling, and tissue repair (Leung et al., 2025; C. Liu, Xia, et al., 2025). It plays a role in infant immunomodulation and gut development (Demmelmair et al., 2017; McClanahan et al., 2024). Casein consists of four types, beta-casein, gamma-casein, alpha-S1-casein, and alpha-S2-casein, of which alpha-S2-casein is the most phosphorylated of the casein molecules, and can be detected in bovine milk but not in human milk (Hassanin et al., 2022). Alpha-S2-casein is highly hydrophilic, which helps to stabilize it in the body. It has many phosphorylation sites and can bind calcium ions to promote bone health (Mohsin et al., 2020; Treweek et al., 2011). However, alpha-S2-casein is an allergen, which binds to IgE antibodies causing the body to become sensitized (Z. Liu et al., 2024). Since alpha-S2-casein is a highly phosphorylated protein, it can be detected. In our study, alpha-S2-casein has been detected with 17 phosphorylation sites.

ALB (Serum albumin) is a typical whey protein detected in human milk, and it has a site at S82. LALBA (Alpha-lactalbumin) and LGB (Beta-lactoglobulin) are typical whey proteins detected in bovine milk. The three phosphorylation sites of LALBA are S89, S88 and S95 respectively, and one phosphorylation site of LGB is S126. ALB belongs to the low molecular weight proteins together with other whey protein components (e.g. LALBA, LGB) to constitute the high nutritional value of whey protein, providing essential amino acids. LALBA is a calcium-binding protein that aids in the absorption of minerals in infants. It functionally focuses on infant neurodevelopment and sleep enhancement (Layman et al., 2018; X. Li et al., 2025). BLG contains all the essential amino acids that the human body needs, especially branched-chain amino acids, which support the growth and development of infants.

However, BLG is one of the major bovine milk allergens that may trigger milk protein allergy in infants (Surucu & Abaci, 2020).

Our motif analysis of the modified peptides showed that all enriched motifs conformed to the SxE pattern, with serine (S) being highly conserved. This pattern is consistent with the classical substrate motifs of protein kinase CK2 (PhosphoSitePlus database), suggesting that CK2 may mediate phosphorylation at these sites. In the future, the phosphorylation function of SxE and its contribution to related signaling pathways need to be verified through kinase activity experiments and site mutations.

GO analysis of whey phosphoproteins was performed to compare the functions of unique phosphoproteins in different species. Signal transduction is the core mechanism of cellular perception of the environment and regulation of life activities, which can transmit external signals, coordinate multicellular functions, regulate development, and participate in diseases (B. S. Chen & Wu, 2012; Morimoto, 2024; Uda & Kuroda, 2016). Dehydroepiandrosterone is a steroid hormone secreted by the adrenal glands and is one of the most abundant steroid hormones in the human body (Miyazaki et al., 2016). It has important roles in anti-aging, anti-tumor, metabolism, regulation of endocrine and immune systems (Krysiak et al., 2021; Wang et al., 2024). In our study, in terms of biological processes, HM is mainly involved in signal transduction, with 33 phosphorylation sites of six phosphoproteins (P01024, P50591, P10451, Q14515, P01042, P01833) in this function annotated. BM is mainly involved in Dehydroepiandrosterone, with 35 phosphorylation sites of five phosphoproteins (P02663, P02668, P00711, P02662, P02666) annotated in this function. The extracellular space is the dynamic microenvironment outside the cell and consists of the extracellular matrix and intercellular fluid. The extracellular matrix is a multifunctional dynamic platform that, at the physical level, maintains structure and transmits substances; at the informational level, stores signals and regulates cells; and at the pathological level, is involved in cancer, fibrosis, and neurodegenerative diseases (Ge et al., 2024; M. Li et al., 2020; Sant et al., 2020; Zamecnik et al., 2004). The extracellular region is the space outside the cell membrane and contains components such as extracellular space, secreted proteins and signaling molecules. Calcium ions are key signaling molecules and structural regulators inside and outside the cell. Calcium ion binding is the binding of calcium ions to specific proteins involved in cell signaling, regulation of enzyme activity, structural stabilization, and pathological processes (Chang et al., 2025; Grzybowska, 2018; Jing et al., 2018). In terms of cellular components, HM is mainly involved in the extracellular space, with 25 phosphoproteins annotated in this function, and BM is mainly in the extracellular region, with 8 phosphoproteins in this function. In terms of molecular function, HM is mainly involved in identical protein binding, with 15 phosphoproteins annotated in this function, and BM is mainly engaged in calcium ion binding, with 7 phosphoproteins for this function. By GO analysis, we can further understand the function of whey phosphoproteins that are exclusive in human and bovine milk.

