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Original Research

Proteomics in Milk: Protein Constituents, Processing, MFGM Proteome, Advances in Proteomics in the Characterization of Milk and Dairy Products

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Abstract:

Proteomics is a relatively new branch of research that has played a significant role in the advancement of medical knowledge over the past few decades. On the other hand, due to its exceptionally high potential for the investigation of proteomes, it can also be applied to other fields of scientific research. Milk is a fluid that is extremely varied and complex, containing a great number of genetic variations and isoforms that have post-translational modifications (PTMs). As a result of the huge number of proteins and peptides that are present in its matrix, proteomics is given as a potent tool for the characterization of milk samples and the products that are derived from them. An exhaustive characterization of the proteins and peptides that are present in milk and dairy products has been made possible by the technology that has been developed to date for the separation and characterization of the milk proteome. This technology, which includes two-dimensional gel electrophoresis (2DE) technology and, in particular, mass spectrometry (MS), has enormous applications in the industry for the control of fundamental parameters, such as microbiological safety, the guarantee of authenticity, or the control of the transformations that are carried out, with the goal of improving the quality of the final product. All of these developments in proteomics have resulted in the creation of novel ways for ensuring the quality, authenticity, and safety of milk and other dairy products. Through the use of the tools that proteomics provides, it is possible to detect microbiological issues at an earlier stage, such as milk from cows who have mastitis or milk and milk products that have received contamination. In a similar manner, proteomics possesses the potential to detect fraud in the industrial sector, such as the use of undeclared milk on the label of a product or that of a protein that is not derived from dairy products. Proteomics, on the other hand, is also capable of monitoring or controlling alterations that appear in the industrial sector. Therefore, a treatment with an excessive amount of heat that results in the production of compounds that degrade proteins and/or the investigation of the typical protein profile of a cheese can be utilised as a means of providing a guarantee of premium quality to the end user.

Keywords: Milk, Advances in Proteomics, Protein Constituents, Dairy Products.

Introduction:

Mammalian mothers rely heavily on milk as a primary source of nutrition for their newborn children. Additionally, throughout the course of their lives, people continue to eat cow's milk in a variety of forms, making it a significant agricultural commodity. In order to maintain good health, growth, and development, milk proteins are a necessary source of nourishment. Other proteins are either partially or minimally broken down and exert functionality according to the structures of their digestive products. For example, whereas many proteins are digested completely in the gut to give essential amino acids, other proteins are only partially or barely broken down. In the case of human milk proteins, some of them help with digestion and the absorption of other nutrients found in milk, while others protect against pathogenic bacteria and viruses, contribute to the strengthening of the acquired immune system, influence cognitive development, affect metabolic development, regulate food intake and satiety, and assist in the development and maturation of the gastrointestinal tract (GIT). There is a lack of information regarding the molecular origins of the impacts that milk proteins have, despite the fact that there is a growing understanding of the numerous health and developmental benefits that milk proteins provide. There is even less evidence available on the development of milk function over the duration of lactation, where milk proteins may assist in a different manner in newborns and early infants [1, 2]. A great number of efforts have been made in order to discover the "secret ingredients" that might be responsible for the benefits that have been pointed out above that are associated with milk proteins. It is envisaged that researchers would be able to obtain more insight into biological processes, signalling pathways, nutritional benefits, and novel functional food ingredients that are either novel or whose significance in milk is not yet understood if they use proteomic methods to investigate milk proteomes. This is one strategy that can be taken. Within the context of human milk, a multitude of proteome and nonproteomic research have been conducted over the course of the last ten years, with the purpose of examining the proteins that are present in milk whey and milk-fat-globule membrane (MFGM). Over the course of time, there has been a consistent rise in the quantity of proteins that have been identified in human milk. Despite the progress that has been made in human milk proteomics, the overall number of proteins that have been found in human milk continues to be rather disheartening. This is especially true when one considers that more than one thousand proteins have been identified in blood serum, which is another human fluid, utilising an experimental setup that is very comparable. The absence of methods that are able to appropriately uncover low-abundance proteins in the presence of high-abundance proteins is the primary cause of the deficiency in the number of proteins that have been found in human milk. If we compare milk to blood serum, we can see that milk is even more characterised by the presence of key proteins. There is yet no comprehensive view of the milk proteome because of the complexity that is associated with the technical aspect. As a consequence of this, the biology and activities of milk proteins continue to be insufficiently understood, which in turn prevents further investigation into the nutritional advantages that milk can offer. Since then, our group has demonstrated that protein fractionation with sample loads that are bigger than the usual offers a method for investigating low-abundance proteins in milk. Together with mixed-bed ion-exchange (IEX) protein fractionation, we were able to determine the identities of 297 proteins that were present in cow's milk. The sample loads that we used were in the range of a few milligrammes. This larger-than-normal load technique was recently applied to the process of protein separation using SDS-PAGE by yours truly. By integrating the results of the IEX and SDS-PAGE analyses, we were able to determine the presence of more than 600 proteins in cow's milk. The same methodology was used to human milk, and the results revealed the presence of almost one thousand proteins. In order to gain a better understanding of the biology and function of milk,

it is essential to first define the milk proteome and then, also of equal or even greater significance, to determine how the milk proteome evolves throughout the process of lactation. It is possible that acquiring this information will result in the discovery of innovative insights in the fields of dairy science and food processing. For example, although it is well knowledge that the complement system that is present in blood plays a significant part in the defence of the host, the role that it plays in milk is still unknown. There is a possibility that the defence system will cause damage to the host tissue [3-5]. However, the regulation of this system and the possible impact it could have on breastfeeding mothers is mainly unknown. Additionally, it has been discovered that milk proteins are beneficial to the development of the gastrointestinal tract; nevertheless, the protein candidates that are responsible for the maturation of the gastrointestinal tract in early infants are still a mystery. Through the construction of human and cow's milk proteomes, it is possible to discover the functions that are shared by cow's milk proteins and their human counterparts. In addition, the comparison can serve as a guide for future trials, which will help us gain a better knowledge of the mechanisms that regulate milk expression as well as the factors that influence milk output and quality. Milk proteins are more than just a premium supply of amino acids; they are also an extensive repertoire of functional food ingredients. This is becoming more obvious as the comprehensive milk proteome and its fluctuations in protein abundances during lactation become more apparent. For the purpose of better preserving the activities of milk proteins, our improved understanding has the potential to stimulate improvements in industrial processing for milk protein. When it comes to the development of improved functional foods based on dairy products, it may also serve as a guide for producers. The purpose of this chapter is to highlight current discoveries in milk proteomes for both humans and cows that can be utilised to strengthen our understanding of novel milk functions. We further explain the use of proteomics in characterising heat-induced protein changes during industrial processes, which may impair the nutritional content and function of milk proteins [6, 7]. This is made possible by the enhanced understanding of milk proteins that we have gained. As a result of the discovery of the human genome, the discipline of biology has seen the emergence of a new area of knowledge known as proteomics. The first time that this term was used was in the middle of the 1990s, when there was a need to develop a specialised language that would explain the work that was being done at that time, which was the study of the proteome. Within the scope of this investigation are all of the proteins that are found in a single cell, as well as all of the isoforms and modifications, the interactions that occur between them, as well as their structure and higher-order complexes. The field of medicine was the primary focus of efforts during the early stages of proteomics. This was because the field of medicine was where significant progress was made in the diagnosis of disorders. On the other hand, the possibility of examining the proteome of a biological sample has been extended to other domains, such as the development of foods for animals. In particular, a significant amount of work has been put in to comprehend the milk proteome, which is characterised as a complex that contains a wide range of chemicals, some of which have been discovered to possess biological activities that are of great interest. The primary objective of this research is to investigate the genetic components, molecular pathways, and cellular functions that are involved in the production of milk, as well as its quality and safety. Approximately five percent of the total protein fraction is comprised of low-abundance proteins, which can be found in the whey or in the milk fat globule membrane (MGMF). Milk is a fluid that contains a large number of these proteins. Proteomic analysis is made even more challenging by the presence of numerous genetic variants and posttranslational modifications (PTMs), which include glycosylation, phosphorylation, disulfide bond formation, and proteolysis. These modifications generate a large number of protein variants from a single gene product, which makes the process of

