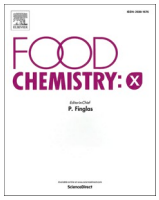
Food Chemistry: X 21 (2024) 101112

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Label-free-based proteomic analysis reveals differential whey proteins of porcine milk during lactation

Huiwen Zhao [a], Shanshan Zhao [a], Qing Zhu [a], Jiali Chen [a], Zhizhong Quan [b], Xiqing Yue [a], Xueyan Cao [a,*]

[a] College of Food Science, Shenyang Agricultural University, Shenyang 110866, China
[b] Liaoning Complete Biotechnology Co., Ltd., Tieling 112600, China

ARTICLE INFO

Keywords:
Porcine milk
Proteomics
Whey proteins
Lactation

ABSTRACT

In this study, label-free proteomic technology was applied to analyze and compare the whey proteomes of porcine colostrum and mature milk. In total, 2993 and 2906 whey proteins were detected in porcine colostrum and mature milk, respectively. A total of 2745 common proteins were identified in the two milk samples, and 280 proteins were found to be significantly differentially expressed whey proteins in porcine milk. Gene Ontology analysis demonstrated that the differentially expressed whey proteins were primarily enriched in lipid homeostasis, oxidoreductase activity, and the collagen trimer. Kyoto Encyclopedia of Genes and Genomes analysis suggested that the phagosome and endocytosis were the crucial pathways. This study provides systematic and in-depth insight into the compositions and functional properties of whey proteins in porcine milk during different periods of lactation, which may be beneficial for the development of porcine whey proteins in the future.

Introduction

As the only nutritional source for infants and newborn mammals, milk contains many valuable nutrients and immunomodulatory factors that can promote their growth and development. Milk is also known as the gold standard for meeting the nutritional needs of newborns. Many functional proteins, including immune globulin, lactoferrin, lactalbumin and lactadherin, are present in milk and can help newborns build up their immune systems and activate gut functions (Eriksen et al., 2018). Bovine milk is widely popular worldwide. In certain areas, people utilize the milk of donkeys, goats, camels, and horses. Various factors (i.e., species, altitudes, physiological conditions, lactation stages and seasons) can affect milk composition (Li et al., 2021; Maity and Ambatipudi, 2019; Zhang et al., 2020; Zhao et al., 2023). However, studies focusing on porcine milk are very limited. Most tissues and organs of pigs are very similar to those of humans in terms of structure and physiology, including the brain, heart, kidney, liver, and lung. Pigs are considered ideal organ donors and important biomedical models (Cooper et al., 2016; Sjostedt et al., 2020). Furthermore, porcine milk contains abundant essential components, such as protein, fat, vitamins, lactose and minerals. Exosomes, oligosaccharides, and bacteria, as well as other components of porcine milk, can regulate the intestinal environment and immune status of piglets (Zhang et al., 2018). Porcine milk has more protein and fat than both human milk and cow milk (Tan et al., 2018). In addition, monogastric animal milk is preferred to ruminant milk for human nutrition in terms of its physicochemical properties. (Trinchese et al., 2015).

Proteins are crucial components of milk and have been the focus of many studies. Whey proteins are major milk proteins that are primarily composed of β-lactoglobulin, α-lactalbumin (LALBA), lactoperoxidase (LPO), lactoferrin and immunoglobulins (Madureira et al., 2007). Several studies have demonstrated that whey proteins have various biological characteristics (i.e., anti-inflammatory, antioxidant and antiviral) (Kerasioti et al., 2014; Veskoukis et al., 2020). Furthermore, whey proteins are widely used as nutritional additives in dairy products such as cheese, sausage, and infant formula. Recent studies have widely investigated the whey proteome of many animal milks. Approximately 600 whey proteins were found in human and bovine milk during lactation (Yang et al., 2017). Whey proteins in donkey milk and bovine milk were studied by LC-MS/MS-based proteomics (Li et al., 2022; Li et al., 2023). Hundreds of goat whey proteins were detected via a data-independent acquisition (DIA) technique (Zhao et al., 2021; Wan et al., 2021). However, there is little research on the proteomic analysis of porcine whey proteins during lactation.

* Corresponding author.
E-mail address: 526287162@qq.com (X. Cao).

https://doi.org/10.1016/j.fochx.2023.101112
Received 14 August 2023; Received in revised form 19 December 2023; Accepted 30 December 2023

Thus, label-free quantitative proteomics was used in this study to identify and compare porcine whey proteins across lactation periods. Bioinformatics analyses were applied to describe the categories of biological functions and related pathways of the porcine whey proteins. In the end, we verified the content of interesting whey proteins in porcine milk using the parallel reaction monitoring (PRM) method. We hope that these findings will effectively expand the porcine whey proteome and bioinformatic dataset, and lay a theoretical and comprehensive basis for future research on porcine whey proteins.

2. Materials and methods

2.1. Porcine milk collection

The porcine colostrum and mature milk were obtained from the same ranch in Shenyang, China. A total of 90 milk samples were collected, 45 of which were porcine colostrum (0–24 h) and 45 of which were porcine mature milk (15–20 days). The pigs were all healthy and between 8 and 9 months old. The milk samples from each category were separated into three groups (each containing 15 samples), a step designed to acquire three biological replicates. Samples from each group were subsequently mixed. This study protocol was approved by Shenyang Agricultural University (China).

2.2. Preparation of whey proteins

To eliminate the upper layer of fat, the mixed samples were initially subjected to centrifugation (10,000 × g, 15 min, 4 °C). After the skimmed samples were collected, 33 % acetic acid (30 μL) was used to adjust the pH of the samples to 4.6, waiting for half an hour. The mixture was subsequently mixed with 3.3 M sodium acetate (30 μL). Casein was separated from the mixture by centrifugation (14,000 × g, 20 min, 4 °C). Finally, the whey protein supernatant was collected. A BCA protein assay kit (Bio-Rad, USA) was used to analyze the concentration of the whey proteins. In preparation for further processing, the whey protein materials were stored at −80 °C.