Our KEGG analysis of whey phosphoproteins revealed that Complement and coagulation cascades were dramatically enriched in HM. Remarkably enriched in BM were Complement and coagulation cascades and Protein processing in endoplasmic reticulum. Complement and coagulation act synergistically in infection and injury, but aberrant activation may lead to thrombosis, inflammation, or autoimmune disease. The Complement system is activated via the classical, lectin and bypass pathways and has a role in immune defense and clearance of pathogens. In addition, it produces allergenic toxins (C3a and C5a) that promote an inflammatory response. Coagulation cascades are beneficial in hemostasis and vascular repair, relying on the coagulation-anticoagulation dynamic balance (Gultom & Rieben, 2024; Strohbach & Busch, 2021). Protein processing in endoplasmic reticulum is a core aspect of cellular life activities, where every step from synthesis, folding, modification to quality control is precisely regulated to ensure the correct function and localization of proteins. Abnormalities in its functioning can lead to neurodegenerative diseases, metabolic disorders and cancer (Beriault & Werstuck, 2013; J. S. Wu et al., 2016). C3(Complement C3) may provide immunoprotection by stabilizing C3 convertase and enhancing complement-mediated pathogen clearance (Sahu & Lambris, 2001). FGA (Fibrinogen alpha chain) is a key component of blood clots. After vascular injury, it is hydrolyzed by thrombin protein and converted into fibrin to participate in the hemostasis process (Asselta et al., 2007). KNG1(Kininogen-1) is closely related to coagulation, inflammation and immune regulation (B. Zhang et al., 2023). CFI (Complement factor I) may negatively feedback inhibit complement activation and prevent excessive inflammatory damage (Hallam et al., 2023). In our study, HM has four phosphorylation sites of three proteins involved in the Complement and coagulation cascades, which are S297 on Complement C3, S364 on Fibrinogen alpha chain, S332 and T327 on Kininogen-1. There are seven phosphorylation sites of four proteins in BM involved in the Complement and coagulation cascades, these are S967 and S1321 on Complement C3, S517, S518 and S578 on Fibrinogen alpha chain, S51 on Uncharacterized protein, and S331 on Kininogen-1. There are six phosphorylation sites of four proteins involved in Protein processing in endoplasmic reticulum, these are S263 on HSP 90-alpha, S532 on Protein OS-9, S148 and T55 on Nucleotide exchange factor SIL1, S604 and S607 on Endoplasmin. In summary, both human and bovine milk whey phosphoproteins showed roles in Complement and coagulation cascades, and more can be learned about the effects of changes in sites on them in the future.

PPI analysis of human and bovine milk whey phosphoproteins to provide clues for functional studies of the proteins. Serum albumin (ALB) is the most plentiful in mammalian plasma and is synthesized primarily by the liver. It safeguards collective homeostasis through mechanisms such as regulation of osmotic pressure, transport of substances, and antioxidants. It is also a marker for disease diagnosis (Jahanban-Esfahlan & Amarowicz, 2025; Malik et al., 2024). Mucin-1 (MUC1) is a transmembrane glycoprotein and belongs to the mucin family. It plays a key role in a number of physiological and pathological processes, maintaining the epithelial barrier and immune homeostasis in physiological states, but is a key factor in promoting malignant progression under pathological conditions (especially cancer) (Cao et al., 2017; Yang et al., 2023). HSP 90-alpha (HSP90AA1) is a central regulator of the cellular stress response, which maintains protein homeostasis and has also been implicated in cancer development and immune regulation (Hazra et al., 2023; J. X. Wu et al., 2020; T. T. Zhang et al., 2021). Nucleobindin 2 (NUCB2) has roles in appetite regulation and energy metabolism (Zhou et al., 2022). In our results, sites S82 on Serum albumin and S1229 on Mucin-1 were found in HM. S263 on HSP 90-alpha and S196 on Nucleobindin 2 were found in BM. Further studies can be conducted in the future for changes in the sites on these phosphoproteins that may affect their function.