proteomic analysis even more challenging. It is for this reason that there is not yet a single procedure that is capable of delivering a comprehensive examination of the milk proteome. The field of analytical chemistry has, over the course of the past few years, developed a number of techniques that involve the fractionation and separation of the sample, the determination of the sample's concentration, and the detection of the proteins that are present with a resolution and sensitivity that are sufficient for a thorough determination of the proteome. Because of the promise that is represented by the application of proteomics in animals, technological advancement has been facilitated. The separation of proteins or protein subunits according to charge is typically accomplished by the use of two-dimensional gel electrophoresis (2DE), which is then followed by another separation based on molecular mass using SDS-PAGE. Mass spectrometry (MS) analysis is used to characterise the proteins that are exhibited in the form of spots after a staining process has been completed [8-11]. In a method that is complimentary, the material can be put through a digestion process, and the peptides that are obtained can be analysed by mass spectrometry (MS) after being differentiated by chromatographic processes. These two approaches are extremely common in the analysis of the milk proteome, and there are numerous operational variations involved, which will be covered further down in this article.

Considerations Regarding Milk Proteins and Their Importance in Human Nutrition:

There is a definition of milk that describes the liquid that is expelled by the mammary glands of mammals in order to provide nourishment for the baby. Milk, on the other hand, has been regarded as a very significant resource by human beings ever since ancient times due to the fact that it is the easiest resource to access and contains a high nutritious content. Because of this contextualised understanding of milk as a product that may be used to satisfy a need, the meaning of milk has significantly shifted. Milk is the product that is obtained by milking mammals [12, 13] that are raised for the exclusive purpose of producing milk. Therefore, from a technological point of view, a more consistent definition of milk would be the product that is obtained by milking mammals. There are many different kinds of animals that are utilised for milk production. At present, cow's milk accounts for over 80% of the global production, with buffalo milk accounting for approximately 15%, goat milk accounting for approximately 2.5%, sheep milk accounting for approximately 1%, and camel milk accounting for approximately 0.4%. On the other hand, the significance of milk in the diet of humans extends beyond the ingestion of milk in its raw form. The production of fermented dairy products has evolved into a history that dates back a thousand years. This tradition encompasses a wide range of foods that differ in terms of their distinct textures, flavours, scents, forms, and sizes. Milk is a food that is excellently balanced in terms of its many components, making it a very comprehensive source of nutrients. These components do not exist in a predetermined quantity; rather, they change depending on a variety of conditions, including the species of animal, breed, length of time the animal is lactating, and food. The fat content of cow milk is 3.3%, while the fat content of goat milk is 3.9%. For instance, the fat content of buffalo milk is 7.5%, while the fat content of sheep milk is 6.4%. The milks of these five species, along with camel milk, are among the most popular milks consumed all over the world. Milk is a product that has been biologically created to meet the nutritional needs of the newborn. In particular, the protein fraction of milk is highly regarded due to the fact that it possesses a high nutritional quality. Consumption of milk supplies the body with all of the needed amino acids as well as additional chemicals, including proteins that attach to substances like vitamins and metals, as well as a variety of protein hormones. The profile of amino acids is comparable to that of the egg, the only difference being that there are fewer amino acids that contain sulphur. On the other hand, the FAO, WHO, and UNU have determined that the number and variety of these molecules that are found in milk are sufficient to

meet the daily requirements found in humans. It was found by Rafiq et al. that cow, sheep, goat, buffalo, and camel milks provide an excellent mix of essential amino acids, particularly branched-chain amino acids (leucine, isoleucine, and valine) in relation to the daily demands that were indicated earlier. A comprehensive investigation and characterization of milk proteins has been carried out as a result of the distinctive qualities of milk proteins and the technological influence they have had by playing a significant part in the manufacturing of dairy products. This particular portion of milk is made up of two different kinds of proteins, namely caseins and whey proteins [14 to 16]. In the casein fraction, which is insoluble in milk at a pH of 4.6, the former is obtained through the process of acid precipitation. A solution of proteins, known as whey proteins, is the liquid that is left behind. This solution also contains traces of lactose, salts, vitamins, and other components throughout its composition. In the realm of caseins, it is possible to differentiate between a total of five distinct varieties, namely αs1-casein, αs2-casein, β-casein, γ-casein, and κ-casein. Approximately eighty percent of the proteins found in cow's milk and other commercial dairy species are comprised of them. They are known as micelles and come in the form of colloidal particles with diameters ranging from 50 to 600 nanometers. This particular protein sub-fraction is responsible for a number of significant and distinctive characteristics of milk, including its white colour, its resistance to high temperatures or ethanol, and its capacity to coagulate when rennet is present. Micelles of casein have a complicated structural makeup. They are produced by caseins that have been strongly phosphorylated, which then bind with calcium phosphate and assemble within themselves. There are numerous features that are determined by the proportion of κ-casein that is positioned on the surface. One of these properties is the stability against aggregation, which is a crucial component in the manufacturing of dairy products. The size of the micelle is also determined by this proportion.

In addition to being referred to as serum proteins, whey proteins account for around 20% of the total proteins and 0.7% of cow's milk. Similar to caseins, whey proteins are further classified into sub-fractions, which include β-lactoglobulin, α-lactoalbumin, and many minor proteins. A significant amount of β-lactoglobulin is the primary protein found in whey. It constitutes more than half of this protein fraction and, as a result, is classified into two genetic variants. These variants are distinguishable from one another by the substitution of a glycine residue (variant A) for another of aspartic acid (variant B). One of the characteristics of the β-lactoglobulin molecule is that it does not include any phosphorous, and it also has two disulfide groups and one free sulfhydryl group. Another protein that is plentiful in whey is α-lactoalbumin, which constitutes around 13% of the total proteins found in serum. The molecule of this protein is composed of four disulfide connections, and similar to β-lactoglobulin, it does not possess phosphorus [17]. Immunoglobulins, which make up 2% of serum proteins and are classified into four classes—IgG1, IgG2, IgA, and IgM—as well as bovine serum albumin (BSA)—are among the other proteins that can be found in whey, but in considerably smaller quantities. The primary milk proteins of a number of species that are ingested in large quantities, including humans. Recent research on milk proteins and associated substances has shown a wide range of health benefits that can be attributed to milk's composition. The use of milk and other dairy products has been hypothesised to have the potential to both prevent and postpone the onset of some types of cancer. It was discovered by Kim et al. that α-caseins, β-caseins, and β-lactoglobulin have the ability to shield cells from the harmful effects of oxidative stress, hence preventing the onset of cellular senescence. These chemicals have the potential to be utilised as dietary supplements for the purpose of preventing disorders that are related with ageing, particularly the development of skeletal muscular atrophy.