2.3. Whey protein digestion

The enzymatic hydrolysis of whey proteins was performed by the filter-aided sample preparation (FASP) method (Wisniewski et al., 2009). Briefly, 200 μg of whey protein was combined with SDT buffer (4 % SDS, 100 mM DTT, 150 mM Tris-HCl, pH 8.0). The samples were centrifuged (14,000 × g, 4 °C, 30 min). Subsequently, the mixtures were mixed with iodoacetamide (100 mM IAA in UT buffer) and then incubated for half an hour in the dark. Ultimately, 4 μg of trypsin (Promega, Madison, WI, USA) was used to digest the proteins in 25 mM NH_4HCO_3 buffer for 12 h. After digestion, the samples were centrifuged (14,000 × g, 4 °C, 15 min). A C18 cartridge was subsequently utilized to desalt the collected peptides.

2.4. LC-MS/MS analysis

The whey proteins were isolated by an EASY-nLC 1200 system (Thermo Scientific Waltham, MA, USA) and identified using a timsTOF Pro mass spectrometer (Bruker, Billerica, MA, USA). The separation system was composed of two phases, mobile phase A (0.1 % formic acid) and mobile phase B (84 % acetonitrile and 0.1 % formic acid). The peptides were injected into an upper column (Thermo Scientific Acclaim PepMap100, 100 μm × 2 cm, nanoViper C18), which was subsequently separated on a Thermo Scientific EASY column (10 cm, ID 75 μm, 3 μm, C18-A2) flowing at 300 nL min^{-1}. The positive ion detection mode was used for the MS analysis. The range of scanning for the parent ions was from 100 to 1700 (m/z). The major parameters are presented: the ion mobility coefficient (1/K0) was in the range of 0.6 to 1.6 vs. cm^2; the cycle window time was 1.17 s; and the dynamic rejection time was 24 s.

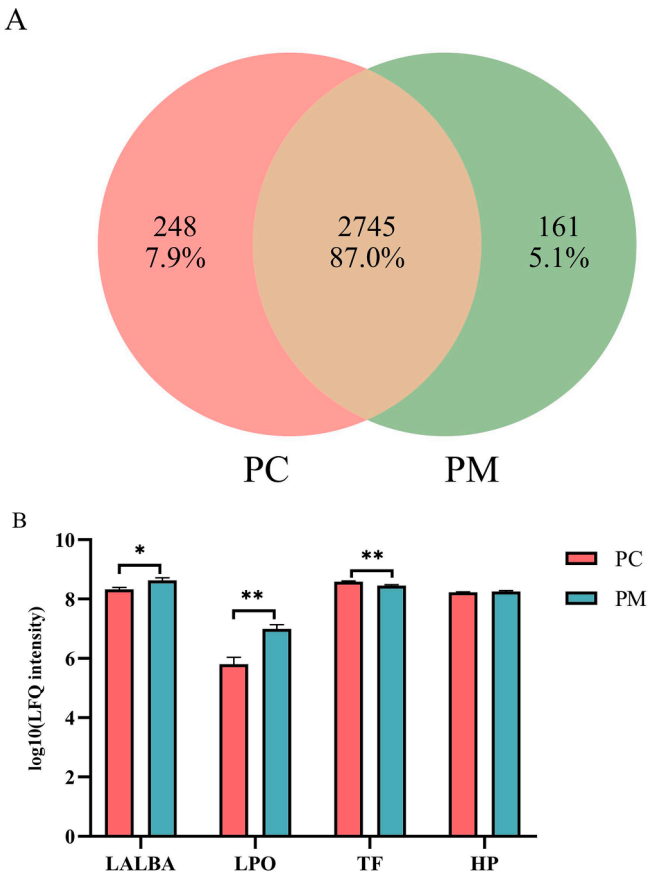


Fig. 1. Venn diagram of the identified whey proteins in porcine colostrum and mature milk (A). The relatively highly abundant whey proteins in porcine colostrum and mature milk (Error bar represents standard deviation of the mean (SD); *represents *t*-test $P < 0.05$; ** represents *t*-test $P < 0.01$) (B). PC, porcine colostrum; PM, porcine mature milk.

2.5. Protein identification and quantitative analysis

The analysis of the original data was conducted via MaxQuant software (version 1.6.14). The related parameters were as follows: trypsin for the enzyme, 2 missed cleavages for the maximum number of missed cleavages, 20 ppm for the first search, and 6 ppm for the main search. The fixed modification was carbamidomethyl (C), while the variable modification was oxidation (M). Proteins and peptides were specified to have a false discovery rate (FDR) ≤ 0.01. The identified proteins with $\geq$ 2 unique peptides were used for comparative analysis.

2.6. Bioinformatics analysis

The differentially expressed porcine whey proteins during lactation were analyzed via Student's *t* test and fold change (FC) values. Cluster 3.0 was applied for hierarchical clustering analysis. Gene Ontology (GO) annotation (http://geneontology.org/) and chiplot software (https://www.chiplot.online/) were used to analyze the annotated functions of the porcine whey proteins and visualize the data, respectively. Pathway analysis of the porcine whey proteins was performed via the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (https://www.genome.jp/kegg/). STRING software (http://string-db.org/) and Cytoscape open source software (version 3.9.1) were used to analyze the protein–protein interactions (PPI) and visualize the results, respectively.