The sites of exclusive phosphoproteins in HM and BM identified in this study, especially the modification sites on immune-related proteins (e.g., Complement C3, Kininogen-1, etc.), may play a key role in immune-protective functions. The results of this study have significant implications for the development of infant formula milk powder. At present, formula milk powder mainly focuses on the simulation of protein composition and content, while ignoring the differences in post-translational modifications such as phosphorylation. Future formula design should consider introducing key whey phosphoproteins or mimicking their function to better replicate the immunoprotective properties of human milk. Meanwhile, the specific phosphorylation profile of bovine milk whey proteins provides a molecular basis for the development of immunomodulatory functional dairy products for adults. The limitation of this study is that functional validation of these phosphorylation modifications needs to be performed *in ex vivo* experiments. It is suggested that future research should: (1) carry out *in vitro* functional experiments on whey phosphoproteins; (2) conduct animal model studies to verify their physiological functions; and (3) explore the effects of industrial processing on the phosphorylation status of whey proteins to provide a basis for optimization of dairy product processing.

5. Conclusion

In this study, label-free quantitative techniques were used for phosphoproteomic analysis of whey proteins in HM and BM. 52 whey phosphoproteins and 128 phosphorylation sites were identified in HM. 151 phosphorylation sites on 59 whey phosphoproteins were identified in BM. The analysis of phosphoproteins from GO, KEGG and PPI showed that in HM they mainly play a synergistic role in immune defense, coagulation regulation and transmembrane signaling, and in BM they are mainly associated with immune regulation. These findings help us to further understand the functional differences between whey phosphoproteins in HM and BM. This study provides valuable information on the processing of functional dairy products and the greater "humanization" of infant formula. The functions of these phosphoproteins can be further verified by *in vivo* and *in vitro* experiments in the future.

CRediT authorship contribution statement

Hong Yu: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Chunshuang Wu:** Visualization, Methodology. **Xinping Chen:** Software, Investigation. **Xue Bai:** Methodology, Investigation. **Zhichi Zhang:** Software, Investigation. **Mei Yang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Yanyu Peng:** Writing – review & editing, Supervision, Project administration.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.107307.

Data availability

Data will be made available on request.

References

Andres, S. F., Scottoline, B., & Good, M. (2023). Shaping infant development from the inside out: Bioactive factors in human milk. *Seminars in Perinatology, 47*(1). https://doi.org/10.1016/j.semperi.2022.151690

Asselta, R., Spena, S., Duga, S., & Tenchini, M. L. (2007). Molecular genetics of quantitative fibrinogen disorders. *Cardiovascular and Hematological Agents in Medicinal Chemistry, 5*(2), 163–173. https://doi.org/10.2174/187152507780363205

Beriault, D. R., & Werstuck, G. H. (2013). Detection and quantification of endoplasmic reticulum stress in living cells using the fluorescent compound, thioflavin T. *Biochimica Et Biophysica Acta-Molecular Cell Research, 1833*(10), 2293–2301. https://doi.org/10.1016/j.bbamcr.2013.05.020

Cao, H. M., Fang, X. E., Li, H. P., Li, H., & Kong, J. L. (2017). Ultrasensitive detection of mucin 1 biomarker by immuno-loop-mediated isothermal amplification. *Talanta, 164*, 588–592. https://doi.org/10.1016/j.talanta.2016.07.018

Chang, Y. Y., Liu, Y. C., Jhang, W. E., Wei, S. S., & Ou, Y. Y. (2025). CaBind_MCNN: Identifying potential calcium channel blocker targets by predicting calcium-binding sites in ion channels and ion transporters using protein language models and multiscale feature extraction. *Journal of Chemical Information and Modeling, 65*(4), 2145–2157. https://doi.org/10.1021/acs.jcim.4c02252