A Proteome of Milk:

Milk Secretome Protein Constituents and Their Functions:

The separation of milk into MFGM, soluble whey, and casein micelles can be accomplished through the process of centrifugation. Whole milk is normally centrifuged at a low speed of roughly 2,000 × g for twenty minutes at a temperature of four degrees Celsius in order to obtain MFGM. Whey proteins and caseins are found in a micellar form in skim milk, which is the remaining component of milk. Skim milk is referred to as "skim milk." Using ultracentrifugation, typically at a speed of 100,000 × g for a duration of one hour at a temperature of 4 degrees Celsius, skim milk can be further separated into soluble milk whey and a casein pellet. Whey is made up of a variety of components that are water-soluble, including proteins, carbs, and other minerals. The majority of the components that make up the casein pellet are casein proteins, minerals, insoluble salts, leukocytes, and cell debris that may be shed from the mammary epithelium during the process of lactation or milk production. During the process of lactation, there is a shifting of the relative abundance of soluble whey proteins and micellar caseins [18, 19]. In adult cow's milk, the ratio of whey protein to casein is roughly 20:80, which is a significant change from the approximately 80:20 ratio found in colostrum. The ratio of whey to casein in human milk decreases from 90:10 during the early stages of lactation to 50:50 during the latter stages of lactation.

Functioning Proteins Found in Milk:

Milk proteins have been suggested as a key source of functional meals, in addition to the inherent nutritional value that they possess. In general, functional foods are ones that are able to provide certain health benefits in addition to or in addition to the nutritional value that they possess. Both human MFGM (hMFGM) and bovine MFGM (bMFGM) have been the focus of a great deal of research over the course of the previous half-century, and they have been receiving an increasing amount of attention in recent times. Whey proteins and soluble caseins make up the remaining portion of milk's protein composition, while MFGM proteins are found in milk in insignificant amounts, accounting for around 1–4% of the total protein content in milk. It has been discovered that MFGM proteins have a variety of beneficial functions in the defence mechanisms of anti-inflammatory, antitumor, immunoregulation, and in lowering blood cholesterol. This is despite the fact that MFGM proteins would probably have less classical nutritional value as a source of essential amino acids in comparison to the major protein constituents of milk whey and caseins. The proteins found in cow's whey are considered to be an exceptional supply of necessary amino acids in comparison to a wide variety of other dietary proteins (Smithers 2008). They were discovered to possess anticancer properties, specifically against malignancies of the colon, breast, and prostate. Cow's whey was known to have favourable effects on host defence as early as the seventeenth century. These effects included the prevention of sepsis, the healing of wounds [20, 21], and the management of "stomach disease." It has been discovered that whey proteins, derived from both humans and cows, have the ability to stimulate cell proliferation and growth. This phenomenon is frequently attributed to the presence of growth factors and cytokines in milk produced by cows. Peptides that are generated from cow's casein, such as phosphopeptides and b-ca-somorphins, have already discovered fascinating applications in the pharmaceutical industry as well as in the field of dietary supplements.

A Secretion of Milk:

The process of milk production and secretion is a complicated one, and it is necessary to conduct additional fundamental study in order to improve our understanding of these processes. This kind of information may provide valuable insights into lactogenesis, which is the process of producing milk. These insights, in turn, may be utilised to fine-tune the amount of milk produced, the amount of fat it contains, and the quality of the protein in both breastfeeding mothers and lactating farm animals. Mammary epithelial cells are responsible for the production and secretion of

milk, which is a highly structured process that incorporates two different physiological routes. The exocytic route that involves the endoplasmic reticulum (ER), the Golgi complex, and secretory vesicles is responsible for the secretion of the aqueous portion of milk, which is comprised of water-soluble whey proteins and casein micelles. The fat component of milk is mostly made up of droplets of triacylglycerols that are encased in plasma membranes and surrounded by them. It is believed that the fat droplets are initially discharged from the ER membranes into the cytosol in the form of microlipid droplets that are rich in triacylglycerol and are partially covered by fat-soluble proteins. Microdroplets combine with one another to form bigger cytoplasmic lipid droplets. These droplets are then carried in a unidirectional manner to the apical plasma membrane, where they are encased by the plasma membrane and released through a process known as budding by the plasma membrane. The formation of a protective coating by the membrane around the lipid globules makes it possible for fat and fat-soluble substances to be dispersed throughout the milk plasma [21, 22]. The lipid droplets that are associated with the plasma membrane are referred to as MFGM, and the proteins that are secreted along with the milk fat are referred to as MFGM proteins. To gain a better understanding of the process behind milk secretion, proteomic methods were utilised. MFGM and its proteins were shown to have originated from the endoplasmic reticulum (ER) in mammary gland epithelial cells, according to the findings of a proteomic review conducted on mice. The actin cytoskeleton may be involved in the process of milk production, according to the findings of an in vitro study conducted on murine mammary epithelial cells.

Investigating the Milk Proteome: The Current State of Things:

A Proteome of MFGM:

There is a lack of thorough comprehension regarding the MFGM proteome and the function of its proteins. Regarding the change in MFGM protein levels and the regulatory mechanism that corresponds to it during breastfeeding, there is a relatively little amount of knowledge that is currently accessible. It is possible that the absence of cell lines that are capable of secreting milk is due, at least in part, to the fact that such information is lacking. Biochemical methods that have been used traditionally often focus on a single protein at a time. With a proteomic technique, on the other hand, the identities of a large number of proteins can be determined in a single experiment. We highlight current investigation of the MFGM proteome, which may throw further insight on some of the signalling and secretory pathways that are utilised by the mammary gland. MFGM is a diverse source of proteins, and we emphasise this analysis. In addition, we discuss several novel health benefits that are attributed to the presence of milk proteins. Reinhardt and Lippolis conducted the first comprehensive proteome study of MFGM by utilising SDS-PAGE to fractionate proteins (Reinhardt and Lippolis 2006). This study was the first of its kind. It was shown that bMFGM included a total of 116 different proteins. In addition, a quantitative method that utilised isobaric tags for relative and absolute quantification (iTRAQ) and was then followed by strong cation exchange (SCX) fractionation of tryptic peptides was utilised in order to assess the developmental alterations that occurred in the bMFGM proteome between day one colostrum and day seven milk [24, 25]. A change that is larger than or equal to a factor of two is observed in 26 of the 138 bMFGM proteins that have been discovered. These proteins are elevated in colostrum, and 19 of them are upregulated in milk. A number of bMFGM proteins that are involved in lipid transport, synthesis, and secretion were discovered to be elevated in day-7 milk in comparison to day-1 colostrum. These proteins include acyl-CoA synthetase, lanosterol synthase, lysophosphatidic acid acyltransferase, and fatty-acid binding protein. Several of the proteins that are elevated by colostrum are linked to processes that are involved in the transport, synthesis, and secretion of lipids throughout the body. On the other hand, we discovered that mucin 1 and 15