2.7. LC-PRM/MS analysis

Briefly, peptide samples (1 μg) were isolated using an EASY-nLC

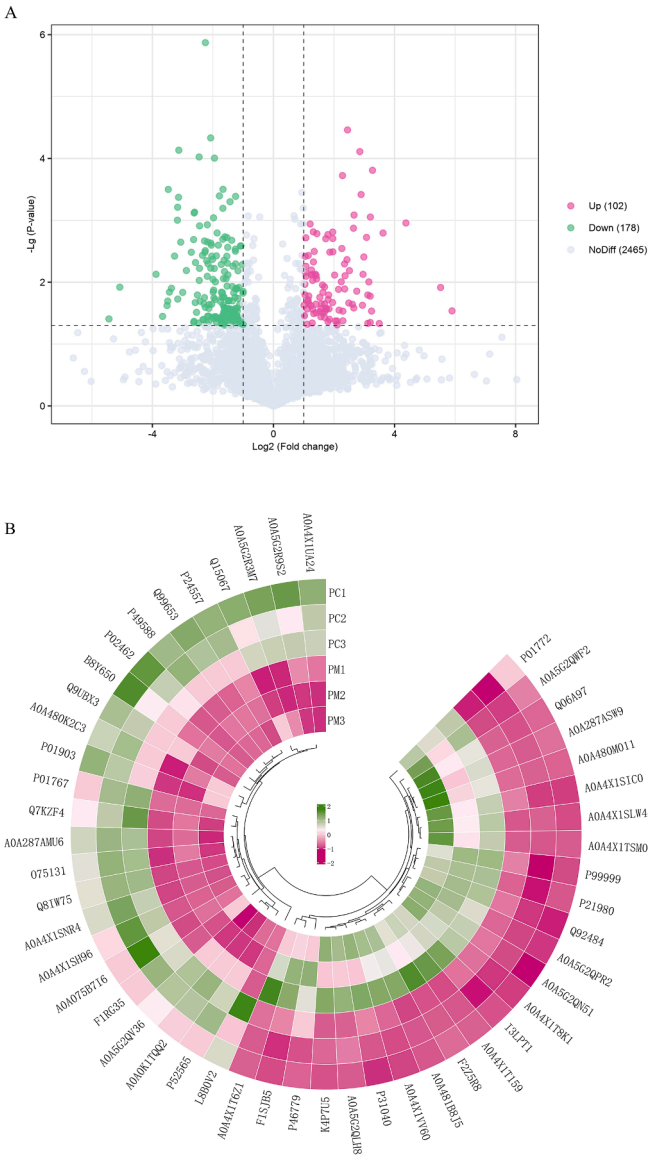


Fig. 2. Volcano plot of the significant differentially expressed whey proteins in porcine colostrum and mature milk (A). Heatmap analysis of the top 50 significant differentially expressed whey proteins in porcine colostrum and mature milk (B). PC, porcine colostrum; PM, porcine mature milk.

1200 system (Thermo Scientific Waltham, MA, USA). The separation system was composed of mobile phase A (0.1 % formic acid) and mobile phase B (0.1 % acetonitrile formic acid). With 95 % mobile phase A, the chromatographic column was equilibrated. Then, the peptides were injected into the trap column (100 μm × 50 mm, 5 μm-C18), which was subsequently separated on an analytical column (75 μm × 200 mm, 3 μm-C18) flowing at 300 nL/min. Then, a Q Exactive HF mass spectrometer (Thermo Scientific Waltham, MA, USA) was utilized for PRM analysis in the mode of detecting positive ions ranging from 300 *m/z* to 1800 *m/z*. The major parameters used were as follows: mass spectrometry resolution: 60,000 (at *m/z* 200); maximum ion injection time (IT): 200 ms; and automatic gain control (AGC) target: 3e6. Following each full MS scan, 20 fragments were captured based on the inclusion list. For the MS/MS scan, the mass spectrometry resolution was set to 30,000 (at *m/z* 200), with an AGC target value of 3e6. The maximum IT was limited to 120 ms. HCD was the activation type of MS2, whereas the isolation window was 1.6 Th. The raw mass spectrometry data were processed via Skyline (version 3.5.0).

3. Results and discussion

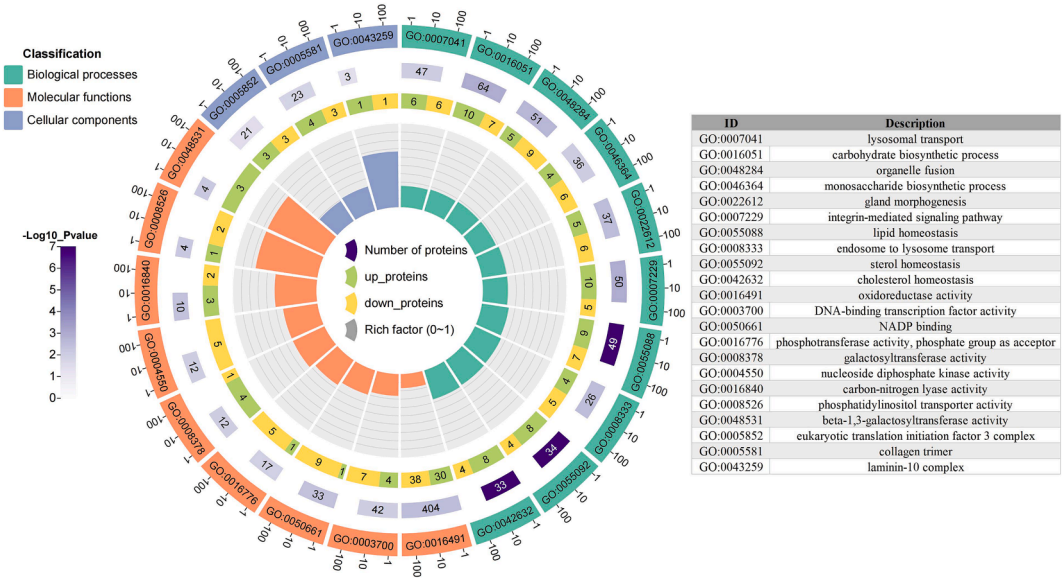
3.1. Identification of whey proteins between porcine colostrum and mature milk

