Whey Proteins and Its Derivatives: Bioactivity, Functionality, and Current Applications

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Abstract: With the increased consumer demand for nutritional foods, it is important to develop value-added products, which will not only catch the attention of a wider consumer group but also provide greater benefits in terms of enhanced nutrition and functionality. Milk whey proteins are one of the most valued constituents due to their nutritional and techno-functional attributes. Whey proteins are rich in bioactive peptides, possessing bioactive properties such as being antioxidant and antihypertensive as well as having antimicrobial activities, which, when ingested, confers several health benefits. These peptides have the potential to be used as an active food ingredient in the production of functional foods. In addition to their bioactivities, whey proteins are known to possess enhanced functional attributes that allow them to be utilized in broad applications, such as an encapsulating agent or carrier materials to entrap bioactive compounds, emulsification, and in edible and active packaging. Hence, over the recent years, several whey protein-based ingredients have been developed and utilized in making formulations for a wide range of foods to harness their beneficial properties. This review highlights the bioactive properties, functional characteristics, associated processing limitations, and applications of different whey protein fractions and derivatives in the field of food formulations, encapsulation, and packaging.

Keywords: bioactive; functional; encapsulation; formulation; whey proteins

1. Introduction

Bovine milk is one of the most nutritious foods and is widely used for human consumption. It is one of the rich sources of nutrients that have several biological properties that impact the biochemical processes in our body, influences the development and functioning of specific organs, and offers protection from diseases. Milk provides a wide range of biologically active components, such as bioactive proteins and peptides, oligosaccharides, immunoglobulins, and fats/lipids, that protect against pathogens and illnesses on regular consumption.

Milk can be sourced from several milch animals, including, cow, buffalo, goat, and sheep. Bovine milk contains approximately a total protein of 3.5%, fats, and essential vitamins, which support growth and development [1]. It is a natural and rich source of well-balanced nutrients that show a diverse range of bio functional properties. These properties are because of the presence of milk proteins/peptides, which support infant development, stimulates growth, improves muscle mass, and confers positive health implications beyond basic nutrition [2]. Besides, the proteins extracted from milk are well characterized for their multiple functional characteristics and are utilized by several industries in food applications. The milk protein system is predominantly constituted by two kinds of proteins: approximately 80% (w/w) casein, which is generally extracted from skim milk through precipitation using either an acid (isoelectric precipitation) or enzymes (rennet coagulation), and 20% whey, which is a leftover byproduct after the casein is extracted [3]. Generally, the whey portion of
milk contains five fractions that altogether make up 85% of the whey protein. These fractions include \(\alpha\)-lactalbumin, \(\beta\)-lactoglobulin, glycomacropeptide, immunoglobulins, protease peptone, and serum albumin, whereas the casein portion of milk contains \(\beta\)-casein, \(\alpha_\text{s1}\)-casein, \(\alpha_\text{s2}\)-casein, and \(\kappa\)-casein [4].

Proteins are macronutrients, and when consumed as supplements may exhibit favorable effects on growth metabolism and health [5,6]. Several reports show that protein deficiency is one of the major health concerns globally [7], and considering this condition, the introduction of dietary protein-rich supplements is of utmost importance. Some of the by-products from agricultural industries, like fruit pomace [8], soy extract [9], cereal brans [10], and milk whey [11], are increasingly getting popular as food ingredients with healthy components. This review focuses on exploiting the bioactive and functional properties of milk whey proteins.

Whey is the yellow-green-colored liquid portion of milk, also called cheese serum, and is obtained after separation of the curd, during coagulation of milk using proteolytic enzymes or acids [12]. It was considered as a major dairy waste for decades because of the disposal issues related to its high biological oxygen demand and high organic matter [13]. However, nowadays whey proteins are recognized as a potential source of nutrients and are exploited for its bioactive ingredients. Because of its high nutritional composition, it is used in several commercial food product applications and is significantly associated with the dairy industry. Generally, fresh liquid whey from cheese-making is composed of 94.2% water and 50% of the total solids of which 0.8% is whey proteins, 0.5% is minerals, 0.1% is fat, and 4.3% is lactose, which is the main constituent [14]. However, the composition and the characteristics of whey may vary with the type of cattle, the diet of the animal, the milk from which it is produced, the processing techniques used, and other environmental factors [15]. Whey proteins are a form of globular proteins, containing a considerable number of \(\alpha\)-helix patterns with evenly distributed hydrophilic and hydrophobic as well as acidic and basic amino acids along their polypeptide chain [16]. The major constituents of whey proteins include \(\alpha\)-lactalbumin (\(\alpha\)-LA), \(\beta\)-lactoglobulin (\(\beta\)-LG), bovine serum albumin (BSA), immunoglobulins (IG), bovine lactoferrin (BLF), bovine lactoperoxidase (LP), and minor amounts of glycomacropeptide (GMP). The composition of each constituent is shown in Table 1. However, the whey protein composition will vary based on the whey type, i.e., sweet whey or acid whey; the type of milk, i.e., bovine, ovine, or caprine; the type of cattle feed; lactation stage; and the type of processing. Whey, acidic in nature, will have a pH of approx. 5.1 and is generally produced through direct acidification whereas sweet whey has a pH of around 5.6 and is produced through rennet-coagulation, particularly during the cheese-making process [17].