were shown to be elevated in day-1 colostrum in comparison to day-7 milk. Proteins that are involved in vesicle transport and protein trafficking were also discovered to exert distinct regulatory alterations between colostrum and milk [26, 27]. These changes may have ramifications for the synthesis and secretion of milk. A total of 95 protein spots were characterised for bMFGM by the utilisation of a two-dimensional gel electrophoresis (2DE) work flow. There were a few proteins that were discovered to have the same molecular weight but variable isoelectric points (pIs), which suggested that MFGM proteins could undergo post-translational modifications. An analogous proteomic system that made use of two-dimensional electrophoresis and isoelectric focusing (IEF) was utilised by Fong et al. (2007). Affolter and his colleagues were able to characterise 133 proteins from buttermilk protein (BMP) concentration, which is an industrial product that is obtained from bMFGM. This was accomplished by the use of a two-dimensional liquid chromatography (LC) technique, namely SCX fractionation of tryptic peptides that were derived from in vitro protein tryptic digestion, followed by reversed-phase LC/MS separation. The results of the label-free relative quantification between BMP and whey protein concentrate revealed that each of the two types of samples had representatives of proteins that were uniquely enriched. However, fatty acid binding protein, butyrophilin, xanthine dehydrogenase/oxidase, lactadherin, and adipophilin are all well-characterized bMFGM proteins that were discovered in increased abundance in BMP. In a recent study, SDS-PAGE was utilised to fractionate bMFGM, and the proteome was further enlarged to include a total of 232 proteins. This methodology is comparable to the approach that Reinhardt and Lippolis previously utilised. Charlwood et al. used two-dimensional enzymatic separation (DDE) to separate human milk MFGM proteins and characterise the primary species that were present. Over one hundred protein spots were analysed by Fortunato et al., who employed a setup that was very comparable. An enrichment of low-abundance proteins was accomplished by a complementary peptide ligand library (CPLL) in a recent work, which resulted in the identification of 191 proteins in human MFGM. It was discovered that a number of proteins that were identified were connected to the process of lipid metabolism and homeostasis. For the purpose of investigating the developmental change of the hMFGM proteome, a number of proteins that were shown to be elevated in either early or late lactation were investigated. In addition to being applied to cows and humans, the proteome analysis of MFGM has also been utilised on goats, sheep, and other agricultural animals. The manner by which MFGM is secreted shows that it may contain a significant amount of cellular proteins throughout the milking process. A small part of the whole MFGM proteome is probably comprised of the approximately 200 MFGM proteins that have been discovered up until this point. In order to acquire a more comprehensive understanding of the MFGM proteomes found in a variety of species, additional exploratory study is required.

Proteomes derived from Whey:

Historically, cheese and casein manufacturers regarded cow's whey as a nuisance. This perception persisted over the years. The transformation of whey proteins and other components from a waste stream into a valuable dairy raw material has been made possible by scientific and technological breakthroughs over the course of time. These advancements have also contributed to the establishment of the basis for the contemporary utilisation of whey in the food and allied sectors. Smithers (2008) has written a review that provides information regarding the significant developmental steps that have been taken in the whey sector. Considering that they are a source of vital amino acids, bovine whey proteins have an excellent nutritional value than other types of proteins. Additionally, whey proteins have been extensively acknowledged as a significant source of functional components that are engaged in a wide range of biological activities. This recognition has been widespread. The determination of the molecular identities of whey proteins is an essential step in the process of

comprehending the physiological advantages that whey proteins offer as a functional meal. Using two-dimensional enzymatic analysis, Smolenski was able to identify more than thirty whey proteins in cow's milk. Several of these proteins are associated with the function of the host's defence system. The use of CPLL to enrich low-abundance milk proteins resulted in the identification of 149 proteins that were found in cow's whey. The whey-derived protein concentrate was analysed with SCX protein fractionation, and the results revealed the presence of 244 proteins. Over the course of the past several years, additional proteins have been able to be discovered in cow's whey as a result of further advancements in proteomics technology. A total of 293 proteins were isolated from cow's whey by our team through the utilisation of mixed-bed IEX for the purpose of fractionation. Based on the comparison between colostrum and mature milk, it can be concluded that the proteome remains rather consistent throughout the process of breastfeeding. Furthermore, recent advancements in protein fractionation have made it possible for us to increase the number of proteins found in cow's milk to over 600 (this information will be published). At the same time that the proteome of cow's whey has been significantly enlarged, a corresponding endeavour has been carried out for human whey. Using immunoprecipitation, Palmer et al. were able to identify 151 proteins in human whey (Palmer et al. 2006). This was accomplished by depleting proteins that were naturally present in high quantities. The identification of 115 human whey proteins was made possible through the utilisation of a similar method that utilised CPLL to enhance low-abundance proteins. A number of proteins, including a-1-antitrypsin, carbonic anhydrase, galectin-3-binding protein, lactadherin, lipoprotein lipase, and tenascin, were discovered to have a greater abundance during the early stages of lactation. On the other hand, it was discovered that CD14, fatty-acid binding protein, lysozyme C, and a number of other proteins appeared to be more abundant during the later stages of breastfeeding. It was discovered by our team that low-abundance proteins can be evaluated with a sample load that is significantly bigger than the norm. By utilising this apparatus, we were able to increase the number of proteins found in human whey to 976. Having a more in-depth understanding of the human whey proteome enables us to outline a number of biological processes and signalling pathways that are either new to us or whose significance in milk was not previously appreciated.

Techniques for Separation The proteome is a complex matrix that contains thousands of different protein molecules. In order to completely characterise the proteome, it is necessary to conduct an analysis of each protein, including its forms and the amount of protein that is present within the matrix. Because of this, a stage of separation that came before it is required in order to correctly identify each animal protein. For many years, scientists have been trying to develop methods of fractionation, separation, concentration, and detection that have sufficient resolution to separate large amounts of proteins, as well as adequate sensibility and dynamic range to detect those proteins that are present in low abundance. The separation of all proteins that are contained in cells, tissues, and biofluids has been and continues to be a challenge for the scientific community. Scientists have relied on electrophoretic and chromatographic technologies, both individually and in combination, offline and online, in order to achieve the goal of protein separation. When it comes to the first of the technologies listed above, electrophoresis, one of the most often used and efficient techniques is the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique, which is based on the technology of two-dimensional gel electrophoresis (2DE).