This study identified 2993 and 2906 whey proteins in porcine colostrum and mature milk, respectively. The Venn diagram in Fig. 1A shows that 2745 proteins (87.0 %) were common to both groups. Additionally, 248 proteins (7.9 %) were uniquely present in porcine colostrum, while 161 proteins (5.1 %) were uniquely present in porcine mature milk. The major whey proteins (i.e., LALBA, β-lactoglobulin, immunoglobulins, and LPO) were identified in two milk samples. LALBA and LPO are highly abundant whey proteins in many animal milks (Han et al., 2023). Han et al. reported that bovine milk contains more LALBA and LPO than does camel and goat milk (Han et al., 2023). Other relatively abundant whey proteins, including serotransferrin (TF), haptoglobin (HP), the IgG heavy chain, albumin, growth/differentiation Factor 8 and peptidoglycan-recognition protein, were also detected in porcine milk. As a serum glycoprotein, TF is involved in iron metabolism (Jamnongkan et al., 2019). HP, an acute-phase plasma protein, is the most significant plasma detoxifier of hemoglobin (Hb) and is known for its antibacterial, antioxidant and angiogenic properties (Wan et al., 2021; Zhao et al., 2021). A recent study showed that the TF and HP contents were greater in donkey colostrum than in mature milk (Li et al., 2020). As shown in Fig. 1B, LALBA and LPO were significantly abundant in porcine mature milk, whereas TF was significantly abundant in porcine colostrum. The abundance of HP was similar in both groups. These results revealed the differences in the composition of porcine whey proteins at different lactation stages, providing a scientific framework for further studies of whey proteins in porcine milk.

3.2. The differentially expressed whey proteins (DEWPs) between porcine colostrum and mature milk

In this study, proteins identified in two replicates were considered screening criteria for quantified proteins. Proteins with a P value < 0.05 and FC > 2.00 or < 0.50 were considered significant DEWPs, and the uniquely expressed proteins were considered DEWPs. Thus, 280 whey proteins were identified as significant DEWPs between porcine colostrum and mature milk (Table S1). A volcanic map (Fig. 2A) showed that 102 proteins were upregulated and 178 proteins were downregulated in porcine colostrum compared to mature milk. The major significant DEWPs were visualized by a heatmap (Fig. 2B).

The whey proteins whose expression in porcine colostrum was significantly upregulated compared to that in mature milk were collagen alpha-1(IV) chain, Ig-like domain-containing protein, aldehyde dehydrogenase, IgG heavy chain, tyrosine-protein kinase SYK, apolipoprotein A-II (APOA2), and apolipoprotein A-I (APOA1). SYK was upregulated 3.64-fold in porcine colostrum compared to mature milk. As a cytoplasmic kinase, SYK plays many roles in the immune system and enhances the ability of cells to survive under stressful conditions (Krisenko et al., 2015). APOA1 and APOA2 were both identified in whey proteins from many animal milks (Han et al., 2023). These two apolipoproteins are the major apolipoproteins of plasma high-density lipoproteins (HDLs), accounting for 70 % and 20 %, respectively, of the total HDL population. APOA1 and APOA2 are both upregulated whey proteins in donkey colostrum compared to mature milk (Li et al., 2020). In the present study, APOA1 and APOA2 were upregulated 2.49-fold and 3.33-fold, respectively, in porcine colostrum compared to mature milk. Several recent studies have demonstrated that APOA1 and APOA2 have multiple biological functions, such as lipoprotein metabolism (Chan et al., 2012; Cochran et al., 2021). Among the downregulated proteins, LPO, secretagogin, dehydrogenase/reductase 3, J domain-containing protein, annexin (ANXA1), cytochrome *c* (CYCS) and EH domain-containing protein 1 were the dominant downregulated proteins in porcine colostrum compared to those in mature milk. LPO was



ID	Description
GO:0007041	lysosomal transport
GO:0016051	carbohydrate biosynthetic process
GO:0048284	organelle fusion
GO:0046364	monosaccharide biosynthetic process
GO:0022612	gland morphogenesis
GO:0007229	integrin-mediated signaling pathway
GO:0055088	lipid homeostasis
GO:0008333	endosome to lysosome transport
GO:0055092	sterol homeostasis
GO:0042632	cholesterol homeostasis
GO:0016491	oxidoreductase activity
GO:0003700	DNA-binding transcription factor activity
GO:0050661	NADP binding
GO:0016776	phosphotransferase activity, phosphate group as acceptor
GO:0008378	galactosyltransferase activity
GO:0004550	nucleoside diphosphate kinase activity
GO:0016840	carbon-nitrogen lyase activity
GO:0008526	phosphatidylinositol transporter activity
GO:0048531	beta-1,3-galactosyltransferase activity
GO:0005852	eukaryotic translation initiation factor 3 complex
GO:0005581	collagen trimer
GO:0043259	laminin-10 complex

Fig. 3. Enriched GO annotation of the differentially expressed whey proteins in porcine colostrum and mature milk.

downregulated 0.07-fold in porcine colostrum compared to mature milk. Yang et al. reported a greater abundance of LPO in human mature milk than in colostrum (Yang et al., 2017). As a crucial component of natural antimicrobial systems in human secretions, LPO has antimicrobial effects on bacteria, fungi and viruses (Al-Shehri et al., 2020). ANXA1, which was previously reported to be expressed in bovine, camel and goat milk (Han et al., 2023), was downregulated 0.13-fold in porcine colostrum compared to mature milk. As an anti-phospholipase protein, ANXA1 has major functions in cholesterol transport, anti-inflammatory effects, and clearance of apoptotic cells (Shen et al., 2020). Furthermore, ANXA1 reduces the progression of atherosclerosis through an anti-inflammatory response (Shen et al., 2020). Among the uniquely expressed proteins, liver carboxylesterase 1 (CES1), β-1,4-galactosyltransferase 5, MACPF domain-containing protein, magnesium transporter protein 1, and sepiapterin reductase were highly expressed in porcine colostrum. The basic proline-rich protein, TRASH domain-containing protein, glutamate receptor, protein GOLM2, dicarbonyl and L-xylulose reductase, and mitochondrial 3-hydroxyisobutyrate dehydrogenase were highly expressed in porcine mature milk. Taken together, these results explored the dynamic changes in porcine whey proteins during lactation and revealed the nutritional requirements of mammalian offspring at different stages.