### Table 1. Whey protein constituents and its composition \(^a\).

| Whey Protein Constituent   | Concentration (g/L) \(^b\)\(^c\) | Molecular Weight in kDa \(^c\)\(^d\) | Number of Amino Acid Residues \(^c\) |
|--------------------------|---------------------------------|-----------------------------------|---------------------------------|
| \(\alpha\)-Lactalbumin   | 1.2                             | 14,175                            | 123                             |
| \(\beta\)-Lactoglobulin  | 1.3                             | 18,277                            | 162                             |
| Bovine serum albumin     | 0.4                             | 66,267                            | 582                             |
| Immunoglobulins          | 0.7                             | 25,000 (light chain) and 50,000–70,000 (heavy chain) | -                               |
| (A, M, and C)            |                                  |                                   |                                 |
| Bovine lactoferrin       | 0.1                             | 80,000                            | 700                             |
| Glycomacropeptide        | 1.2                             | 6700                              | 64                              |
| bovine Lactoperoxidase   | 0.03                            | 70,000                            | 612                             |

References: \(^a\) [3], \(^b\) [18], \(^c\) [19], \(^d\) [20], \(^e\) [21].

2. Whey Protein Derivatives: Concentrates, Isolates, and Hydrolysates

With the rising popularity of healthy eating, there is a worldwide demand for food products formulated with high protein [22]. The daily average protein intake for a sedentary person should be 0.8 g per kg of body weight per day (g/kg/day) [23]. This amount of protein is required to maintain a positive nitrogen balance and healthy metabolic function in the body. There are various forms of
supplemental proteins available, such as egg, soy, hemp, whey, and casein. Among these, milk whey contains the maximum concentration of amino acids that are readily available and easy to digest, making it effectively incorporate into body cells [24].

Besides, milk whey proteins are recognized as healthy ingredients because of their several advantages associated with their regular intake, including appetite control, exercise recovery, and promoting satiety [25]. In recent years, several applications of membrane filtration have enabled the use of different whey protein components as food additives. Using selective membranes, after the milk is coagulated the whey protein is extracted in two main forms: whey protein concentrates (WPCs), having ~34–89% protein, and whey protein isolates (WPIs), having at least 90% protein [26,27]. Passing the whey proteins through various processing treatments leads to the formation of whey products (Figure 1) with different qualitative and quantitative protein profiles, minerals, lipids, and sugars. Application of selective membranes to fractionate whey proteins include ultrafiltration (UF) to concentrate proteins or the use of the diafiltration (DF) method to exclude the molecular compounds like minerals, lactose, and other low-weight components. This leads to the production of whey protein concentrates (WPC) [24]. It is the most concentrated form of protein supplement, which has high calories and contains all the macro- and micro-nutrients derived from the manufacturing process. However, based on the protein concentration, it can be of several types, like a WPC of 35%, 50%, 65%, and 80% (w/w) protein. When most of the components are removed, i.e., the whey undergoes an additional purification step to eliminate or minimize the extraneous carbohydrates and fats to obtain a protein threshold of 90% (w/w), it is referred as whey protein isolate (WPI). Though being a high-quality protein, the disadvantage of an isolated form of whey protein is that the purification leads to the elimination of some of the important micro-nutrients and protein fractions like lactoferrins, β-lactoglobulins, and immune-globulins.

The concentrates and isolates are composed of large intact protein structures, hence, during digestion, the enzymes in our digestive tract break down these proteins, targeting the amino acid bonds, to generate smaller peptides with amino acid sequences. To facilitate this process and make the protein absorption faster, the manufacturers pre-digest the protein to produce protein hydrolysates.

**Figure 1.** Production of whey protein derivatives.
When whey protein concentrates or isolates are treated with acids, enzymes, or heat, the intact form of protein breaks down into peptides and amino acids, leading to the formation of whey protein hydrolysates (WPH). These pre-digested forms of whey protein are effectively absorbed in the gut, and the hydrolysates that are produced through enzymatic hydrolysis using protease enzyme contain the identical amino acid composition to that of the concentrate and isolate; thus, on ingestion, they can rapidly increase the amino acid concentration in the plasma as compared to intact forms of protein [28]. The final composition of the hydrolysate largely depends on the type of process implied to break the proteins, the type of enzymes used, reaction or hydrolysis conditions applied, and the number of amino acid bonds that are targeted and broken. Therefore, the degree of hydrolysis is measured to determine the release of the amino acids. The greater the degree of hydrolysis, the smaller the amino acids per peptide, resulting in the generation of more bitter peptides [29]. However, all these forms of proteins are enriched with several benefits and used as food additives to exhibit biological properties.