The combination of two orthogonal separation techniques, namely isoelectric focusing (IEF) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS) PAGE, is the concept behind this method. In the first step of the IEF approach, proteins are sorted according to the distinct isoelectric points (pI) that they possess

(first dimension factor). After that, proteins are separated once more, but this time using SDS-PAGE (second dimension), which is based on the electrophoretic mobility of the proteins respectively. Coomassie, silver, or fluorescent stains are some of the methods that are utilised in the final step of the process, which involves visualising and quantifying the proteins. For the most part, the two-dimensional SDS-PAGE technique is not particularly effective when it comes to resolving proteins that have a molecular mass that is comparable. In a similar vein, proteins that have a comparable pI are difficult to resolve using IEF gels. This is especially true when attempting to analyse samples that include significant amounts of specific proteins, such as milk. In contrast, these constraints are largely alleviated when a two-dimensional technique is utilised, which results in a significant improvement in resolution. In spite of the fact that 2D-PAGE demonstrates a high level of effectiveness in the separation of proteomes, it has been discovered that it has a number of drawbacks. These include a limited capacity to identify proteins with low abundance, a limited efficiency in separating polypeptide chains with molecular masses that fall outside the range of 150-8 kDa, and the challenge of resolving proteins with a pH that is either extremely acidic (pI 3) or extremely basic (pI > 12).

It has been claimed that the efficacy of 2D-PAGE, as well as its potential for protein resolution, can be utilised in the analysis of milk proteomes. Within the scope of a study conducted by Hsieh et al. [40], a total of fifteen proteins were discovered in cow's milk. These proteins included three αs1-caseins, three αs2-caseins, three β-caseins, three κ-caseins, two β-lactoglobulins, and one serum albumin. The purpose of this study was to conduct a proteomic analysis of the effects of chymosin on the coagulation of individual milk proteins. Two-dimensional polyacrylamide gel electrophoresis (PAGE) was utilised by D'Auria and colleagues in order to identify the existence of four primary protein spots in milks derived from various animal species, including human milk. These spots corresponded to albumin, caseins, β-lactoglobulin, and α-lactalbumin. Milk from cows and milk from humans were found to contain up to eight and nine distinct proteins, respectively, after being isolated and identified. Capillary electrophoresis (CE) and liquid chromatography (LC) are two examples of procedures that are often employed in liquid phase separation, which is another form of separation that is becoming increasingly popular. Because of its multiple advantages over 2DPAGE, such as its great sensibility, superior dynamic range, easy automatization, and speed, its application in proteomic analysis has been increasing. This is owing to the fact that it is more efficient. On the other hand, this technique is extremely flexible because it makes use of a variety of separation mechanisms (such as size exclusion, reverse phase, and ion exchange), and as a result, it may be used to a wide range of situations. capable of analysing proteins of any molecular mass, whether they are basic or acidic. On the other hand, liquid phase separation has a poorer resolution than two-dimensional polyacrylamide gel electrophoresis (PAGE). When it comes to the separation of camel milk proteins, however, Omar et al. highlighted the superior resolution of the CE approach, which was found to be greater than that of the 2D-PAGE technique. All of the major caseins, including α-casein, β-casein, and κ-casein, as well as whey proteins, including α-lactoalbumin, serum albumin, and lactoferrin, were successfully identified and quantified by these authors through the use of this particular technique.

Immune-globulins (Ig):

Among the functional roles that whey proteins play, the one that is arguably the most well-understood is the defence of the host. Milk whey is loaded with a wide range of antimicrobial proteins, which serve two purposes: first, they protect the mammary gland that is responsible for lactation, and second, they offer protection to infants who are still nursing, when their immune systems are still developing. The results of a gene ontology (GO) analysis revealed that milk proteins associated to immune system functions are over-represented in comparison to the complete human

genome. This finding is in line with the fact that milk plays a protective role through immunological processes. Immunoglobulins (Igs) found in whey are almost certainly the most extensively researched of the several protective milk proteins that are available. During milk consumption, they protect the gut from pathogenic infections and improve the health of the gut. For instance, immunoglobulin A (IgA) can be found in milk in a secretory form that is referred to as secretory IgA (sIgA). In addition to giving its direct nutritional benefit as a source of amino acids, sIgA has the ability to combat pathogenic infections that are present in the lumen of the digestive tract. In contrast to sIgA, which is another immunoglobulin that has been found in human whey, immunoglobulin G (IgG) binds to antigens that may be present in the intestinal lumen. It then transfers these antigens across the epithelial layer by binding to the epithelial neonatal Fc receptor (FcRn), which recognises the Fc region of IgG. This process is referred to as transcytosis. Following this, antigens are discharged into the basal propria, which is the epithelial layer beneath the basal propria. This, in turn, causes B-cell proliferation, immunological activation, or tolerance. Different proteomic analyses were able to successfully identify immunoglobulins (Igs). In point of fact, proteomics uncovered previously unknown patterns of Igs expression. It is possible to employ a proteomic technique in order to evaluate the degree to which the expression levels of a particular protein fluctuate under different circumstances, such as between the stages of early and mature breastfeeding. In the early stages of breastfeeding, the levels of immunoglobulin A (sIgA) and immunoglobulin M (IgM) are higher, but the levels of immunoglobulin G (IgG) are higher in the later stages of lactation. The difference in expression between sIgA and IgG shows that milk's role shifts from providing direct pathogen-killing, as sIgA does in early lactation, to providing antigen intake, which is aided by IgG, with the goal of developing an infant's own immunity as breastfeeding proceeds. This shift in function is caused by the fact that sIgA is responsible for providing this function. According to the phenomenon known as transplacental transport of maternal IgG, it is interesting to note that the levels of IgG in infants are comparable to those of their mothers. After birth, infants do not begin producing their own IgG until approximately six months have passed. Infants experience a temporary lack of IgG during the first year of their lives due to a combination of factors, including the delayed generation of endogenous IgG and the catabolism of maternal IgG. This occurs between the ages of birth and one year. When compared to the simultaneous decline in IgG that occurs during early infancy, the rising supply of IgG that occurs from transitional milk to mature milk may give a mechanism to supplement this decrease. It is possible that the greater understanding of developing immune functions might be utilised to the production of functional foods that have optimal defence function at different stages of human development.

System that Complements:

Proteomic analysis can be utilised for a variety of purposes, including the measurement of expressional changes of a specific protein, as well as the comparison of relative abundances among proteins. There is a correlation between a higher protein content and a greater number of multiple spectral occurrences (spectral counts) in ordinary circumstances. It was determined that the relative protein abundances in milk could be determined by using the spectral counts of each protein. With the help of this method, we discovered that the proportion of sIgA detected in milk is significantly larger than that of IgG and IgM. Additionally, the method was utilised in order to investigate the relative abundance of many additional proteins that are members of the complement system. When it comes to the immune system, the complement system is an essential component that aids in the defence of the body against infectious agents. There are two primary mechanisms that have the potential to kickstart the complement system. In the process that is referred to as the "classical" pathway, the

complement system collaborates with antibodies that are able to identify the surface of pathogens. In the "alternative" pathway, the complement system is activated directly by the surfaces of the microorganisms, and the presence of antibodies is not necessary (Murphy 2012). We discovered that the spectral counts for the complement system proteins that were identified span a large range, ranging from around 6,000 counts for C3 to 2 counts for C1S. This indicates that these species likely have a vast dynamic range of concentration. According to the findings of KEGG pathway analysis (Huang et al. 2009a, b), the complement cascade is one of the pathways that is considerably abundant in human milk (Fig. 13.1b). C1r, C1s, and C2 are the complement components that are unique to the classical system, whereas CFB, CFI, and CFH are the complement components that are distinctive of the alternative pathway. When compared to C1r, C1s, and C2, the abundance of CFB, CFI, and CFH was shown to be significantly greater in our proteomic analysis. When compared to the classical system that is mediated by C1 and C2, the higher levels of CFB, CFI, and CFH in respect to C1 and C2 indicate that the alternative pathway plays a more significant role.

