# Comparison of extraction methods and nutritional benefits of proteins of milk and dairy products: A review

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Abstract: Proteins are the second most essential macromolecules after nucleic acids. This article aimed at the comparative analysis of extraction methods and nutritional benefits of milk and dairy products. Proteins form the body mass and perform several crucial tasks that include acting as a catalyst and carrying out different metabolic reactions in the body. Furthermore, protein acts as a transporter, transmits nerve impulses, provides mechanical support or immune protection, and controls growth. Several sources of proteins are present, but milk holds an important place due to its biological activities. The considerable health benefits of milk and its products are due to proteins. Yoghurt and cheese have significant importance among milk products. Proteins of milk and its products can be extracted by pH adjustment through homogenisation, centrifugation, and deproteinisation. There are several techniques for identifying and quantifying milk and product proteins. The Kjeldahl and spectrophotometric methods are the most widely used methods for quantifying proteins in milk and its products. Furthermore, these techniques include electrophoresis and chromatographic methods, including native gel electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), urea-PAGE, capillary electrophoresis, and isoelectric focusing. A few are chromatographic methods like reverse-phase high-performance liquid chromatography (RP-HPLC), size exclusion chromatography, and ion-exchange chromatography. Each technique has its advantages as well as disadvantages. The selection of the process depends upon the type of protein. The extracted proteins from milk and its products have many health or therapeutic effects that exhibit antimicrobial, antiproliferative, antioxidant, antihypertensive, anticancer, antiviral, and immunomodulatory effects. Yoghurt has prime importance among milk products because of its therapeutic effects and more protein.

**Keywords:** antioxidant effect; cheese; yoghurt; Kjeldahl method; urea-PAGE; antiproliferative effect; immunomodulatory effect

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Proteins are among the three macromolecules essential for the survival of living organisms, and they carry out crucial biological functions or processes in the living system. Proteins are complex molecules formed by combining amino acids in long chains (Branden and Tooze 2012). The 3D structure of the protein determines its function. The protein's crucial tasks include acting as enzymes and carrying out different reactions in the body, acting as a transporter, transmitting nerve impulses, providing mechanical support or immune protection, and controlling growth. It plays a vital role in building or repairing tissues and in the hair, skin, muscle, nails, and organs (Awata et al. 2020). Approximately 20% of the human body is composed of proteins (Hui et al. 2008).

According to the international Recommended Dietary Allowance (RDA), the protein intake should be 0.8 g per kg of body weight (Lonnie et al. 2018). The human body should take in a sufficient amount of protein. Otherwise, its deficiency poses serious problems such as swelling, degeneration of skin, stunt growth in children, high risk of infections, fatty liver, and abnormal blood coagulation (Patel et al. 2013; Khan et al. 2017). The sources of protein include meat, dairy products (milk, yoghurt, cheese), vegetables, rice, fish, eggs, cereal, or cereal-based products, and other animal products (Nadathur et al. 2016). The daily protein intake from plant sources is about 57%. The other references for the daily protein intake include meat (18%), fish (6%), dairy products (10%), and other animal products (9%) (Lonnie et al. 2018). In many countries, the daily protein intake is mainly from animal dairy products (Verbeke et al. 2015).

#### DAIRY PRODUCTS

Dairy products are also known as milk products, defined as products obtained or made from milk. Milk is considered the food that has importance in every stage of life. The milk composition is 4.6% lactose, 3.3% protein, 4% fat, 0.7% minerals, and 87.7% water (Jost 2000). The milk composition varies from mammal to mammal, principally to meet the developmental paces of the individual species (Domínguez Salas et al. 2019). The proteins in a mother's milk play an important role in the growth and development of infants or young. The protein amount in human milk is lower than in other mammals, such as cows and goats (Saarinen et al. 2002; Schaechter 2009). Examples of dairy products include milk, yoghurt, cheese, butter, cream, and ice cream, which are obtained using different methods or techniques (Aït-Kaddour et al. 2021).

Types of dairy products. Yoghurt is the most popular milk product all around the world. The production of this dairy product uses thermophilic bacteria in the fermentation process. The yoghurt composition is 4% carbohydrates, 5% fats, 9% protein, and 81-82% water (Lee and Lucey 2010). Yoghurt has many therapeutic effects: it increases appetite, lowers body weight, and decreases body fat (Chandan 2017). It has been observed that yoghurt consumption is reduced compared to other dairy products. It was noticed that most yoghurt consumers were healthy, young, fair, white females, non-diabetic, non-hypersensitive, and non--smokers (Samara et al. 2013; Wang et al. 2014). Yoghurt enhances the nutritional value of the diet, providing probiotic bacteria and, as a result, maintaining the natural microflora in the intestine. It plays an influential role in preventing fungal growth in the vagina (Gómez--Gallego et al. 2018b).