3. Biological Properties of Whey Proteins Associated with Bioactive Peptides

The biological properties of whey proteins (Figure 2) are widely recognized and have been increasingly exploited in scientific research studies and food applications by various industries. β-lactoglobulins contribute to 50% of the whey protein, which helps to bind minerals like zinc and calcium. It also has partial sequence homology to retinol-binding proteins. α-Lactalbumin, on the other hand, is strongly advised to be added in infant formulas or into foods to develop protein-rich dietary intakes [30]. Serum albumin can bind fatty acids and immunoglobulins like IgA, IgM, IgG1, and IgG2, which helps to develop passive immunity in consumers [31]. Other protein fractions like lactoferrin is an iron-binding protein that increases the iron absorption in the digestive tract to inhibit enteric microorganisms and promote the growth of desirable microorganisms. It also modulates the immune system and is considered as the major non-specific disease resistance factor in the mammary gland. Lactoferricin is a peptide derived from lactoferrin and is used against intestinal pathogens. Lactoperoxidase is an enzyme with antibacterial properties that is used as a natural preservative to control acid development in milk during refrigerated storage [32]. Whey proteins are a rich source of essential amino acids like cysteine, branched-chain amino acids like leucine, isoleucine, and valine, and in bioactive peptides [33]. Leucine plays an important role in regulating the synthesis of skeletal muscle protein and is 50–75% higher in whey proteins as compared to other sources [34]. It is also high in sulphur-rich amino acids, i.e., cysteine, which is a precursor of glutathione [35]. Glutathione is a non-enzymatic thiol obtained from the diet, which acts as an antioxidant. It helps to protect from diseases by reducing the antioxidative stress and regulating the cellular processes [36]. Glycomacropeptide (12%), released during the rennet coagulation of cheese, is a casein-derived whey peptide that has many health benefits, including satiety and phenylketonuria management [37]. Specific biological functions of the whey protein components are given in Table 2.

Depending on the protein concentration and characteristics, whey proteins are marketed in forms of whey protein concentrates, isolates, and hydrolysates (partially broken down through digestion) [38]. These derivatives have a broad range of biological functions, including reducing oxidative stress, promoting muscle growth and synthesis, suppressing appetite, hypoglycemia, phenylketonuria management, reducing risks related to cardiovascular diseases, and protecting from ultraviolet (UV) radiation damage [11].
phenylketonuria management, reducing risks related to cardiovascular diseases, and protecting from ultraviolet (UV) radiation damage [11].

**Figure 2.** Biological properties of the whey protein derivatives.

**Table 2.** Biological activities of the major whey protein constituents based on Madureira et al. (2007) [3].

| Whey Protein Constituent | Biological Activities                                      | References |
|--------------------------|------------------------------------------------------------|------------|
| α-Lactalbumin             | Anticancer activity                                        | [18]       |
|                          | Lactose metabolism and synthesis                           | [39]       |
|                          | Treatment of chronic stress-induced disease                | [40]       |
| β-Lactoglobulin          | Transporter of retinol, fatty acids, palmitate, vitamin D and cholesterol [41–43] |           |
|                          | Increase in pregastric esterase activity                    | [44]       |
|                          | Mammary gland phosphorus synthesis and metabolism          | [45]       |
|                          | Passive immunity transfer                                   | [46]       |
| Bovine serum albumin     | Bind fatty acids                                            | [47]       |
|                          | Anti-mutagenic activity                                     | [48]       |
|                          | Anti-cancer activity                                        | [49]       |
|                          | Immune system modulation through passive immunity           | [50,51]    |
| Immunoglobulins (A, M, and C) | Antimicrobial activity                                    | [52]       |
|                          | Antifungal activity                                          | [53]       |
|                          | Opioid activity                                              | [54]       |

### 3.1. Whey Protein-Associated Bioactive Peptides

The isolated protein fragments, i.e., those containing 2 to 20 amino acid residues, that influence health by delivering beneficial effects on body functions are referred to as bioactive peptides. Mellander in 1950 derived phosphorylated peptides from casein and showed an enhanced effect on rachitic infants in Vitamin-D-independent bone calcification [55]. Thereafter, numerous bioactive peptides have been isolated, identified, and studied [56]. BIOPEP consists of both sequence databases and tools for the evaluation of protein as precursors of bioactive peptides. Using this database, several peptides with biological functions have been identified [57], from which ACE inhibitory
peptides are the most identified ones [13,58,59]. However, other bioactive peptides with opioid, antioxidant, immunomodulatory, and anticancer properties have also been presented. Bioactive peptides can be isolated from different food proteins either through gastrointestinal digestion or through fermentation using proteolytic lactic acid bacteria. Depending on their amino acid chains, bioactive peptides, on ingestion, may significantly affect the body functions related to the digestive, immune, cardiovascular, or nervous system. These amino acid sequences are specific to their actions in delivering health effects. For example, peptides exhibiting antioxidative, antimicrobial, ACE inhibition, and immunomodulation will possess specific known peptide sequence [56,60–63]. Some of these peptides also exhibit multi-functional activities [64]. Hence, these bioactive peptides have been recently used in several food applications for the development of pharmaceutical, nutraceutical, and functional foods [65].