3.3. GO enrichment analysis of DEWPs between porcine colostrum and mature milk

The DEWPs between porcine colostrum and mature milk were classified into three categories, biological process (BP), cellular component (CC) and molecular function (MF), by GO enrichment analysis. As shown in Fig. 3, in terms of BP, porcine whey proteins were primarily enriched in the lipid homeostasis, cholesterol homeostasis, sterol homeostasis, integrin-mediated signaling pathway, carbohydrate biosynthetic process, organelle fusion, gland morphogenesis, endosome-to-lysosome transport, lysosomal transport and monosaccharide biosynthetic

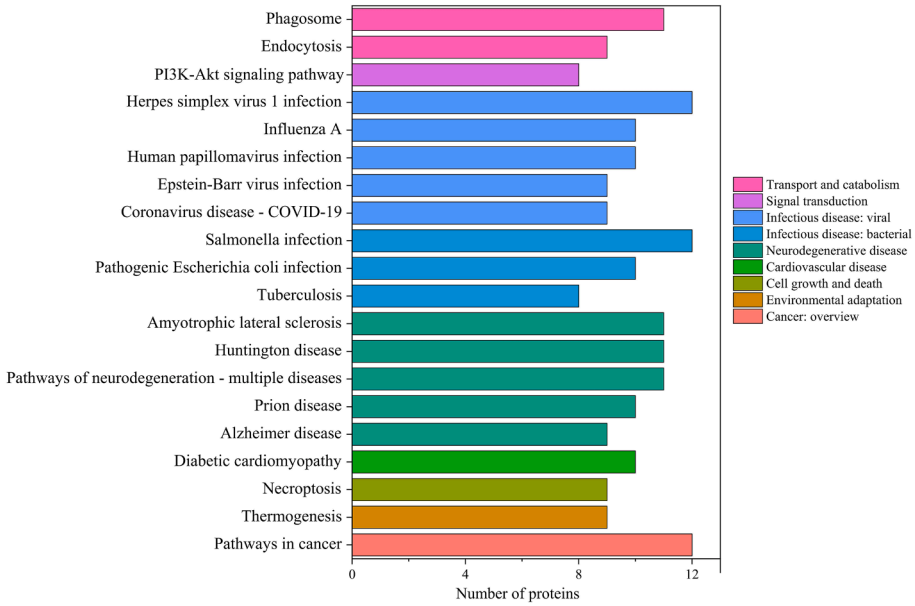


Fig. 4. KEGG pathway analysis of the differentially expressed whey proteins in porcine colostrum and mature milk.

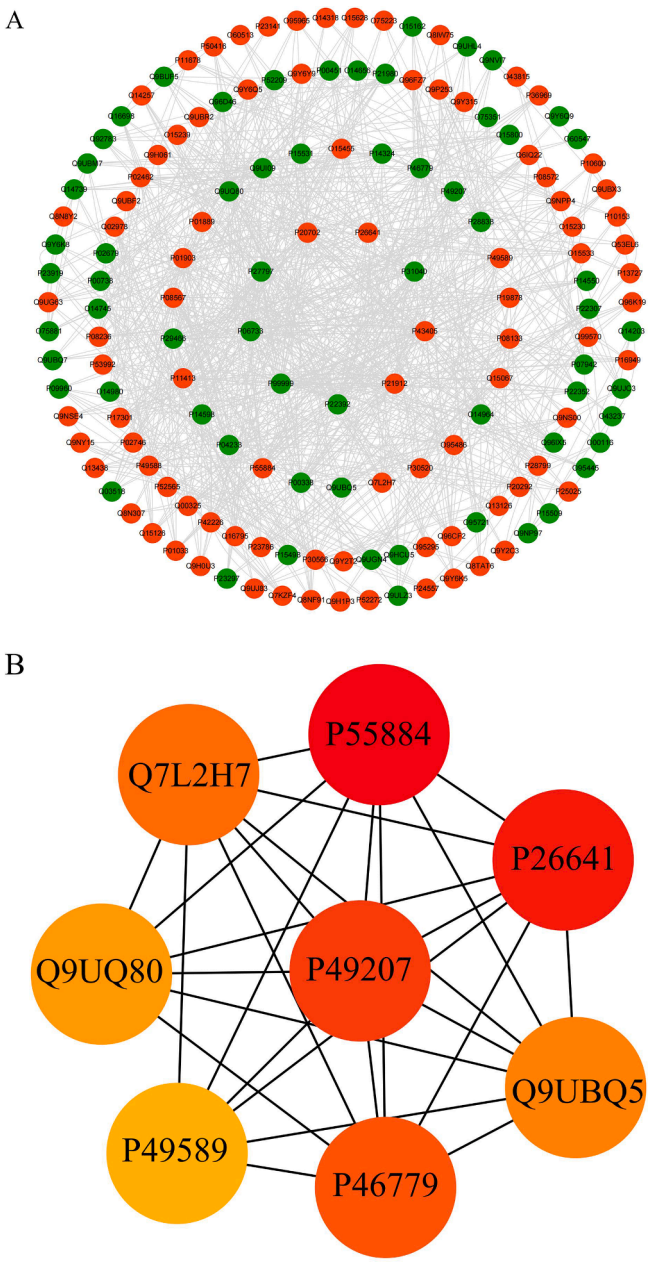


Fig. 5. PPI network analysis of the differentially expressed whey proteins between porcine colostrum and mature milk. Red nodes represent upregulated proteins; green nodes represent downregulated proteins. Proteins with degree ≦ 2 are hidden (A). The top 8 hub proteins network. The closer the color of protein to red, the higher its MCC score (B).