Cheese is a fermented dairy product with a variety of flavours and textures. It has a high nutritional value and is formed by milk coagulation. The cheese formation is done by using lactococcus, streptococcus, and lactobacillus bacteria through milk coagulation (Bachmann et al. 2009). Cheese has many types, and the composition of the cheeses varies according to its type. The most utilised cheddar cheese composition is 1.3% carbohydrates, 33.1% fat, 24.9% protein, and 36% water (Deshmukh and Vyas 2016). Almost a third of the world's milk is used in cheese manufacturing. The major therapeutic effect of cheese is its antioxidant or radical scavenging activities due to the antioxidant peptides it contains (Santiago-López et al. 2018). It is an excellent source of calcium and, as a result, protects the bones and enamel from demineralisation (Kashket and DePaola 2002).

Another example of dairy products is butter. It is a yellowish-whitish primary solid composed of fats, and it contains little protein. Butter is formed by concentrating the milk. For butter formation, a large amount of milk is required to produce a small amount of butter compared to the milk used in its formation (Couvreur et al. 2006). The butter composition is 80% fat, 1.5-2.0% salts, 16% water, and about 2% other solids. Butter has low nutritional importance and can cause diabetes, obesity, and cardiovascular diseases (Baer et al. 2001; Pimpin et al. 2016). A study was carried out to evaluate the nutritional value of butter made from cow's milk that was free of adulterants. According to the findings of this study, the inclusion of sodium chloride as a preservative in butter inhibits rancidity. It also helps to reduce the moisture level while increasing the solid content and ash

weight of the butter. It also prevents the denaturation of butter proteins and lipids, hence preserving their integrity (Rashid et al. 2017).

Cream is a dairy product with low nutritional value. The yellowish colour of the cream is due to the presence of carotenoids. A high-fat layer is skimmed from the milk before homogenisation. The general composition of the cream is 3.5% lactose, 30% fat, 2.4% protein, and 64% water (Liu et al. 2021a). It does not have any therapeutic effects and uses in foods; thus, it plays a role in gaining weight (Dougkas et al. 2019).

Importance of dairy products. Milk is a widely used refreshment and an essential component of the diet. Milk production is 730 million tons per year (Hemme and Otte 2010; FAO 2012). Various dairy products such as butter, cream, yoghurt, kefir, cheese, etc. have their uses and drawbacks depending upon their composition. The dairy products that have been discussed above are the most widely utilised products all around the world. But based on the nutritional value, yoghurt, milk, and cheese have prime importance over cream and butter (Baer et al. 2001; Pimpin et al. 2016). The supreme importance of milk, yoghurt, and cheese over butter and cream is the wide-ranging protein. In contrast, cream and milk contain a large amount of fat and little or no protein (Baer et al. 2001; Pimpin et al. 2016; Chandan 2017).

## ANALYTICAL CONSIDERATION OF MILK, CHEESE, AND YOGHURT PROTEINS

Several techniques can obtain proteins from milk, cheese, and yoghurt. Every technique has its advantages as well as disadvantages. The techniques mostly used to extract proteins from milk, cheese, and yoghurt are homogenisation, deproteinisation, and centrifu-

gation (De Noni and Cattaneo 2010). The separation and fractionation techniques include electrophoresis and chromatographic methods. The electrophoresis and chromatographic techniques are further divided into types (Arakawa 2006). Spectrophotometer and Kjeldahl methods are the most widely used methods for quantifying milk protein (Olson and Markwell 2007). Figure 1 shows the involved techniques and steps from protein extraction to its quantification. Figure 2 indicates the techniques with advantages and disadvantages.

#### IMPORTANCE OF MILK PROTEINS

Milk is the most widely consumed food all around the world. Milk is famous not only for its high nutritional value but also due to its medicinal properties. Milk proteins play a crucial role in preventing several diseases, such as cardiovascular diseases, obesity, and cancer. The proteins of the milk are renowned for both pharmacological and therapeutic properties. Two types of proteins are present in milk: major milk proteins and minor milk proteins (Goulding et al. 2020). Major milk proteins are further divided into casein and whey protein. Casein comprises 80% of protein, while whey contains 20% of the total proteins; other proteins are present in trace amounts. Whey and casein proteins differ based on their physiological and biochemical properties. The different major and minor milk proteins and their functions are shown in Figure 3.