3.2. Manufacture of Bioactive Peptides from Whey Proteins

Bioactive peptides or biologically active peptides are mostly produced through the use of different enzymes through enzymatic hydrolysis. They can also be generated through food processing and microbial fermentation using proteolytic lactic acid bacteria.

3.2.1. Enzymatic Hydrolysis of Whey Proteins

Bioactive peptides are mostly produced using different enzymes through enzymatic hydrolysis. The enzymes that are most widely used are proteases and they can be specific or non-specific to their target protein. Hydrolysis of whey proteins using enzymes is mostly preferred by food manufacturers due to their short reaction time, the specific site of enzyme action, and the availability of wide sources of enzymes (from animal, plant, and microorganisms). The most used enzymes are trypsin, pepsin, chymotrypsin, and bromelain, and they have their specific reactions conditions (temperature, pH, and time) [66,67]. However, for the maximum activity, the type of enzymes to be used, the enzyme:substrate ratio, and their reaction conditions should be optimized before the hydrolysis. The selection of enzymes is essential as it influences the cleavage site and patterns of the peptide bonds. Enzymatic modifications are also known to produce peptides with more consistent molecular weights and improved functional and biological properties of the hydrolysates. Various proteases are commercially produced and used for generating bioactive peptides on a laboratory scale [68]. Sometimes, these enzymes, when used in combination, are shown to release more stable and effective peptides [69]. Yamamoto and coworkers used enzymes to hydrolyze protein-rich food materials such as fish, milk, meat, cereal, eggs, and soybean to extract bioactive peptides [70]. Those peptides exhibited properties like being antihypertensive, opioid, immunomodulatory, antimicrobial, and promoting mineral binding. Sarmadi and Ismail showed that hydrolysis of β-conglycinin and glycinin using enzymes can lead to the production of active antioxidant peptides with R group amino acids [71]. They also reported that digestion through enzymes can also produce bioactive peptides with a low molecular weight (below 1000 Da).

3.2.2. Microbial Fermentation and Food Processing of Whey Proteins

Food-grade bioactive peptides are mostly preferred to be produced through microbial fermentation using proteolytic lactic acid bacteria (LAB). These microorganisms are commonly found in our digestive system and are widely spread in nature. Lactic acid bacteria are generally used in food fermentation because of their physiological significance and their role in influencing the texture and flavor of the product [72]. During the fermentation process, these LAB are also able to break down food proteins to produce biologically active peptides. Their proteolytic system comprises proteinases, which break down the proteins to generate numerous oligopeptides (4–8 amino acids), an oligopeptide transport system, a route to provide entry for nitrogen into the cells and peptidases, which completely break down the accumulated peptides [73]. Compared to enzymatic hydrolysis, microbial fermentation is considered to be more economical and is recognized as safe. LAB, being an efficient source of proteases,
require minimal nutrition and expresses proteases on the cell membrane, which makes the enzyme extraction and purification convenient and cost effective [66].