Both cytokines and chemokines:

The extracellular matrix (ECM) not only plays a role in the development of functions by way of interactions between the ECM and receptors, but it also serves as a reservoir for cytokines, which are essential for the processes of development. There are a wide variety of cytokines and chemokines that are known to be present in milk. Interleukins (ILs) and growth factors, such as transforming growth factor b (TGF-b), are examples of the types of cytokines that are commonly detected. Cytokines are not only utilised for the purpose of protecting the host, but they are also transmitted by the immune system to newborns and babies in order to assist them in mounting a defence against illness. In the case of milk cytokines, they play a role in the regulation of critical immunoregulatory processes, such as the maturation of the infant's intestinal immune system and the regulation of the secretion of sIgA. A proteomic technique has been utilised to discover a number of cytokines, cytokine receptors, and chemokines that have not been previously known to exist in human milk. In the case of tumour necrosis factor ligand superfamily members 11B, –13 (TNFSF11B, –13) and nicotinamide phosphor-ribosyltransferase (NAMPT), the results of GO analysis indicate that these proteins play a significant part in the development of immune cells. Through the utilisation of a proteomic technique, the low-abundance protein TGF-b2 and its receptor were successfully identified in cow's whey.

The Characterization of Milk and Other Dairy Products, Using Recent Developments in Proteomics Analysis:

During the 1980s, large amounts of 2DE methods were utilised for the mapping of milk proteomes, which included the mapping of the proteomes of some dairy products. Due to the fact that the protein patterns of various milks were obtained through the use of the aforementioned IEF and SDS-PAGE procedures, it has become feasible to compare the proteins that are most abundant in each of these milks. Quantification of protein expression in images makes it possible to compare the results of different studies, which has led to the widespread usage of two-dimensional gels. Nevertheless, proteins with a low abundance in the sample were not detected. Since the development of immobilised pH gradients (IPGs), this issue has been partially resolved, which has resulted in an increase in resolution, which has led to a more accurate visualisation of the protein profile. The researchers Conti et al. were able to successfully isolate and subsequently characterise a genetic variant of bovine β-lactoglobulin. This was accomplished by that particular method. Through the utilisation of preparative HPLC gel filtration and preparative IEF-IPG, they were able to achieve a protein that was extremely pure. Despite the fact that the utilisation of two-dimensional electron microscopy (DDE) technology was a significant step in the early stages of proteomics and yielded excellent outcomes in the past, the fact of the matter is that when it is used as the sole technique for protein

characterization, it is only possible to identify a portion of the protein from the analytical sample. When it comes to characterising protein profiles, the utilisation of 2DE technology in conjunction with particular detection techniques has resulted in an increase in sensitivity. In the field of milk proteomics, one of the most significant developments was the connection of two-dimensional enzymatic analysis to mass spectrometry. This combination of technologies, in which identification is accomplished by mass spectrometry (MS) after proteins have been separated by two-dimensional electrophoresis (DDE) and digested with trypsin, is an intriguing technology that has a great application in the research of dairy products. This method was successful in identifying up to thirty proteins in the aqueous extract of Swiss-type cheese during the ripening stage. These proteins included those that were expressed by the microorganisms that were utilised as starters, which were Streptococcus thermophilus ITG ST20 and Lactobacillus helveticus ITG LH1. The proteome and peptidome of kashz, a typical Iranian dairy product made from sour milk, were characterised by Pourjoula et al. [60]. This was accomplished by combining technologies such as SDS-PAGE and HPLC separation techniques coupled to mass spectrometry. The results demonstrated a protein profile that was comparable to that of yoghurt. Furthermore, exposure to in vitro plasmin hydrolysis revealed that kashz is actually a product that is the result of acid coagulation in the absence of γ-caseins. As a result of the presence of a great number of protein isoforms, the proteome of milk has been discovered to be quite diverse. Alternate mRNA splicing, single point mutations, and polymorphisms in the genome are also potential sources of these alterations. It is common for these modifications to the structure of proteins to result in alterations in the molecular mass and the net charge. Some of these isoforms, which are referred to as PTMs, are extremely significant when it comes to the process of characterising the proteome since they have an effect on a structural or functional feature of the protein that is being studied. The majority of the time, these changes are produced on cellular ribosomes after the process of protein synthesis. PTM is the second mechanism, following genetic polymorphism, that contributes to the expansion of the milk proteome, which in turn significantly increases its complexity. Numerous proteins go through a wide variety of post-translational modifications (PTMs), which include phosphorylation, acetylation, glycosylation, disulfide crosslinking, lipid conjugation, and proteolytic cleavages. These PTMs are essential for fundamental functions of milk proteins, such as micellar stability, which is essential for the production of cheese. It was discovered that a number of these unique alterations are influenced by a variety of various characteristics. As a result, Fang et al. [65] observed that the relative quantities of αs-casein phosphorylation isoforms in cow milk varied depending on the presence of both intrinsic and extrinsic variables. Parity, lactation stage, and genetic variation among animals positive were found to be contributors to phenotypic variation in the relative concentrations of individual αs-casein phosphorylation isoforms and in the phosphorylation degree of αs-caseins, as demonstrated by the utilisation of LC-ESI MS for the purpose of proteome characterization. Variations in the degree of phosphorylation of this type of caseins play a significant effect in the industrial qualities of milk. During their investigation into the factors that distinguish good coagulation from poor coagulation, as well as its absence, Frederiksen and colleagues discovered that the presence of a lower proportion of two less phosphorylated variants of α-casein (αs1-casein-8P and αs2-casein-11P) in Danish Holstein milk is associated with poorer coagulation when compared to other milk samples where its presence was higher. While the level of phosphorylation in κ-casein appears to be less critical for milk coagulation, it is still important. An investigation was conducted using the Holstein and Jersey cow breeds, which resulted in the identification of six distinct isoforms of κ-casein. These isoforms exhibited varying degrees of phosphorylation and glycosylation, with 95 to 96% of the total κ-casein being phosphorylated

and 34 to 35% of the κ-casein isoforms being glycosylated. Nevertheless, in spite of this, the authors of the study observed that the κ-casein PTM patterns were remarkably consistent, regardless of the milk's ability to coagulate.