#### IMPORTANCE OF WHEY PROTEINS

The biological functions that are performed by the whey proteins are as follows:

**Anticancer effects.** Several studies have shown the effect of whey proteins against cancer by promoting humoral and cell-mediated immunity. The fractionation

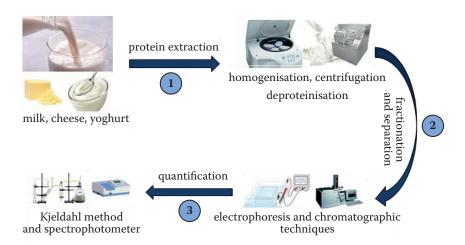


Figure 1. Techniques involved in protein extraction

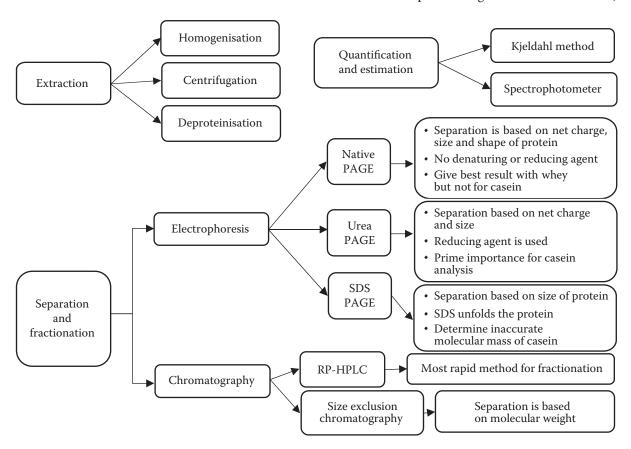


Figure 2. Techniques involved from protein extraction to quantification with advantages and disadvantages RP-HPLC – reverse-phase high-performance liquid chromatography; SDS – sodium dodecyl sulfate; PAGE – polyacrylamide gel electrophoresis

of whey proteins such as lactoferrin (LF),  $\beta$ -lactoglobulin ( $\beta$ -LG),  $\alpha$ -lactalbumin ( $\alpha$ -LA), and immunoglobulins (Igs) has shown anticarcinogenic properties (Cuevas-Gómez et al. 2021; Khan et al. 2021). The liver pro-

duces a tripeptide known as glutathione that protects the intestine from tumours.  $\beta$ -LG is rich in cysteine and helps in the synthesis of glutathione; as a result, it protects against intestinal tumours (Le Maux et al. 2012).

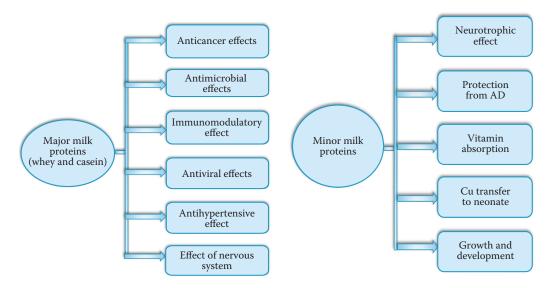


Figure 3. Importance of milk major and minor proteins

 $\alpha$ -LA has shown antiproliferative and apoptotic activity in cancer cell lines (Sternhagen and Allen 2001). LF has been shown to reduce several cancers, such as breast, liver, colon, oesophagus, and bladder cancer. It alters the genetic expressions of the cell cycle and apoptosis by interacting with receptors (Davoodi et al. 2013).

**Antibacterial effect.** LF has shown antimicrobial properties by binding with the lipid of the lipopoly-saccharides of the bacterial membrane and, as a result, increasing the permeability of the membrane (Liu et al. 2021b).

Antiviral effect. LF,  $\beta$ -LG, and  $\alpha$ -LA have shown an antiviral role against human immunodeficiency virus (HIV) by inhibiting the activity of reverse transcriptase, integrase, and protease (Ng et al. 2015).

**Immunomodulatory effect.** Several studies have shown that several immunomodulators can be promoted by the intake of milk whey fraction. A significant increase was observed by the intake of whey fraction, CD4+, and CD8+ lymphocytes (Ford et al. 2001).

### IMPORTANCE OF CASEIN PROTEINS

#### **Anticancer effects**

The study has shown that casein protein is effective against colon cancer. It protects against cancer by inhibiting the conversion of procarcinogen glucuronides to carcinogens by inhibiting the beta-glucuronides, an enzyme produced by the intestine bacteria. It is involved in this conversion (Parodi 2007). In an analysis, a significant reduction in colon tumours was observed in rats by feeding them casein or whey protein mixture (Barick et al. 2021).