3.3. Bioactive Properties Associated with the Bioactive Peptides Isolated from Whey Proteins and Derivatives

3.3.1. Antioxidant Activity of the Bioactive Peptides

Oxidative stress in the body can lead to several disorders, such as diabetes, cancer, cystic fibrosis, atherosclerosis, aging, and numerous other degenerative diseases. Whey protein is a precursor of the antioxidant glutathione and exhibits antioxidant activity by suppressing the adverse effects of stress factors. The release of bioactive peptides from whey proteins is shown to raise the intracellular glutathione level and reduced the generation of in vitro interleukin IL-8 (cytokine responsible for mediating pathogenesis in the respiratory tract) [74]. Supplementation of pressurized whey (20 g/day) for a month was shown to reduce the C-reactive protein serum level in patients with cystic fibrosis [75]. Whey protein hydrolysates treatment, produced from alcalase enzymes, was found to contain two peptide fragments, P4 and P4c (a pentapeptide containing amino sequence of Val-His-Leu-Lys-Pro). These peptides exhibited antioxidant activity by significantly reducing the hydrogen peroxide exposure to human lung fibroblast MRC-5 cells [76]. A diet (MHN-02) formulated with antioxidants and whey peptides was tested for anti-inflammatory activity in rats. It was observed that the rats that received this diet showed higher survival (90%) as compared to the ones fed with the control (55%). This was due to the high superoxide dismutase activity (conversion of superoxide radicals to hydrogen peroxide and oxygen) and less pathological lesions in the MHN-02 diet group [77]. The role of whey protein derivatives in improving the glutathione synthesis in neurons and reducing the neuro-system disorders was studied [78]. It was reported that whey protein isolates and native hydrolysates with antioxidant and anti-inflammatory peptides, when added to human epithelial colorectal adenocarcinoma Caco-2 cells that was exposed to H$_2$O$_2$, both inhibited production of IL-8 and reactive oxygen species (ROS) [74]. However, the effect was comparatively higher for whey protein isolate treatment, which suggested that the whey protein hydrolysates from isolates are more effective in alleviating inflammation and oxidative stress in intestinal cells. Besides, these activities were observed to be elevated following hyperbaric treatment. In one of the studies, rats were subjected to a high concentration of iron followed by treating them with a placebo or whey protein diet to determine the effect on oxidative stress. After 6 weeks, the test animals showed an increase in lipid peroxidation and a reduction in the radical scavenging activity. Whereas, rats that were fed with a whey protein diet exhibited a higher blood glutathione level as compared to the control (iron overload) group. This suggested the ability of the whey proteins to alter the high iron-induced DNA-damage and reduce ROS in cells [79]. *Pseudomonas aeruginosa* is one of the known pathogens responsible for lung colonization and pulmonary infection, leading to difficulty in breathing [80]. Kishta and his team studied the effect of whey protein in lowering pulmonary infection and found that mice fed with a pressurized-whey protein diet showed a reduced level of oxidative stress, inflammation, and lung damage [81]. The potential reason was the ability of the peptides to stimulate the leucocytes to kill the pathogens and protect the airway proteins from oxidation. A whey protein hydrolysate, when administered in mice with paracetamol-induced hepato-nephrotoxicity, was found to increase the level of antioxidant enzymes, like catalase, glutathione peroxidase, and superoxide dismutase, but reduced the production of thiobarbituric acid reactive substances (TBARS) and the oxidative biomarkers like phosphatase, glutathione pyruvate transaminase, and creatinine [82]. It was also observed that peptide generated from chymotrypsin-hydrolyzed whey protein exhibited a higher ferrous chelating capacity and DPPH radical-scavenging activity as compared to the whey protein isolates [83].

3.3.2. Antihypertensive Activity of the Bioactive Peptides

The bioactive peptides from whey protein concentrates, isolates, and hydrolysates that have angiotensin-converting enzyme (ACE) inhibitory or antihypertensive activity is strongly associated
Whey protein hydrolysates (WPH) containing peptides derived from the α-lactalbumin whereas when β-lactoglobulin in inhibiting the ACE enzyme is relevant [87]. ACE-inhibitory bioactive peptides are with the renin–angiotensin system. Therefore, foods with antihypertensive peptides should be regularly (f 104–108), and (f 105–110). It was reported that the whey protein fraction (f 104–108), and (f 105–110). It was reported that the whey protein fraction (α-lactalbumin (f 99–110) fraction has been shown to demonstrate ACE inhibitory activity, specifically in the sequences (f 99–108), (f 104–108), and (f 105–110). It was reported that the whey protein fraction (α-lactalbumin (f 50–53) exhibited antihypertensive activity at IC₅₀ = 733.3 μM. Other dipeptides that demonstrated similar ACE inhibition at an IC₅₀ = 1522.6 μM and IC₅₀ = 349.1 μM include Tyr-Gly and Leu-Phe, respectively [85]. Triptides (Try-Gly-Leu) (α-lactalbumin f 50–52) are also shown to demonstrate ACE-inhibition in the same range as that of the dipeptides. Whey protein hydrolysates derived from the β-lactoglobulin chain consist of a mixture of peptides that are shown to demonstrate antihypertensive activity. Whey proteins treated with trypsin enzyme liberated peptides with moderate antihypertensive activity, including β-Lg (f 22–25), (f 32–40), and (f 81–83). β-Lactoglobulin peptide (f 142–148) generated from trypsin as reported by Mullaly and his team, exhibited higher ACE-inhibition effects with an IC₅₀ = 42.6 μM as compared to the peptides that showed ACE inhibition effects with a range of IC₅₀ = 77–1682 μM [85]. Studies from Philanto-Leppala showed the most active antihypertensive whey protein peptide to be from α-lactalbumin (f 104–108) with an IC₅₀ = 77 μM [86].

Neutrase enzyme hydrolysis of cheese whey protein generated a mixture of peptides, which was shown to exhibit strong antihypertensive or ACE-inhibition activity. ACE is responsible for regulating several biological processes and is strongly associated with cardiovascular disorders; hence, the role of whey proteins in inhibiting the ACE enzyme is relevant [87]. ACE-inhibitory bioactive peptides are generally below 1 kDa and hold 38% of the total protein content in the whey protein hydrolysate [88].