process. In terms of MF, porcine whey proteins were highly enriched in the oxidoreductase activity, NADP binding, DNA-binding transcription factor activity, carbon–nitrogen lyase activity, galactosyltransferase activity, and nucleoside diphosphate kinase activity. In terms of CC, porcine whey proteins were mainly enriched in the collagen trimer, eukaryotic translation initiation Factor 3 complex, and laminin-10 complex. According to the GO enrichment analysis, the DEWPs were notably enriched in lipid homeostasis (GO:0055088). Lipid homeostasis is necessary for normal cells to maintain physiological activity. In this study, 16 porcine whey proteins (9 upregulated proteins and 7 downregulated proteins) were enriched in lipid homeostasis, namely, APOA1, APOA2, ORM1-like protein (ORMDL3), low-density lipoprotein receptor adaptor protein 1, EH domain-containing protein 1, CES1, and tetratricopeptide repeat protein 39B. APOA1 and APOA2 have positive effects on the maintenance of cellular lipid homeostasis (Chan et al., 2012; Cochran et al., 2021). ORMDL3 is involved in sphingolipid homeostasis and Ca^{2+} homeostasis (Bugajev et al., 2021). CES1, a serine hydrolase, is abundant in hepatocytes and adipocytes, which is able to hydrolyze a wide range of endogenous esters (e.g., triacylglycerols and cholesteryl esters) and is involved in cholesterol homeostasis, lipid metabolism, and fatty liver disease (Wang et al., 2018). Therefore, these mentioned porcine whey proteins may be vital proteins that regulate lipid homeostasis. Additionally, these results revealed the biological significance of porcine whey proteins and contributed to exploring the potential health-related properties of whey proteins.

3.4. KEGG pathway analysis of the DEWPs between porcine colostrum and mature milk

The top 20 pathways related to the DEWPs between porcine colostrum and mature milk are presented in Fig. 4. According to KEGG analysis, many porcine whey proteins participated in several disease pathways, such as herpes simplex virus 1 infection, Salmonella infection, amyotrophic lateral sclerosis, diabetic cardiomyopathy, and coronavirus disease-COVID-19, possibly through passive immunization (Cavaletto et al., 2004). The porcine whey proteins participating in these pathways may have the ability to defend newborns against infection, especially viral infection. These results were similar to those of many recent studies on whey proteins from many types of mammalian milk (Han et al., 2023). Moreover, several whey proteins participated in phagosomes, which play important roles in the maintenance of tissue homeostasis and host immunity (Garin et al., 2001). Endocytosis was also a significant pathway in this study and included 5 upregulated proteins and 4 downregulated proteins. Previous research has confirmed that human vascular endothelial cells carry human milk exosomes and other components to surrounding tissues through endocytosis, which is mediated by the surface glycoproteins of cells and exosomes in human intestinal cells (Kusuma et al., 2016; Wolf et al., 2015). The present study indicated the intrinsic biological functions of porcine whey proteins during lactation, which could be beneficial for the development of whey proteins.

3.5. PPI network analysis of the DEWPs between porcine colostrum and mature milk

A total of 272 whey proteins participated in the PPI network, which generated 551 interactions (Fig. 5A). P99999 (CYCS) and P06733 (ENO1), which both interacted with 20 proteins, were the nodes with the highest degrees of interactions, followed by P27797 (CALR) with 19 interactions, and P20702 (ITGAX) with 18 interactions. CYCS was downregulated 0.16-fold in porcine colostrum compared to mature milk. As a mitochondrial protein, CYCS is involved in the life-supporting process of ATP synthesis and plays vital roles in canonical intrinsic apoptosis (Ow et al., 2008). As shown in Fig. 5B, we used the maximal clique centrality (MCC) method to calculate the top 8 hub proteins (4 upregulated and 4 downregulated proteins). Among these, the highest MCC score was P55884 (EIF3B), followed by P26641 (EEF1G), P49207 (RPL34), P46779 (RPL28), Q7L2H7 (EIF3M), Q9UBQ5 (EIF3K), Q9UQ80 (PA2G4), and P49589 (CARS1). EIF3B (eukaryotic translation initiation Factor 3B), a key scaffold protein, is crucial for translation regulation, cell growth, and tumorigenesis (Feng et al., 2018). These results reflected intrinsic connections between whey proteins from porcine milk during lactation and contributed to excavating hub whey proteins in porcine milk.

3.6. PRM analysis of whey proteins between porcine colostrum and mature milk

The 5 selected whey proteins (HP, LPO, peptidoglycan-recognition protein, ribonuclease inhibitor, and LALBA) were confirmed by PRM

Table 1
Results of selected whey proteins identified by PRM and LFQ analyses.

Uniprot Accession	Protein Name	Log_2FC (PRM) PC/PM	Log_2FC (LFQ) PC/PM
A0A4X1SL78	Haptoglobin	−0.36	−0.09
A0A4X1SLW4	Lactoperoxidase	−3.73	−3.88
A0A4X1W2B2	Peptidoglycan-recognition protein	0.74	0.78
P10775	Ribonuclease inhibitor	−0.91	−0.77
P18137	α-lactalbumin	−1.58	−1.01

Abbreviation: FC, fold change; PRM, parallel reaction monitoring; LFQ, label-free quantification; PC, porcine colostrum; PM, porcine mature milk.
Table S1. Significant differentially expressed whey proteins in porcine colostrum and mature milk.

analysis (Table 1). LPO and LALBA, which were significantly upregulated in porcine mature milk compared to porcine colostrum according to LFQ analysis, were also confirmed to be expressed at high levels in porcine mature milk via PRM analysis. The fold changes in the other chosen proteins analyzed via PRM were approximately similar to those analyzed via LFQ, which verified the accuracy of our identification results.

4. Conclusions

Using label-free quantitative proteomics, 2993 and 2906 whey proteins were characterized in porcine colostrum and mature milk, respectively. Among these proteins, 2745 common proteins were identified in two milk samples, 248 proteins were uniquely present in porcine colostrum, and 161 proteins were uniquely present in porcine mature milk. A total of 280 whey proteins were characterized as significant DEWPs in porcine milk. Moreover, GO annotation, KEGG pathway and PPI analyses revealed the biological importance of the porcine whey proteins. The comprehensive comparison of whey proteins between porcine colostrum and mature milk indicated dynamic variations in whey protein composition during different periods of lactation. Furthermore, this study provides in-depth insight into the biological functions of porcine whey proteins and may help develop whey proteins based on porcine milk in the future.