#### Hypocholesterolaemia effects

A study showed that casein and soy protein intake reduces low-density lipoprotein (LDL) (Meinertz et al. 1989). Another crossover study observed that casein intake reduces lipoprotein concentration by 50% compared to soy protein. It showed a reduction in the concentration of LDL, high-density lipoprotein (HDL), and cholesterol (Nilausen and Meinertz 1999). An Australian study showed that the daily intake of 25 g of casein could reduce blood cholesterol concentration and lower the risk of developing heart diseases (Chin-Dusting et al. 2006).

#### **Antimicrobial effects**

The fragment of casein,  $\alpha$ S1-casein, has shown activity against both gram-positive and gram-negative

bacteria. Studies have demonstrated that casein acts against the bacteria by enhancing or promoting the activity of macrophages through the upregulation of the major histocompatibility complex class 2 (MHC 2) and then increasing the phagocytic activity (Sandré et al. 2001). Another peptide of casein has shown activity against many bacteria such as *Diplococcus pneumoniae*, staphylococci, *Streptococcus pyogenes*, and *Sarcina* spp. (Lahov and Regelson 1996).

#### **Antihypertensive effects**

An analysis of mildly hypertensive and normal people showed that 10 g of casein intake for four weeks had an antihypertensive effect (FitzGerald and Meisel 2000). In a crossover study, it has been observed that casein-derived peptides lower blood pressure in mildly hypertensive people (Turpeinen et al. 2011).

#### Minor milk proteins

The importance or role of minor milk proteins is as follows.

*Vitamin-binding proteins.* These proteins can bind with many vitamins, such as biotin, cobalamin, folic acid, and retinol. Binding with vitamins allows the absorption of the vitamin from the intestine. Also, it shows the activity against intestinal bacteria by inhibiting the availability of vitamins to bacteria (Wynn et al. 2011).

Metal-binding proteins. In milk, several metal-binding proteins include ferroxidase, LF, transferrin, and glutathione oxidase that bind with Fe, Se, and Cu. An iron-binding glycoprotein of milk is known as LF. The cleavage of LF releases lactoferricin, which plays an essential role against the activity of bacteria compared to LF. LF is also rich in sialic acid and, as a result, provides protection against Alzheimer's disease and plays an essential role in brain and memory development (Fox 2003).

Another metal-binding protein is ceruloplasmin. This protein binds with the six atoms of Cu and plays an essential role in transferring the Cu to the neonates (O'Mahony et al. 2013).

#### **Growth factors**

The peptide hormones present in milk are known as growth factors, such as insulin and insulin-like growth factors 1 and 2. These growth factors play a crucial role in the development and growth of neonates and newborns. In humans, three growth factors are present such as  $\alpha 1$ ,  $\alpha 2$ , and  $\beta$ . They play an essential role in developing neonate and mammary glands (Gauthier et al. 2006).

#### **Glycoproteins**

Besides LF, another glycoprotein in milk is known as prosaposin. It has a neurotrophic effect and plays a vital role in repairing, developing, and maintaining nerve tissue. This protein also plays an anticarcinogenic role and protects from breast cancer development (Campana et al. 1999; Patton 1999).

#### IMPORTANCE OF CHEESE PROTEINS

Cheese is a rich source of essential nutrients and has been consumed by people worldwide. It has greater health importance because of protein, fat, minerals, and vitamins. The highest amount of protein and fat in cheese is its high nutritional value. Recent advances in nutrition science have mentioned the health and nutritional benefits of cheese (Walther et al. 2008). The proteolysis of cheese protein secretes several bioactive peptides. Among all the dairy products, cheese has greater nutritional importance because of the higher amount of protein. The proteins of cheese are better digestible and also contain a significant amount of lysine. The absence of Maillard reactions is the primary reason for the bioavailability of lysine. The cheese protein has several nutritional benefits and health importance such as antimicrobial, antiproliferative, antioxidant, opioid, and antihypertensive effects, as shown in Figure 3 (Expósito and Recio 2006). The function or significance of these bioactive peptides and proteins are shown in Figure 4.