3.3.3. Opioid Activity of the Bioactive Peptides

Bovine whey protein fractions like α-lactalbumin (f 50–53) and β-lactoglobulin (f 102–105) contain certain peptides that exert opioid activity. These peptides are referred to as α- and β-lactorphins [89]. These peptides have an affinity towards the opiate receptor, inhibited by naloxone. These peptides have an amino acid sequence of Tyr-Gly-Gly-Phe in their N-terminal and exhibit their activity to the target cell by binding to the specific opiate receptors. The presence of tyrosine residue at the N-terminal and the aromatic amino acids at the other positions play an important role in forming the peptide structure motif that perfectly binds to the opiate receptors [90]. These receptors play a role in several physiological responses, like the μ-receptor for emotions and a reduction in intestinal motility; the κ-receptor for food consumption and sedation; and the σ-receptor for emotional behavior.

Several process treatments can be applied to generate lactorphins from bovine whey proteins. α-Lactalbumin, when treated with enzymes like pepsin, liberates α-lactorphin through proteolysis, whereas when β-lactoglobulin is treated with pepsin followed by trypsin, or with a combination of trypsin and chymotrypsin, yields β-lactorphin. Furthermore, hydrolysis of β-lactoglobulin (f 146–149) using chymotrypsin alone led to the production of β-lactotensin (His-Ile-Arg-Leu). Considering the receptor-binding affinity, α-lactorphin exhibits a weak but consistent affinity whereas the β-lactorphin exhibits non-opioid activity when tested on the ileum of the guinea pig. Overall, these peptides belong to the μ-type receptor ligands, which displayed a low receptor-binding affinity towards opioid receptors. Both these peptides, when added in micromolar concentrations, were found to inhibit 3H-naxolone from binding to the receptor sites. In contrast, morphine, which is a standard opioid peptide, was found to inhibit 3H-naxolone in the range of IC₅₀ = 23 ± 13 nM nanomolar concentrations [91]. Approximately
0.9 g/L of $\alpha$-lactalbumin and 3.0 g/L of $\beta$-lactoglobulin is present in bovine milk, which contributes to the production of 30 mg of $\alpha$-lactorphin and 90 mg of $\beta$-lactorphin. During in vitro digestion of milk, these peptides get released to exhibit in vitro opioid effects. For hydrolysates, it was observed that the release of lactorphins at concentrations of 5–14% was sufficient to exhibit opioid activity in vitro.

### 3.3.4. Antidiabetic Property of the Bioactive Peptides

Diabetes is one of the critical health issues that causes several disorders, including vision loss, angiopathy, and blood flow restriction, leading to tissue hypoxia and ulcers with reduced healing [92]. Consumption of hypoglycemia chemical drugs with a controlled diet can help to treat type 2 diabetes. Dietary supplements with added whey proteins have been shown to demonstrate anti-diabetic effect by reducing the serum glucose level in healthy individuals, improve muscle mass, and increase the secretion of satiety hormones (cholecystokinin, leptin, and glucagon-like peptide 1(GLP-1)) and reduce the release of ghrelin (the hormone responsible for hunger) [11]. It was observed that the presence of cysteine, plays an important role in treating glycemia and controlling inflammation in people with diabetes [93]. A study from Badr and coworkers showed the effects of whey protein on type I diabetes-induced wounds in a mouse model. Compared to the untreated mice, it was found that whey proteins significantly lowered the diabetic inflammation and wounds by restricting the production of inflammatory cytokines and expression of chemokines (MIP-1$\alpha$, MIP-2, KC, CX3CL1, and TGF-$\beta$) [94]. Salehi and coworkers investigated the effects of whey protein and found that an increase in the levels of insulin and amino acids, like valine, leucine, isoleucine, threonine, and lysine, are the major causes for antidiabetic activity [95]. Whey protein derivatives (isolate and hydrolysate) when supplemented in a fat-rich diet were found to improve the secretion of insulin, leading to the lowering of postprandial triglyceride responses in type 2 diabetes subjects [96]. After feeding rats with a diet rich in whey protein hydrolysate for a month, an increase in the leucine content and insulin level was observed [97]. Results showed that the whey protein is metabolized in the gut and as result peptides and amino acids are released, which are responsible for inducing the insulin level along with the secretion of the gut and incretin gastric hormones. When the whey protein action was investigated for reducing the glucose concentration, it was observed that the protein was able to strongly lower the levels of plasma glucose, insulin, and C-peptide. However, it increased the levels of GLP-1 and PYY, which tells that consumption of whey protein before a meal can lower the post-meal glycemia by both insulin-dependent and insulin-independent pathways [98]. Tong and coworkers demonstrated the effect of both whey protein and its hydrolysate fraction to exhibit anti-diabetic effects by improving the insulin resistance in rat subjects [99].