CRediT authorship contribution statement

Huiwen Zhao: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Shanshan Zhao:** Resources, Software. **Qing Zhu:** Investigation. **Jiali Chen:** Investigation. **Zhizhong Quan:** Resources. **Xiqing Yue:** Funding acquisition, Project administration. **Xueyan Cao:** Conceptualization, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by Distinguished Professor of Liaoning Province, and Key Laboratory of Agricultural Product Processing Safety and Risk Assessment, Liaoning Province (22-106-2-25).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101112.

References

Al-Shehri, S. S., Duley, J. A., & Bansal, N. (2020). Xanthine oxidase-lactoperoxidase system and innate immunity: Biochemical actions and physiological roles. *Redox Biology, 34*. https://doi.org/10.1016/j.redox.2020.101524, 101524.

Bugajev, V., Paulenda, T., Utekal, P., Mrkacek, M., Halova, I., Kuchar, L., Kuda, O., Vavrova, P., Schuster, B., Fuentes-Liso, S., Potuckova, L., Smrz, D., Cernohouzova, S., Draberova, L., Bambouskova, M., & Draber, P. (2021). Crosstalk between ORMDL3, serine palmitoyltransferase, and 5-lipoxygenase in the sphingolipid and eicosanoid metabolic pathways. *Journal of Lipid Research, 62*. https://doi.org/10.1016/j.jlr.2021.100121, 100121.

Cavaletto, M., Giuffrida, M. G., & Conti, A. (2004). The proteomic approach to analysis of human milk fat globule membrane. *Clinica Chimica Acta, 347*(1–2). https://doi.org/10.1016/j.cccn.2004.04.026

Chan, D. C., Ng, T. W., & Watts, G. F. (2012). Apolipoprotein A-II: Evaluating its significance in dyslipidaemia, insulin resistance, and atherosclerosis. *Annals of Medicine, 44*(4), 313–324. https://doi.org/10.3109/07853890.2011.573498

Cochran, B. J., Ong, K. L., Manandhar, B., & Rye, K. A. (2021). APOA1: A Protein with Multiple Therapeutic Functions. *Current Atherosclerosis Reports, 23*(3). https://doi.org/10.1007/s11883-021-00906-7, 11.

Cooper, D. K., Ekser, B., Ramsoondar, J., Phelps, C., & Ayares, D. (2016). The role of genetically engineered pigs in xenotransplantation research. *Journal of Pathology, 238*(2), 288–299. https://doi.org/10.1002/path.4635

Eriksen, K. G., Christensen, S. H., Lind, M. V., & Michaelsen, K. F. (2018). Human milk composition and infant growth. *Current Opinion in Clinical Nutrition and Metabolic Care, 21*(3), 200–206. https://doi.org/10.1097/MCO.0000000000000466

Feng, X., Li, J., & Liu, P. (2018). The Biological Roles of Translation Initiation Factor 3b. International Journal of Biological Sciences, 14(12), 1630-1635.Doi: 10.7150/ijbs.26932.

Garin, J., Diez, R., Kieffer, S., Dermine, J. F., Duclos, S., Gagnon, E., Sadoul, R., Rondeau, C., & Desjardins, M. (2001). The Phagosome Proteome: Insight into Phagosome Functions. *Journal of Cell Biology, 152*(1), 165–180. https://doi.org/10.2307/1620271

Han, B., Zhang, L., & Zhou, P. (2023). Comparative proteomics of whey proteins: New insights into quantitative differences between bovine, goat and camel species. International Journal of Biological Macromolecules, 227, 10-16. Doi: 10.1016/j.ijbiomac.2022.12.103.

W. Jamnongkan C.B. Lebrilla M. Barboza A. Techasen W. Loilome P. Sithithaworn N. Khuntikeo C. Pairojkul N. Chamadol R. Thanan P. Yongvanit Discovery of Serotransferrin Glycoforms: Novel Markers for Diagnosis of Liver Periductal Fibrosis and Prediction of Cholangiocarcinoma Biomolecules 9(10).Doi: 10.3390/biom9100538 2019.

Kerasioti, E., Stagos, D., Priftis, A., Aivazidis, S., Tsatsakis, A. M., Hayes, A. W., & Kouretas, D. (2014). Antioxidant effects of whey protein on muscle C2C12 cells. *Food Chemistry, 155*, 271–278. https://doi.org/10.1016/j.foodchem.2014.01.066

Krisenko, M. O., Higgins, R. L., Ghosh, S., Zhou, Q., Trybula, J. S., Wang, W. H., & Geahlen, R. L. (2015). Syk Is Recruited to Stress Granules and Promotes Their Clearance through Autophagy. *Journal of Biological Chemistry, 290*(46), 27803–27815. https://doi.org/10.1074/jbc.M115.642900

Kusuma, R. J., Manca, S., Friemel, T., Sukreet, S., Nguyen, C., & Zempleni, J. (2016). Human vascular endothelial cells transport foreign exosomes from cow's milk by endocytosis. American Journal of Physiology Cell. *Physiology, 310*(10), C800–C807. https://doi.org/10.1152/ajpcell.00169.2015

Li, M., Dong, Y., Li, W., Shen, X., Abdlla, R., Chen, J., Cao, X., & Yue, X. (2022). Characterization and comparison of whey proteomes from bovine and donkey colostrum and mature milk. Lwt, 158.Doi: 10.1016/j.lwt.2022.113113.

Li, M., Li, Q., Yu, H., Zhang, X., Li, D., Song, W., Zheng, Y., & Yue, X. (2023). Differentially expressed whey proteins of donkey and bovine colostrum revealed with a label-free proteomics approach. *Food Science and Human Wellness, 12*(4), 1224–1231. https://doi.org/10.1016/j.fshw.2022.10.004

Li, M., Yu, H., Chen, J., Abdlla, R., Liu, A., Song, W., Zhang, J., Zhang, X., Yue, X., & Li, Q. (2021). Novel insights into whey protein differences between donkey and bovine milk. Food Chemistry, 365. Doi: 10.1016/j.foodchem.2021.130397.