Antioxidant effects. Many studies have shown the importance of peptides or proteins derived from cheese because of their antioxidant effect. Oxidative stress produces many reactive oxygen species or reduces the antioxidant capability of cells and contributes to many diseases such as cancer, inflammation, cardiovascular disease, ageing, metabolic, and neurogenerative conditions (Erdmann et al. 2008). Among the food-derived peptides, cheese-derived peptides have more importance. In 2006, the first study was reported on cheese-derived peptide significance against the oxygen reactive species (Silva et al. 2006, 2012). The three peptides from  $\alpha$ s1- and  $\beta$ -casein in cheese have shown radical scavenging or antioxidant activity (Timón et al. 2014).

Antiproliferative effects. Tremendous publications show the importance or effect of LF and lactoferricin against cancer (De Leblanc et al. 2005; Mader et al. 2005). In an experiment, 12 commercial cheese products were used to check the growth in promyelocytic leukemia cells, and among them, a few cheese types showed the highest activity (Yasuda et al. 2010). It was

observed that cheese containing whey had a more antiproliferative potential. Mozzarella containing whey has shown a 43% reduction in cell proliferation in cancer cell lines (De Simone et al. 2009). No such more significant effect of whey was observed in milk. This indicates that this bioactivity of whey occurred during the production of cheese. The peptides derived from  $\kappa$ -casein and  $\beta$ -casein have also shown antiproliferative potential (De Simone et al. 2011).

**Antihypertensive effects.** Hypertension is a chronic disease affecting more than 30% of the population in developed countries that can further lead to stroke and cardiovascular diseases (Chen et al. 2009). The pathway that can control blood pressure is the renin--angiotensin-aldosterone system. The vital component of this pathway is the angiotensin-converting enzyme (ACE) involved in the conversion of angiotensin I into angiotensin II. This results in the release of aldosterone, increased sodium ion concentration, contraction of blood vessels, and finally, increased blood pressure (Nussberger 2007). The studies have shown that the purified form of the cheese-derived peptides shows a more significant effect in reducing blood pressure by lowering or inhibiting the ACE activity (Gómez--Ruiz et al. 2006). The peptides derived from the αs1and β-casein have shown moderate potential inhibition of ACE activity. By inhibiting the activity of ACE, these peptides help in the lowering of blood pressure and prevention from diseases that are due to high blood pressure (del Mar Contreras et al. 2009).

Antimicrobial effects. It has been observed that antimicrobial peptides are mainly obtained from milk and its products (López-Expósito et al. 2012). In several studies, the antimicrobial role of cheese-derived peptides has been observed against several bacteria

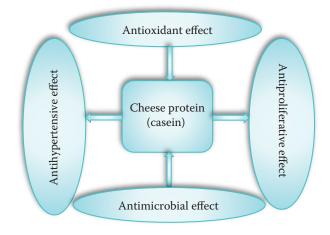


Figure 4. Major therapeutic effects of cheese

such as *Listeria ivanovii*, *Escherichia coli*, and *Listeria monocytogenes* (Lignitto et al. 2012). These antibacterial peptides were mostly derived from  $\alpha$ s1-casein and  $\alpha$ s2-casein (López-Expósito et al. 2012).

**Effect on the nervous system.** Opioid receptors are present in the endocrine, immune, and nervous systems. The ligand for these receptors is derived chiefly from the milk-derived peptides, i.e. β-casomorphins (β-BCMs). These opioid receptors are involved in several effects and functions: euphoria, sedation, appetite and eating behaviour, nausea and vomiting, and respiratory depression. It has been observed that BCMs have analgesic activity (De Noni et al. 2009). Many studies have shown that BCMs are the agonist of the  $\mu$ -receptor. The BCMs can be derived from the β-casein. The β-casein is present in milk and its products. In addition to the effect of BCMs on the nervous system, it has also shown an effect in intestinal mucus secretion, increasing insulin level, and decreasing glucose level (Yin et al. 2010). Only a few studies have shown the presence of BCMs in cheeses. BCM5 and BCM7 presence has been observed in Gouda, Brie, Cheddar, and Gorgonzola cheeses (De Noni and Cattaneo 2010).

Role in mineral absorption. *In vitro* and *in vivo* studies have shown that the enzymatic hydrolysis of casein fractions releases many bioactive peptides such as casein phosphopeptides (CPPs). These bioactive peptides play an essential role as immunomodulatory, antimicrobial, antioxidant, and gastric secretion regulators. Several studies have shown that these peptides can be obtained from different cheeses. In cheese, CPPs can be obtained from the fraction of casein such as  $\alpha$ s1-casein,  $\alpha$ s2-casein and  $\beta$ -casein (López-Expósito et al. 2017).