### 3.3.5. Anticancer Activity of the Bioactive Peptides

Intake of whey protein has been shown in several studies to exhibit beneficial effects on cancer patients. Reports have stated that whey protein hydrolysates confer an improved anticancer effect as compared to other forms of whey protein. A study showed that rats with colon cancer, when fed with a whey protein hydrolysate, demonstrated a reduction in the appearance of macroscopic and microscopic tumors as compared to the rats that belonged to the control group fed with un-hydrolyzed whey protein [100]. Whey protein was also reviewed for anticancer properties against the melanoma B16F10 cell model and it was observed that expression of caspase-3 increased significantly in the media containing whey protein isolate [101]. Caspase-3 is known to play an important role in mediating apoptotic cell death [102]. In a 48-year-old Caucasian female, when whey protein at a dosage of 10 g (three times daily) was administered in combination with a weekly injection of testosterone enanathate before and during chemotherapy, an improvement in the lean body mass, physical movement, and overall quality of life were observed [103]. Another study demonstrated the protective effect of whey protein hydrolysate on rat pheochromocytoma PC 12 cells with oxidative damage. A 20–30% increase in the cell viability was observed at a dosage level of 100–400 $\mu$g hydrolysate/mL as compared
to the ones that were incubated with an infusion of \( \text{H}_2\text{O}_2 \). This suggests the potentiality of the whey protein hydrolysates to exhibit antioxidant activities [104].

3.3.6. Immunomodulatory Activity of the Bioactive Peptides

Whey protein derivatives in the form of concentrates are known to improve the innate mucosal immunity and deliver protection from immune disorders [105]. Public concern for atopic dermatitis (a condition where the skin is swollen, scaly with itchy rashes) is continuously increasing worldwide with infants being more susceptible to it. Recently, a meta-analysis showed that infants fed with a hydrolyzed form of whey protein developed reduced symptoms of atopic dermatitis as compared to the control subjects that were given plain bovine milk [106]. These results suggested that diets included with whey protein might play an effective role to protect the infants from atopic dermatitis. Another study in mice models showed reduced levels of the plasma (interleukin) IL-1\(\alpha\), IL-1\(\beta\), IL-10, (tumor necrosis factor) TNF-\(\alpha\), ROS (reactive oxygen species), and cholesterol after they were treated with whey protein concentrate and examined for blood parameters, plasma cytokine profiles, proliferation, and migration of immune cells [94]. Mice subjects given the whey protein diet displayed significantly improved concentrations of IL-2, IL-4, IL-7, IL-8, and glutathione. Besides, an improvement in the response of leucocytes, macrophages, and monocytes to different antigens was observed. As compared to the control group, in the treated group it was observed that the cytokine CC chemokine ligand-21 (CCL-21) and CXC chemokine ligand-12 (CXCL12) can attract the immune cells and migrate the B cells, T cells, and dendritic cells towards them [94]. The bioactive effect of the whey protein isolate was determined against psoriasis (a skin condition with thick skin, dry scales, and red patches) and an intake dose of 20 g/day was provided to the patients. It was found that the glutathione level increased and, following the inflammation due to the psoriasis, decreased with the consecutive intake of whey protein [107].

3.3.7. Muscle Protein Synthesis by Bioactive Peptides

Heavyweight exercise and resistance training, as well as eccentric (muscle lengthening), concentric (muscle shortening), and isometric (muscle non-lengthening) exercises can cause skeletal muscle tear and damage and possibly can lead to internal inflammation (due to the production of inflammatory muscle protein markers) [108]. Resistance training (heavy weightlifting) can lead to the accumulation of oxidation products in blood plasma, resulting in leukocyte functionality [109]. Regular intake of whey protein supplements containing amino acids like hydrolysates has been reported to repair muscle damage. Ingestion of \( \beta \)-hydroxy-\( \beta \)-methyl butyrate, generated from leucine has been shown to improve muscle recovery. Expression of glucose transporters in skeletal muscle in the form of cell-surface glucose transporter 4 (GLUT-4) is known to control the rate of glucose transport in the cell membrane, in response to insulin and muscle contraction. Hence, whey protein was investigated for its capability to accumulate GLUT-4 in the membrane, which can lead to a reduction in glucose entrapment in the muscles. The major amino acid and bioactive peptide in the whey hydrolysate contributing to the process was found to be L-isoleucine and L-leucyl-L-isoleucine, respectively [110]. The effects of whey protein on muscle functionalities like contraction, elasticity, extensions ability, and excitability as compared to a casein-diet were reviewed. It was observed that the whey protein-fed rats with isometric and concentric exercise injury recovered faster as compared to the ones fed with casein [111]. A whey protein beverage supplemented with different doses of leucine and branched-chain amino acids was shown to stimulate the synthesis of myofibrillar protein both at a high and low protein content and at a 5 g leucine content [112]. Leucine contributes to 10% of the total whey protein amino acid and, therefore, is more important for muscle protein synthesis and recovery. In a study, whey protein hydrolysate was consumed for 12-week and it was observed that protein played a role in reducing the muscle damage markers like creatine kinase and lactate dehydrogenase [113]. As compared to soy protein, it was found that whey protein supplements are more effective in expressing leucine in subjects undergoing resistance training exercises [114].
3.4. Identification of Bioactive Peptides Isolated from Whey Proteins and Derivatives