Li, W., Li, M., Cao, X., Han, H., Kong, F., & Yue, X. (2020). Comparative analysis of whey proteins in donkey colostrum and mature milk using quantitative proteomics. *Food Research International, 127*. https://doi.org/10.1016/j.foodres.2019.108741

Madureira, A. R., Pereira, C. I., Gomes, A. M. P., Pintado, M. E., & Xavier Malcata, F. (2007). Bovine whey proteins – Overview on their main biological properties. *Food Research International, 40*(10), 1197–1211. https://doi.org/10.1016/j.foodres.2007.07.005

Maity, S., & Ambatipudi, K. (2019). Quantitative proteomics of milk whey reveals breed and season specific variation in protein abundance in Holstein Friesian cow and Murrah buffalo. *Research in Veterinary Science, 125*, 244–252. https://doi.org/10.1016/j.rvsc.2019.07.001

Ow, Y. P., Green, D. R., Hao, Z., & Mak, T. W. (2008). Cytochrome c: Functions beyond respiration. *Nature Reviews: Molecular Cell Biology, 9*(7), 532–542. https://doi.org/10.1038/nrm2434

Shen, X., Zhang, S., Guo, Z., Xing, D., & Chen, W. (2020). The crosstalk of ABCA1 and ANXA1: A potential mechanism for protection against atherosclerosis. *Molecular Medicine, 26*(1), 84. https://doi.org/10.1186/s10020-020-00213-y

E. Sjostedt W. Zhong L. Fagerberg M. Karlsson N. Mitsios C. Adori P. Oksvold F. Edfors A. Limiszewska F. Hikmet J. Huang Y. Du L. Lin Z. Dong L. Yang X. Liu H. Jiang X. Xu J. Wang H. Yang L. Bolund A. Mardinoglu C. Zhang K. von Feilitzen C. Lindskog F. Ponten Y. Luo T. Hokfelt M. Uhlen J. Mulder An atlas of the protein-coding genes in the human, pig, and mouse brain Science 367(6482). Doi: 10.1126/science.aay5947 2020.

Tan, C., Zhai, Z., Ni, X., Wang, H., Ji, Y., Tang, T., Ren, W., Long, H., Deng, B., Deng, J., & Yin, Y. (2018). Metabolomic Profiles Reveal Potential Factors that Correlate with Lactation Performance in Sow Milk. *Scientific Reports, 8*(1), 10712. https://doi.org/10.1038/s41598-018-28793-0

Trinchese, G., Cavaliere, G., Canani, R. B., Matamoros, S., Bergamo, P., De Filippo, C., Aceto, S., Gaita, M., Cerino, P., Negri, R., Greco, L., Cani, P. D., & Mollica, M. P. (2015). Human, donkey and cow milk differently affects energy efficiency and inflammatory state by modulating mitochondrial function and gut microbiota. *Journal of Nutritional Biochemistry, 26*(11), 1136–1146. https://doi.org/10.1016/j.jnutbio.2015.05.003

Veskoukis, A. S., Kerasioti, E., Skaperda, Z., Papapostolou, P. A., Nepka, C., Spandidos, D. A., Asprodini, E., Taitzoglou, I., & Kouretas, D. (2020). Whey protein boosts the antioxidant profile of rats by enhancing the activities of crucial antioxidant enzymes in a tissue-specific manner. *Food and Chemical Toxicology, 142*, 111508. https://doi.org/10.1016/j.fct.2020.111508

Wan, B. N., Zhou, S. G., Wang, M., Zhang, X., & Ji, G. (2021). Progress on haptoglobin and metabolic diseases. *World Journal of Diabetes, 12*(3), 206–214. https://doi.org/10.4239/wjd.v12.i3.206

Wang, D., Zou, L., Jin, Q., Hou, J., Ge, G., & Yang, L. (2018). Human carboxylesterases: A comprehensive review. *Acta Pharmaceutica Sinica B, 8*(5), 699–712. https://doi.org/10.1016/j.apsb.2018.05.005

Wisniewski, J. R., Zougman, A., Nagaraj, N., & Mann, M. (2009). Universal sample preparation method for proteome analysis. *Nature Methods, 6*(5), 359–362. https://doi.org/10.1038/nmeth.1322

Wolf, T., Baier, S. R., & Zempleni, J. (2015). The Intestinal Transport of Bovine Milk Exosomes Is Mediated by Endocytosis in Human Colon Carcinoma Caco-2 Cells and Rat Small Intestinal IEC-6 Cells. *Journal of Nutrition, 145*(10), 2201–2206. https://doi.org/10.3945/jn.115.218586

Yang, M., Cao, X., Wu, R., Liu, B., Ye, W., Yue, X., & Wu, J. (2017). Comparative proteomic exploration of whey proteins in human and bovine colostrum and mature milk using iTRAQ-coupled LC-MS/MS. *International Journal of Food Sciences and Nutrition, 68*(6), 671–681. https://doi.org/10.1080/09637486.2017.1279129

Zhang, F., Pu, J., Gu, Z., & Mao, H. (2020). DIA proteomics reveals hypotensive and immune-enhancing constituents in buffalo whey from different altitudes. *International Journal of Biological Macromolecules, 164*, 4146–4154. https://doi.org/10.1016/j.ijbiomac.2020.08.213

Zhang, S., Chen, F., Zhang, Y., Lv, Y., Heng, J., Min, T., Li, L., & Guan, W. (2018). Recent progress of porcine milk components and mammary gland function. *Journal of Animal Science and Biotechnology, 9*. https://doi.org/10.1186/s40104-018-0291-8

Zhao, H., Li, M., Zhu, Q., Liu, A., Bi, J., Quan, Z., Luo, X., Zheng, Y., Yang, N., Yue, X., & Cao, X. (2023). Label-free quantitative proteomic analysis of milk fat globule membrane proteins in porcine colostrum and mature milk. *Food Chemistry, 426*. https://doi.org/10.1016/j.foodchem.2023.136447

Zhao, Z., Liu, N., Wang, C., Cheng, J., & Guo, M. (2021). Proteomic analysis of differentially expressed whey proteins in Saanen goat milk from different provinces in China using a data-independent acquisition technique. *Journal of Dairy Science, 104*(10), 10513–10527. https://doi.org/10.3168/jds.2020-19877