Chen, X. B., Lin, Y. X., Jin, X., Zhang, W. Z., Guo, W. H., Chen, L. L., … Wang, C. (2022). Integrative proteomic and phosphoproteomic profiling of invasive micropapillary breast carcinoma. *Journal of Proteomics, 257*. https://doi.org/10.1016/j.jprot.2022.104511

Chen, B. S., & Wu, C. C. (2012). On the calculation of signal transduction ability of signaling transduction pathways in intracellular communication: Systematic approach. *Bioinformatics, 28*(12), 1604–1611. https://doi.org/10.1093/bioinformatics/bts159

Demmelmair, H., Prell, C., Timby, N., & Lönnerdal, B. (2017). Benefits of lactoferrin, osteopontin and milk fat globule membranes for infants. *Nutrients, 9*(8). https://doi.org/10.3390/nu9080817

Duan, H. Z., Nie, Z. K., Li, Y., & Chen, Y. X. (2020). Unremitting progresses for phosphoprotein synthesis. *Current Opinion in Chemical Biology, 58*, 96–111. https://doi.org/10.1016/j.cbpa.2020.07.009

Dullius, A., Goettert, M. I., & de Souza, C. F. V. (2018). Whey protein hydrolysates as a source of bioactive peptides for functional foods - Biotechnological facilitation of industrial scale-up. *Journal of Functional Foods, 42*, 58–74. https://doi.org/10.1016/j.jff.2017.12.063

Dutta, H., & Jain, N. (2023). Post-translational modifications and their implications in cancer. *Frontiers in Oncology, 13*. https://doi.org/10.3389/fonc.2023.1240115

Ge, X. Q., Xu, X. Q., Cai, Q., Xiong, H. J., Xie, C., Hong, Y., … Qin, Z. P. (2024). Live mapping of the brain extracellular matrix and remodeling in neurological disorders. *Small Methods, 8*(1). https://doi.org/10.1002/smtd.202301117

Grzybowska, E. A. (2018). Calcium-binding proteins with disordered structure and their role in secretion, storage, and cellular signaling. *Biomolecules, 8*(2). https://doi.org/10.3390/biom8020042

Gultom, M., & Rieben, R. (2024). Complement, coagulation, and fibrinolysis: The role of the endothelium and its glycocalyx layer in xenotransplantation. *Transplant International, 37*. https://doi.org/10.3389/ti.2024.13473

Hallam, T. M., Sharp, S. J., Andreadi, A., & Kavanagh, D. (2023). Complement factor I: Regulatory nexus, driver of immunopathology, and therapeutic. *Immunobiology, 228*(5). https://doi.org/10.1016/j.imbio.2023.152410

Hassanin, A. A., Osman, A., Atallah, O. O., El-Saadony, M. T., Abdelnour, S. A., Taha, H. S. A., … Eldomiaty, A. S. (2022). Phylogenetic comparative analysis: Chemical and biological features of caseins (alpha-S-1, alpha-S-2, beta- and kappa-) in domestic dairy animals. *Frontiers in Veterinary Science, 9*. https://doi.org/10.3389/fvets.2022.952319

Hazra, J., Vijayakumar, A., & Mahapatra, N. R. (2023). Emerging role of heat shock proteins in cardiovascular diseases. *Advances in protein chemistry and structural biology, 134*, 271–306. https://doi.org/10.1016/bs.apcsb.2022.10.008

Hu, B., Zhang, J. S., Jiang, Y. R., Tong, W. F., Lai, S. Y., & Ren, Y. P. (2021). Quantitative determination of osteopontin in bovine, buffalo, yak, sheep and goat milk by ultra-high performance liquid chromatography-tandem mass spectrometry and stable isotope dimethyl labeling. *Food Chemistry, 343*. https://doi.org/10.1016/j.foodchem.2020.128489

Jahanban-Esfahlan, A., & Amarowicz, R. (2025). Optical sensing of albumin in human serum and urine-A historical review of the transition from classical dye-binding assays to advanced technologies. *International Journal of Biological Macromolecules, 287*. https://doi.org/10.1016/j.ijbiomac.2024.138593