It has been observed that CPPs are released in the gut and pile up in the ileum of the small intestine, where the absorption of several minerals takes place (Zidane et al. 2012). *In vivo* studies have demonstrated that the presence of CPPs helps in the absorption of minerals, especially calcium absorption. Several studies on the role of CPPs in the absorption of minerals were conducted, but the results are still controversial. This controversy is due to different experimental approaches to assessing the bioavailability of minerals (Scholz-Ahrens and Schrezenmeir 2000; Meisel and FitzGerald 2003; Mills et al. 2011).

#### IMPORTANCE OF YOGHURT PROTEINS

Yoghurt is a fermented dairy product and has importance due to its high nutritional value in the food industry. It aids in the digestion of food and also

strengthens the immune system. It also helps manage weight and protects against several bone-related diseases such as osteoporosis. The protein content of yoghurt is higher than in milk. The whey protein of the yoghurt helps in managing weight better than the milk whey. Casein also plays an influential role in absorbing minerals in the body. Both of these proteins have several properties that include antioxidant, antiproliferative, antimicrobial, and antitumor properties (Gómez-Gallego et al. 2018a). The role or importance of yoghurt protein is shown in Figure 5.

Antioxidant effect. Oxidative stress produces many reactive oxygen species or reduces the antioxidant capability of cells and contributes to many diseases such as cancer, inflammation, cardiovascular disease, ageing, metabolic, and neurogenerative diseases (Erdmann et al. 2008). It has been reported that the LF protein of the yoghurt has antioxidant or radical scavenging activity (Gjorgievski et al. 2014).  $\alpha_{S1}$ -casein has also shown an antioxidant effect by chelating the ions (Jiménez et al. 2008; Perna et al. 2018).

Antiproliferative effect. Several studies have shown the effect of whey proteins against cancer by promoting humoral and cell-mediated immunity. The fractionation of whey proteins such as LF,  $\beta$ -LG,  $\alpha$ -LA, and immunoglobulin (Igs) has shown anticarcinogenic properties (McIntosh et al. 1998). Tremendous publications show the importance or effect of LF and lactoferricin against cancer (De Leblanc et al. 2005; Mader et al. 2005). Studies have shown that yoghurt proteins play an essential role in inhibiting tumour cell proliferation. Antiproliferative or anticancer effects have also been shown by  $\beta$ -LG and  $\alpha$ -LA (Ayyash et al. 2018).

**Mineral absorption.** Casein-derived biopeptides can absorb the minerals from the small intestine (Park

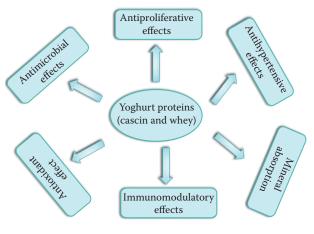


Figure 5. Therapeutic effects of yoghurt

and Nam 2015). The CPP are released in the gut and pile up in the small intestine ileum, where several minerals occur (Zidane et al. 2012). *In vivo* studies have demonstrated that the presence of CPPs helps in the absorption of minerals, especially calcium absorption (Scholz-Ahrens and Schrezenmeir 2000).

Antimicrobial effect. Milk and cheese-derived peptides and proteins have shown antibacterial effects like the fragment of casein, αS1-casein has demonstrated the activity against both gram-positive and gram-negative bacteria. Studies have shown that casein acts against the bacteria by enhancing or promoting the activity of macrophages through the upregulation of the MHC 2 and then it increases phagocytic activity (Sandré et al. 2001). The antibacterial effect has also been shown in yoghurt casein (Perna et al. 2013, 2018).

**Immunomodulatory effect.** Several studies have shown that several immunomodulators can be promoted by the intake of the whey fraction of milk or fermented milk product. A significant increase was observed by the intake of whey fractions in CD4+ and CD8+ lymphocytes (Ford et al. 2001; Ebringer et al. 2008).

Effect on nervous system. Opioid receptors are present in the endocrine, immune, and nervous systems. These opioid receptors are involved in several effects and functions: euphoria, sedation, appetite and eating behaviour, nausea and vomiting, and respiratory depression. Lactoferroxin acts as an opioid antagonist while  $\alpha$ -lactorphin and  $\beta$ -lactorphin act as opioid agonists (Ebringer et al. 2008).