Control of the Nutrients Found in Milk:

Milk proteins have been hypothesised to have new functional functions in the control and development of the immune system, maturation of the gastro-intestinal tract, and glucose homeostasis in recent decades. These hypotheses originate from research conducted in the twentieth century. It is possible that the question "Can the constituent proteins of milk regulate milk's own nutritional content?" is one of the most important questions to consider when discussing milk as a source of functional food.It has been suggested that variations in the quantity of MFGM proteins, which are involved in the process of lipid metabolism, could potentially have a role in the regulation of the amount of fat that is present in milk. Additionally, our proteomic data point to the possibility of a feedback regulatory mechanism that is provided by whey proteins in the process of controlling the amount of carbohydrates found in milk. It is common knowledge that milk contains a high concentration of carbohydrates, which rises from the transitional milk to the mature milk stage. As a result of the pathway study of milk proteins, it was discovered that a wide variety of them are involved in the metabolism of carbohydrates, with many of them being elevated in mature milk. The transformation of metabolic activity in breast-feeding mothers, who provide their infants with a higher milk carbohydrate content throughout later stages of lactation, is reflected in the overexpression of metabolic proteins implicated in diverse carbohydrate pathways in mature milk.

Milk proteins' capacity to be digested and their bioavailability:

There is evidence that immunoglobulins and immunoregulatory proteins found in milk are active in the digestive tract of neonates. IgG, IgA, and IgM immunoglobulins from bovine milk were used as an example. It was discovered that more than twenty percent of the immunoglobulins that were swallowed by humans were able to survive the process of gastric digestion and maintain their antibacterial properties. Additionally, it was discovered that the human digestive tract is resistant to digestion of a number of the proteins found in cow's milk. We used an in vitro model that was designed to simulate the human stomach in order to quantify the digestion of milk proteins. This was accomplished through the utilisation of a proteomics technique. Over two hundred different proteins were analysed in order to identify the rates of protein breakdown. After being subjected to digestion conditions for an extended period of time, a number of different quantities of proteins were discovered, which points to the common theme of incomplete hydrolysis of milk proteins throughout the process of stomach digestion (to be published). Milk contains a high number of bioactive peptides that have a variety of biological activities, despite the fact that there is growing evidence of the functional activities that are exerted by proteins that are either intact or badly broken down. Alternatively, the peptides can be produced from protein precursors through the process of hydrolysis, or they can be found naturally in milk. It is possible that these peptides behave as potential physiological modulators during the process of milk digestion in the gastrointestinal tract. This might occur most likely by receptor binding, transcytosis, or transepithelial transport. For instance, apelin peptides, which were recently discovered in cow's colostrum and milk, have been considered to play a role in the development of the gastrointestinal tract in neonates. There have been a number of extensive reviews published that cover a wide variety of milk-derived bioactives. In spite of the fact that the specifics of the activation mechanism are yet unknown, proteolytic digestion under gastric conditions has also been proposed as a method for the production of bioactive components. These components include proteins and peptides that have been partially digested but are still active. It was discovered through proteomic analysis of both human and cow's milk that the proteases that are present in milk proteins are abnormally high.

It has been suggested through in vitro models that certain cytokines and chemokines remain dormant and can be triggered through proteolytic processes throughout the process of gastric digestion. Recent research has shown that the TGF-b protein contained in milk is able to be retained during in vitro digestion, and as a result, it can improve the integrity of the gut.

Manufacturing of Milk:

modifications of proteins brought about by heat:

Milk whey is an excellent source of protein because it contains a high proportion of necessary amino acids in a ratio that is comparable to the composition that is required for human nutrition. Furthermore, several proteins are resistant to digestion by the stomach and contribute to a wide range of physiological activities in the intestinal lumen, blood stream, and organ tissues while also being resistant to digestion by the stomach. Cow's whey proteins have garnered a lot of attention due to the fact that whey is a significant component of both the human diet and the agricultural economy. In the course of industrial processing, however, liquid whey is typically heated in order to produce sterile liquid products or to get milk powder, such as whey protein concentrate (WPC), by the use of high-temperature spray-drying. The use of heat can have an effect on the nutritional value of whey proteins, particularly with regard to the bioavailability of amino acids and the functions of proteins and peptides it contains. In order to investigate the effects of thermal processing on the reactivity of milk proteins with lactose, the main whey proteins a-lactalbumin and b-lactoglobulin have been utilised as models. It has been demonstrated by a number of different groups that the Maillard reaction, which is a nonenzymatic reaction, is the principal change that takes place to milk proteins during the heat processing. Within the context of the reaction, the carbonyl group of milk lactose has the potential to react with lysine residues in proteins, which ultimately results in the synthesis of Amadori products. It is possible for the Amadori products to go through additional reactions, which can result in a wide range of different chemical structures, specifically advanced glycation end-products (AGEs). The breakdown of lactulosyl-lysine into advanced glycol-oxidation products, such as N-carboxymethyl-lysine, oxalic acid monolysinylamide, pentosidine, and galactosyl-b-pyranone, is one of the additional chemical changes that can be caused by heat treatment. While milk samples were being stored, it was discovered that protein crosslinking of a- and b-caseins as well as deamidation of as1-casein occurred. This discovery was made more recently. The inaccessibility of blocked lysine residues in Amadori products to enzymatic digestion in the gastrointestinal tract may, as a result, have an effect on the digestibility of proteins. In vitro enzymatic protein glycosylation is a typical pattern that can be detected in raw milk proteins. This motif is comparable to the heat-induced lactosylation of lysine residues. Through the use of GO analysis, for instance, we were able to identify 976 whey proteins in human milk, and more than 300 of those proteins are classed as glycoproteins. When compared to nonglycosylated proteins, glycoproteins have a lower digestibility, which may make them a source of a wide variety of bioactive peptides. These peptides have the potential to perform positive biological functions throughout the process of consuming dietary protein.

Proteomic Analysis of Protein Alterations and Their Characterization:

As one moves from pasteurisation (at temperatures ranging from 75 to 85 degrees Celsius for 15 to 30 seconds) to ultra-high temperature (UHT) sterilisation (at temperatures ranging from 138 to 145 degrees Celsius for 2 to 5 seconds) to spray-drying, the degrees of thermal treatment increase. In order to evaluate the quality of milk proteins following a variety of heat procedures, one of the most significant metrics to consider is the amount to which the Maillard reaction occurs. It is possible that the degree of lactosylation of proteins is substantially determined by the heat processes that are utilised during the processing of industrial milk. There has