Javitt, A., & Merbl, Y. (2023). Capturing the diversity of protein modifications on presented tumor antigens. *Nature Biotechnology, 41*(2), 195–196. https://doi.org/10.1038/s41587-022-01465-1

Jing, Z. F., Liu, C. W., Qi, R., & Ren, P. Y. (2018). Many-body effect determines the selectivity for Ca^{2+} and Mg^{2+} in proteins. *Proceedings of the National Academy of Sciences of the United States of America, 115*(32), E7495–E7501. https://doi.org/10.1073/pnas.1805049115

Krysiak, R., Szkróbka, W., & Okopien, B. (2021). Impact of dehydroepiandrosterone on thyroid autoimmunity and function in men with autoimmune hypothyroidism. *International Journal of Clinical Pharmacy, 43*(4), 998–1005. https://doi.org/10.1007/s11096-020-01207-w

Layman, D. K., Lönnerdal, B., & Fernstrom, J. D. (2018). Applications for α-lactalbumin in human nutrition. *Nutrition Reviews, 76*(6), 444–460. https://doi.org/10.1093/nutrit/nuy004

Leung, J., Qu, L., Ye, Q. F., & Zhong, Z. B. (2025). The immune duality of osteopontin and its therapeutic implications for kidney transplantation. *Frontiers in Immunology, 16*. https://doi.org/10.3389/fimmu.2025.1520777

Li, M., Zhang, A. Q., Li, J. J., Zhou, J., Zheng, Y. N., Zhang, C., … Zhao, J. Y. (2020). Osteoblast/fibroblast coculture derived bioactive ECM with unique matrisome profile facilitates bone regeneration. *Bioactive Materials, 5*(4), 938–948. https://doi.org/10.1016/j.bioactmat.2020.06.017

Li, X., Zhang, X. M., Zhang, M. H., Liu, X. J., Gao, Z. Y., Zhao, J. Y., … Chen, L. J. (2025). Human milk whey proteins: Constituents, influencing factors, detection methods, and comparative analysis with other sources. *Food Chemistry, X*, 25. https://doi.org/10.1016/j.fochx.2024.102082

Li, J. C., & Zhu, F. (2024). Whey protein hydrolysates and infant formulas: Effects on physicochemical and biological properties. *Comprehensive Reviews in Food Science and Food Safety, 23*(3). https://doi.org/10.1111/1541-4337.13337

Liu, X. Y., Bai, X., Li, B. F., Qiu, J. H., Sun, J. L., & Yang, M. (2025). Comparison and analysis for exclusively expressed sites of milk fat globule membrane phosphoproteins between human and bovine milk based on label-free phosphoproteomic. *Journal of Food Composition and Analysis, 141*. https://doi.org/10.1016/j.jfca.2025.107328

Liu, Z., Gao, X. C., Ruan, H., Xu, X. Y., Zhu, B. Q., Dang, Y. L., & Hua, Y. (2024). Determination of αs1-Casein and αs2-Casein in hypoallergenic infant formula by extended specific peptide -isotope-labeled internal standard dilution-LC-MS/MS. *Food Bioscience, 62*. https://doi.org/10.1016/j.fbio.2024.105197

Liu, C., Xia, S. J., Wang, B., Li, J. Y., Wang, X. Y., Ren, Y., & Zhou, X. (2025). Osteopontin promotes tumor microenvironment remodeling and therapy resistance. *Cancer Letters, 617*. https://doi.org/10.1016/j.canlet.2025.217618

Lönnerdal, B. (2016). Bioactive proteins in human milk: Health, nutrition, and implications for infant formulas. *The Journal of Pediatrics, 173*, S4–S9. https://doi.org/10.1016/j.jpeds.2016.02.070

Malik, N. A., Nazir, N., Manzoor, M., & Gull, F. (2024). Fungicide-albumin interactions: Unraveling the complex relationship-a comprehensive review. *Biophysical reviews, 16*(4), 417–439. https://doi.org/10.1007/s12551-024-01190-w

McClanahan, K. G., Reese, J., Weitkamp, J. H., & Olivares-Villagómez, D. (2024). Effects of pasteurization on osteopontin concentrations in human breastmilk. *Pediatric Research, 95*(3), 641–646. https://doi.org/10.1038/s41390-023-02838-1