Antihypertensive effect. Hypertension is a chronic disease affecting more than 30% of the population in developed countries that can further lead to stroke and cardiovascular diseases (Chen et al. 2009). The pathway that can be used to control blood pressure is the reninangiotensin-aldosterone system. The critical component of this pathway is ACE involved in the conversion of angiotensin I into angiotensin II. This results in the release of aldosterone, increased sodium ion concentration, contraction of blood vessels, and finally, increased blood pressure (Nussberger 2007). Studies have shown that  $\beta$ -LG-derived peptides can lower blood pressure by inhibiting ACE activity (Sipola et al. 2002).

#### **DISCUSSION**

Several techniques extract the protein from the milk or its products. These techniques include homogenisation, centrifugation, and deproteinisation by adjusting pH (Nguyen et al. 2015). This technique can extract protein from milk, cheese, and yoghurt. The protein from

the milk can also be extracted using other methods that include the urea method, trichloroacetic acid (TCA)/ acetone method, and chloroform/methanol method. The chloroform/methanol method is at a high advantage among the milk protein extraction methods because it allows polar and non-polar protein recovery in a single step. This method is less time-consuming because it enables protein extraction in a single step (Vincent et al. 2016). In an experiment, results have shown that the extraction of protein by using these methods and then the analysis of these proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have demonstrated that the chloroform/ methanol method is superior to other methods because it has shown the best result by extracting the vital milk protein such as  $\alpha$ -S1-,  $\alpha$ -S2-,  $\beta$ -, and  $\kappa$ -forms,  $\beta$ -LG, α-LA, LF, lactoperoxidase, and immunoglobulins (Igs). So, this protein extraction method is the most preferable, and TCA/acetone is the least preferable method (Vincent et al. 2016).

Several methods or techniques for separating and identifying milk or dairy product proteins include electrophoresis methods and chromatographic techniques. These techniques are further divided into different types. Electrophoresis is further divided into native gel electrophoresis, SDS-PAGE, urea-PAGE, isoelectric pH, and electrophoresis. In contrast, chromatographic techniques are divided into ion-exchange electrophoresis, size exclusion chromatography, mass spectrometry-liquid chromatography (MS-LC), and reverse-phase high-performance liquid chromatography (RP-HPLC). All of these techniques can potentially separate or identify the milk and its product proteins, but every method has its pitfalls (Goulding et al. 2020). Among electrophoresis methods, the most utilised techniques for extracting milk or its product proteins are native gel electrophoresis, urea-PAGE, and SDS--PAGE, with its advantages and disadvantages. In native gel electrophoresis, proteins do not denature and remain inactive (Sharma et al. 2021). It has been observed that this technique is not suited for separating the casein protein. But this technique can be used for fractionation, separation, and identification of whey protein. This technique is not suitable for cheese because it contains only casein proteins (McSweeney and Fox 1997).

In contrast, it can be used to separate the whey protein from milk and yoghurt. As the molecular mass ladder cannot be used in this technique, identifying proteins becomes difficult. So, this is not preferable for fractionation and protein identification (Sharma et al. 2021).

In urea-PAGE electrophoresis, separation of proteins occurs based on the size of proteins. This technique can separate or identify the casein protein fractionation (Sharma et al. 2021). So, this technique is preferable for the fractionation and identification of cheese protein. In this method, whey proteins appear as a smear and challenging to determine the molecular masses. So, this technique is not preferable for the fractionation of milk or yoghurt proteins (Sharma et al. 2021).

The most preferred technique for fractionation and identification of milk or milk products (yoghurt, cheese) is SDS-PAGE. If there is a reducing agent in this technique, it would be known as SDS-R-PAGE or the absence of a reducing agent (non-reducing) is known as SDS-NR-PAGE (Sharma et al. 2021). The denaturation of the protein occurs in SDS-R-PAGE. This disadvantage leads to the determination of the inaccurate molecular weight of β-casein and αs1-casein. This anonymous behaviour is due to the binding of β-casein with large SDS and to electrophoretic mobility rather than to the actual mobility. The separation of proteins of similar molecular masses is difficult using this technique (Sharma et al. 2021). The literature has shown that SDS-PAGE is better for separating milk or yoghurt proteins compared to the native-PAGE or urea-PAGE (Gauvin et al. 2018; Jansson et al. 2019; Nurup et al. 2020). This is because SDS can determine casein and whey protein simultaneously. However, urea-PAGE is the preferable method for determining only casein (cheese) protein.

In comparison, native-PAGE is the preferable method for determining whey protein (milk or yoghurt) (Sharma et al. 2021). Among the chromatographic techniques, the most widely used process for the fractionation or identification of milk, yoghurt, and cheese protein is reversed phase-high pressure liquid chromatography. According to the literature, this technique is more preferable for cheese, milk, or yoghurt fractionation or identification than ion exchange chromatography or size exclusion chromatography (Veloso et al. 2002; Bramanti et al. 2003; Crowley 2016; Ma et al. 2017).