been a long-standing interest in utilising proteomics to evaluate the impact of heat treatment on protein lactosylation and milk quality. This interest stems from the fact that proteomics has the ability to determine the modifications of proteins. The thermal history of bovine milk proteins has been evaluated using a variety of proteomic techniques that measure protein lactosylation. These approaches have also been utilised to discern the commercial quality of the proteins. The enrichment of lactosylated protein motifs and the subsequent proteomic analysis of these amino acid sequences is one method. An affinity chromatography technique that utilised m-aminophenylboronic acid-bound agarose was utilised in order to preferentially capture lactosylated peptides and to characterise the lactosylation sites of these peptides. The immobilised boronic acid moiety on the agarose beads was utilised to recognise cis-diol groups on lactosylated peptides, which were later characterised by mass spectrometry. This was done after the milk proteins were broken down into peptides by the endoproteinase LysC. High-abundance milk proteins that had a significantly elevated lactosylation level were found to have a number of lactosylation sites. The extent increased from milk that had been pasteurised to milk that had been treated with ultra-high temperature (UHT) to milk powder, followed by a trend that was consistent with a greater amount of heat treatment that each of the sample types underwent. In point of fact, it has been proven that the lactosylated protein forms are responsible for as much as three percent, thirty percent, and seventy percent of the b-lactoglobulin content in milk samples that have been pasteurised, treated with ultra-high temperature, and spray-dried, respectively. As part of their bottom-up proteomic technique, Hong et al. utilised trypsin in order to discover proteins that were present in milk-based infant formula. Due to the fact that trypsin selectively cleaves unmodified N-termini at lysine residues, it has been utilised for the purpose of investigating unmodified lysine residues. However, it does not cleave lactosylated sites. The study resulted in the identification of 154 peptides that were attributed to 31 different proteins that were found in infant formula. Based on the data, it appears that a specific number of protein domains do not undergo any modifications following severe heat treatment. During the storage process, when proteins are exposed to temperatures that are higher than normal, modifications can also take place. In order to better understand the processes of protein cross-linking, deamidation, and lactosylation that occur in UHT-treated milk samples while they are being stored, proteomics methods were utilised. It is possible for milk samples to be exposed to a wide range of temperatures while they are being stored, transported, or moved to other geographic regions. The stability of milk samples was investigated at a range of storage temperatures and for a variety of lengths of time. When stored at a higher temperature, such as forty degrees Celsius, a longer period of time results in a greater degree of protein cross-linking of a- and b-caseins, deamidation of as1-casein, and lactosylation of major whey proteins. This is most likely due to the accelerated thermodynamics that occur at moderately elevated thermal conditions in comparison to mild conditions.

To Ensure the Safety of Milk and Other Products Derived from Dairy:

The analysis of samples and the early detection of diseases that can affect the entire production chain in the dairy sector, such as mastitis, have been made possible by proteomics, which has been a revolutionary technique. Tof MALDI-TOF The use of MS has been essential in resolving this issue by enhancing the detection of bacteria in samples through the identification of biomarkers that are representative of ribosomal proteins from a variety of bacteria. In the case of microbiological procedures, where there is an inherent fault in the sample preparation and conditions of the culture medium, this methodology has been enhanced to the point where it can now be solved. When it comes to the mastitis disease that was discussed before, it typically results in the loss of a significant amount of milk on farms if it is not prevented or

controlled in the appropriate manner. Furthermore, if it is not recognised, it can also be considered an industrial problem. It is characterised as an inflammatory disease that is brought on by an infection of the udder tissue. Although it can be brought on by a wide variety of pathogenic microorganisms, the most common culprits are staphylococci, streptococci, and enterobacteria. Staphylococcus aureus and Streptococcus agalactiae are the most contagious and are responsible for the vast majority of infections. When the condition manifests itself, milk no longer possesses the capacity to undergo transformation, which results in alterations to the nutritional profile. According to the findings of Ogola and colleagues, the levels of non-casein components, salt, chloride, and free fatty acids increased, whereas the amounts of casein, lactose, caseins, potassium, and calcium fell in comparison to the usual levels that are seen in the milk of a healthy cow. The existence of a large number of somatic cells, which are in turn associated with a fluctuating proteome, is one of the defining characteristics of this disease. In light of this, proteomics is a significant step forward in the field of early detection of this aberration in milk. After utilising SDS-PAGE and LC-MS/MS, Pisanu et al. discovered that buffalo milk with mastitis had an increase in the number of 119 proteins that were associated with innate immune defence or structural activities. These proteins included vimentin, cathelicidins, histones, S100 and neutrophil granule proteins, haptoglobin, and lysozyme. During the same experiment, the authors discovered up to 33 lowering proteins that were determined to be involved in lipid metabolism. These proteins included butyrophilin, xanthine dehydrogenase/oxidase, and enzymes that are responsible for the production of lipids on the cellular level. Within the same vein, Abdelmegid et al. utilised proteomics to discover alterations in the protein profiles found in the whey of cows that were afflicted with mastitis. The goal of this research was to identify biomarkers that aid in the diagnosis of the condition. By combining two-dimensional differential gel electrophoresis (2D-DIGE) with liquid chromatography-mass spectrometry (LC-MS/MS), a total of 28 proteins that are extremely abundant were found in milk that was contaminated with S. aureus (Figure 2). Nine of these proteins were involved in host defence functions. These proteins include acute phase proteins, which are involved in innate immunity and antimicrobial functions (for example, serotransferrin, complement C3, fibrinogen γ-B chain, and cathepsin B). Additionally, there are proteins that are associated with the immune response to pathogens, such as polymeric immunoglobulin receptor-line protein, MHC class I antigen, and β-2-microglobulin.

Conclusion:

Over the course of several decades, investigations into the proteome of biological samples have been carried out, and numerous advancements have been made in order to acquire a comprehensive understanding of the full network of molecules that make up the proteome. There are up to five percent of proteins in milk that are found in low quantities and are difficult to detect. The milk proteome is obviously complex. In the beginning, the two-dimensional electrophoresis separation techniques were a technological revolution that made it possible to quantify proteins through the use of a milky matrix. On the other hand, just a portion of that matrix was managed to be successfully identified. The combination of two-dimensional electron microscopy (DDE) and mass spectrometry (MS) has resulted in an increase in the sensitivity to characterise protein profiles of samples, which has constitutes a significant advancement in the investigation of the milk proteome. Recent developments in mass spectrometry, such as the ESI and MALDI techniques, have made it possible to characterise proteomes in a more expedient and comprehensive manner. The current developments in milk proteomics have the potential to serve as a foundation for future research into milk proteins that are involved in physiological processes that are essential to human health. Numerous compelling pieces of data imply that proteomics may be able to offer direction in the production of

high-quality protein components derived from dairy milk processing. Proteomic studies of milk from humans and other animal species are predicted to yield significant contributions to this field. These studies are likely to be conducted systematically. A better understanding of human milk, for instance, would provide a reference point for the development of functional foods that are sourced from farm animals. A comprehensive proteome analysis that quantifies milk proteins at several phases of lactation is not yet accessible, despite the fact that the mammary gland continues to be incredibly active throughout the entire process of lactation. With the use of proteomic assessments of milk subproteomes, which have the potential to traverse the intestinal epithelium through either transcytosis or transepithelial transport, the bioavailability of milk proteins could be better understood. In the field of milk proteomics, the implementation of quantitative methods, such as stable isotope labelling by amino acids in cell culture (SILAC), continues to be extremely limited. Analysis of stable-isotopelabeled intestinal epithelial cell lines would provide a means to differentiate proteins that are either taken up from foods or synthesised internally by intestinal epithelial cells. This would also provide insights into the underlying molecular mechanisms that are involved in cellular responses when they are stimulated by different food sources.

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