Miyazaki, H., Takitani, K., Koh, M., Inoue, A., & Tamai, H. (2016). Dehydroepiandrosterone alters vitamin E status and prevents lipid peroxidation in vitamin E-deficient rats. *Journal of Clinical Biochemistry & Nutrition, 58*(3), 223–231. https://doi.org/10.3164/jcbn.15-133

Mohsin, A. Z., Sukor, R., Selamat, J., Hussin, A. S. M., Ismail, I. H., Jambari, N. N., & Jonet, A. (2020). A highly selective two-way purification method using liquid chromatography for isolating α_{S2}-casein from goat milk of five different breeds. *Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences, 1160*. https://doi.org/10.1016/j.jchromb.2020.122380

Moreno-Navarrete, J. M., Ortega, F. J., Ricart, W., & Fernandez-Real, J. M. (2009). Lactoferrin increases ^{172Thr}AMPK phosphorylation and insulin-induced ^{p473Ser}AKT while impairing adipocyte differentiation. *International Journal of Obesity, 33*(9), 991–1000. https://doi.org/10.1038/ijo.2009.143

Morimoto, Y. V. (2024). Ion signaling in cell motility and development in Dictyostelium discoideum. *Biomolecules, 14*(7). https://doi.org/10.3390/biom14070830

Muneer, G., Chen, C. S., & Chen, Y. J. (2025). Advancements in global phosphoproteomics profiling: Overcoming challenges in sensitivity and quantification. *Proteomics, 25*(1–2). https://doi.org/10.1002/pmic.202400087

Olvera-Rosales, L. B., Cruz-Guerrero, A. E., García-Garibay, J. M., Gómez-Ruíz, L. C., Contreras-López, E., Guzmán-Rodríguez, F., & González-Olivares, L. G. (2023). Bioactive peptides of whey: Obtaining, activity, mechanism of action, and further applications. *Critical Reviews in Food Science and Nutrition, 63*(30), 10351–10381. https://doi.org/10.1080/10408398.2022.2079113

Rout, P. K., & Verma, M. (2021). Post translational modifications of milk proteins in geographically diverse goat breeds. *Scientific Reports, 11*(1). https://doi.org/10.1038/s41598-021-85094-9

Sahu, A., & Lambris, J. D. (2001). Structure and biology of complement protein C3, a connecting link between innate and acquired immunity. *Immunological Reviews, 180*, 35–48. https://doi.org/10.1034/j.1600-065X.2001.1800103.x

Sant, S., Wang, D., Agarwal, R., Dillender, S., & Ferrell, N. (2020). Glycation alters the mechanical behavior of kidney extracellular matrix. *Matrix Biology, 8*, Article 100035. https://doi.org/10.1016/j.mbplus.2020.100035

Strohbach, A., & Busch, R. (2021). Predicting the in vivo performance of cardiovascular biomaterials: Current approaches in vitro evaluation of blood-biomaterial interactions. *International Journal of Molecular Sciences, 22*(21). https://doi.org/10.3390/ijms222111390

Surucu, O., & Abaci, S. (2020). Electrochemical determination of β-lactoglobulin in whey proteins. *Journal of Food Measurement and Characterization, 14*(1), 11–19. https://doi.org/10.1007/s11694-019-00262-w

Treweek, T. M., Thorn, D. C., Price, W. E., & Carver, J. A. (2011). The chaperone action of bovine milk α_{S1}- and α_{S2}-caseins and their associated form α_S-casein. *Archives of Biochemistry and Biophysics, 510*(1), 42–52. https://doi.org/10.1016/j.abb.2011.03.012

Tsakali, E., Aggarwal, R., Houhoula, D., Konteles, S., Batrinou, A., Verheyen, D., … Chatzilazarou, A. (2023). Lactoferrin in breast milk-based powders. *Journal of Dairy Research, 90*(4), 409–412. https://doi.org/10.1017/s0022029923000778