There are also several methods for quantifying protein in milk, cheese, and yoghurt. These methods include the Kjeldahl method, Lowry method, biuret, infrared spectroscopy, and spectrophotometric method. The Lowry technique, which is quick and sensitive, was used to examine the protein level in milk. The Lowry assay has three primary advantages: *i*) convenience due to reagent formulation stability, *ii*) detection of protein in both colourless and coloured biological materials without sacrificing sensitivity, and *iii*) assaying proteins

at extremely low concentrations. This innovative assay will be relevant to the quantitative detection of protein in both colourless and coloured biological sample homogenates, including lipid-rich (e.g. avocado) and difficult-to-homogenise samples (Upreti et al. 2012; Arif et al. 2018, 2021).

But the literature has shown that the most widely used method for the quantification of milk, cheese, and yoghurt protein is the Kjeldahl method compared to the other methods and it has given the best results (Ilirjana and Gentiana 2013; Nielsen and Ogden 2015; Margolies and Barbano 2018).

The literature has shown that cheese contains more protein than milk or yoghurt. The percentage of protein in different milk or milk products is milk 3.3%, cheese 24.9%, and yoghurt 9% (Jost 2000; Lee and Lucey 2010; Deshmukh and Vyas 2016). The protein in milk or its products plays several crucial roles in maintaining good health and preventing several diseases. The roles played by the milk proteins include anticancer effect, immunomodulatory effect, antiviral effect, hypercholesterolaemic effect, antihypertensive effect, neurotrophic effect, and binding with vitamins or their absorption in bodies; and preventing from various diseases such as Alzheimer's disease. Milk plays a crucial role in the development and growth of newborn or young children (Davoodi et al. 2016). The therapeutic effects of the cheese protein include antioxidant effect, antihypertensive effect, antimicrobial effect, and antiproliferative effect. The intake of cheese affects the nervous system. Its  $\beta$ -casein protein binds with an opioid receptor and controls the pain, appetite, or euphoric state. Cheese protein also plays an essential role in absorbing minerals from the small intestine (López-Expósito et al. 2017). Another dairy product with significant therapeutic effect or importance is yoghurt. It also plays several crucial roles in maintaining health and preventing several diseases. These roles include immunomodulatory effect, antihypertensive effect, antiproliferative effect, antioxidant effect, antitumor or anticancer effect, antimicrobial effect, and neuropathic effect. The peptides obtained from the proteolytic cleavage of yoghurt peptides have shown their role in mineral absorption from the small intestine (Marette and Picard-Deland 2014).

According to the literature, cheese contains more protein than milk or yoghurt. But cheese has only one protein (casein) and is deprived of whey protein. So, this fact decreases the nutritional value or importance of cheese protein. At the same time, milk and yoghurt contain both types of protein and have a higher

nutritional reputation than cheese. But yoghurt has supreme importance over milk due to the more immense amount of protein. Besides the therapeutic effect of the yoghurt protein, it also plays an essential role in the digestion of food and it digests quickly in the body (Marette and Picard-Deland 2014).

## CONCLUSION AND FUTURE PERSPECTIVES

Milk and its products play a crucial role in maintaining good health and preventing several diseases. Cheese contains more protein than milk or yoghurt. But it has only one kind of protein (casein) and is deprived of whey protein that lessens its nutritional value. However, milk and yoghurt contain both types of protein and have more nutritional importance than cheese. Yoghurt has supreme importance over milk due to the more considerable amount of protein. Besides the therapeutic effect of the yoghurt protein, it also plays a vital role in the digestion of food and is also digested quickly in the body. Several techniques can be used for the extraction, separation, fractionation, and quantification of protein, and each has its advantages and disadvantages. The selection of the extraction method depends upon the type of protein.

As the proteins from milk and milk products offered therapeutic effects, they can cure several diseases. For example, LF and lactoferricin have shown antiviral activity against HPS and the hepatitis C virus. These can be combined with ribavirin to treat patients suffering from viral diseases. Because of the therapeutic effects, peptides and proteins from milk and milk products can be used as a nutraceutical to treat several conditions. However, the exact mechanisms of some proteins and peptides are not known, and some are controversial. So, there is a dire need to determine these mechanisms and be used in the nutraceutical and pharmaceutical industry against diseases.

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