Uda, S., & Kuroda, S. (2016). Analysis of cellular signal transduction from an information theoretic approach. *Seminars in Cell & Developmental Biology, 51*, 24–31. https://doi.org/10.1016/j.semcdb.2015.12.011

Wang, Y. F., Li, R. L., Yuan, R. S., Wang, L., Qiao, Q., Han, Z. Y., … Guo, C. B. (2024). Dehydroepiandrosterone attenuated the immune escape of oral squamous cell carcinoma through NF-KB p65/miR-15b-5p/B7-H4 axis. *International Immunopharmacology, 137*. https://doi.org/10.1016/j.intimp.2024.112480

Wang, Y. F., Zhou, X. H., Gong, P. M., Chen, Y. J., Feng, Z., Liu, P., … Song, L. (2020). Comparative major oligosaccharides and lactose between Chinese human and animal milk. *International Dairy Journal, 108*. https://doi.org/10.1016/j.idairyj.2020.104727

Wu, J. X., Chen, S. X., Liu, Y. T., Liu, Z. Q., Wang, D. W., & Cheng, Y. Y. (2020). Therapeutic perspectives of heat shock proteins and their protein -protein interactions in myocardial infarction. *Pharmacological Research, 160*. https://doi.org/10.1016/j.phrs.2020.105162

Wu, J. S., Li, W. M., Chen, Y. N., Zhao, Q., & Chen, Q. F. (2016). Endoplasmic reticulum stress is activated in acute pancreatitis. *Journal of Digestive Diseases, 17*(5), 295–303. https://doi.org/10.1111/1751-2980.12347

Yang, F., Li, Z., Shao, J. B., Gao, W., & Sun, Z. G. (2023). Mucin 1 expression correlation with lymph node metastasis and micrometastasis and poor prognosis in patients with esophageal squamous cell carcinomas. *Archives of Medical Science, 19*(3), 638–644. https://doi.org/10.5114/aoms.2019.89219

Yu, H., Shang, J. W., Wu, C. S., Bai, X., Chen, X. P., & Yang, M. (2025). Sequencing analysis of bovine milk exosomal miRNA and the effect of Bta-miR-141 on the biological function of HepG2 cells. *Food Bioscience, 64*. https://doi.org/10.1016/j.fbio.2025.105993

Zamecnik, J., Vargova, L., Homola, A., Kodet, R., & Sykova, E. (2004). Extracellular matrix glycoproteins and diffusion barriers in human astrocytic tumours. *Neuropathology and Applied Neurobiology, 30*(4), 338–350. https://doi.org/10.1046/j.0305-1846.2003.00541.x

Zhang, Y. M., Guo, Y. Z., Jiang, C. X., Xie, A. J., Yue, X. Q., & Li, M. H. (2024). A review of casein phosphopeptides: From enrichment identification to biological properties. *Food Bioscience, 59*. https://doi.org/10.1016/j.fbio.2024.104217

Zhang, B., Han, H. J., Zhao, X. Y., Li, A. N., Wang, Y., Yuan, W. J., … Li, M. D. (2023). An HBV susceptibility variant of KNG1 modulates the therapeutic effects of interferons α and 11 in HBV infection by promoting MAVS lysosomal degradation. *EBioMedicine, 94*. https://doi.org/10.1016/j.ebiom.2023.104694

Zhang, T. T., Li, Q. Q., Zhang, Y. Y., Wang, Q. L., Wang, H., & Gu, K. S. (2021). Diagnostic and prognostic value of heat shock protein 90α in malignant melanoma. *Melanoma Research, 31*(2), 152–161. https://doi.org/10.1097/cmr.0000000000000716

Zhou, Q., Liu, Y., Feng, R. R., & Zhang, W. L. (2022). NUCB2: Roles in physiology and pathology. *Journal of Physiology & Biochemistry, 78*(3), 603–617. https://doi.org/10.1007/s13105-022-00895-4

Zhu, F. (2024). Modifications of whey proteins for emulsion based applications: Current status, issues and prospectives. *Food Research International, 178*. https://doi.org/10.1016/j.foodres.2024.113935

Zhu, J., & Dingess, K. A. (2019). The functional power of the human milk proteome. *Nutrients, 11*(8). https://doi.org/10.3390/nu11081834