Bioactive properties of the peptides are determined based on their amino acid sequence and molecular weight. Mostly, the peptides are of short-chain length with 2 to 6 amino acid sequences; however, some peptides with high molecular weight are made of 30 amino acids. Hence, to isolate these peptides, firstly they can be passed through an ultra-filtration membrane of varying molecular weights, such as 10 kDa, 5 kDa, or 3 kDa. Roblet and coworkers recovered peptides from a soy-protein hydrolysate through ultra-filtration and screened them for their bioactive properties. He found an improvement in the bioactivities following fractionation [115]. Another technique that has been commonly used for separating and purifying these bioactive peptides is High-Performance Liquid Chromatography (HPLC). Other methods, such as sodium dodecyl sulfate–polyacrylic gel electrophoresis (SDS-PAGE) and ultra-centrifugation, are also implied for the characterizing the protein and identifying the amino acid composition of the peptides. Recently, several other methods like electrospray ionization (ESI), mass spectrometry (MS), matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) are being used to isolate, identify, and characterize the bioactive peptides. Among these methods, mass spectrometry has been used to generate the peptide profile and determine the molecular mass and amino acid sequences of the protein hydrolysates. It is also used to analyze the protein conformational changes and protein degradation products [116]. The peptides generated can be concentrated using ultra-filtration and size exclusion chromatography. In addition, to obtain the protein structure-based functional properties, reverse phase HPLC is used to segregate the peptides depending on their hydrophobic properties [117].

Haileselassie and coworkers extracted peptides from an enzyme-modified cheese. The peptides were then passed through reverse phase HPLC using a Delta Pak C18 column [118]. In total, eight peaks were generated (one from neutrase digest, five from neutrase debitrase digest, and two from microbial enzyme from Lactobacillus digest). These peptides were purified and identified through API mass spectrometry. In another study, antihypertensive peptides were extracted from soy milk and fractionated into four parts (A–D) using a size exclusion chromatography [119]. It was found that, among the fractions obtained, B showed the highest antihypertensive property. Using proteolytic fermentation, three antimicrobial peptides were generated, and these peptides were later separated using reverse-phase HPLC. The fractionated peptides were identified for their properties using a mass spectrometry combined with MALDI-TOF [120]. Electrospray LC-MS was used to purify and identify peptides from buckwheat protein that exhibited ACE-inhibitory activity. A UHPLC-Q-TOF MS/MS method was developed to identify peptides from Mactraveneriformis hydrolysates. Four nucleobases and one nucleoside were in total identified through the de novo sequencing based on the MS/MS fragmentation [121]. There are also several databases, such as the BIOPEP bank, available that can be used to generate information related to proteins and peptides [122].

4. Functional Properties of Whey Proteins

Functional properties of proteins refer to the physicochemical properties that play an important role in imparting a specific behavior and performance to proteins when added in food systems. The properties of whey proteins include thermal stability, hydration, gelling, and emulsification properties, which influence the final quality of foods. These properties vary with the interaction among proteins or with other food components and are strongly affected during preparation, processing, storage, and consumption of the foods. Some of the processing conditions and extrinsic and intrinsic parameters that influence the functionalities of whey proteins are shown in Table 3. Whey proteins and derivatives vary in their composition and, hence, possess different functional properties. As a result, they are used in different food applications.
Table 3. Processing conditions and extrinsic and intrinsic parameters affecting the functional properties of whey proteins (sourced from [123]).

| Processing conditions | Heating | Acidification | Counter ions | Ionic strength | Reducing conditions | Drying | Storage conditions | Modifications related to physical, chemical, enzymatic, and genetic |
|-----------------------|---------|---------------|--------------|----------------|---------------------|--------|-------------------|----------------------------------------------------------------|
| Extrinsic parameters | Temperature | pH    | Oxidation-reduction potential | Salts or ions | Water | Carbohydrates | Lipids | Gums | Surfactants | Tannins |
| Intrinsic parameters | Protein composition | Monomeric oligomeric | Protein blends | Rigidity/flexibility | Hydrophobicity or hydrophilicity | Surface charge | Bound flavor ligands |

4.1. Thermal Denaturation of Whey Proteins

The thermal processing of food is greatly influenced by processing as well as compositional factors. Processing parameters include temperature, pH, ionic strength, and rate of heating, whereas the compositional factors include the lactose and protein content. Foods with whey proteins when exposed to mild heating (40 °C) lead to denaturation of β-lactoglobulin, whereas heating beyond that (50 °C to 60 °C) leads to unfolding and exposure of the thiol group [124]. During the cooling of β-lactoglobulin, in the presence of calcium, the protein–protein linkages are formed via disulfide bonding and entropic forces, leading to aggregation [125]. At a low pH of <3, denaturation of whey proteins can develop unique physicochemical and functional behaviors. It was reported that whey protein concentrates obtained through ultra-filtration at pH 2.5–3 yielded proteins with different viscosity and gelling properties before or after heat treatment [126]. Heating treatment at 90 °C for 15 min at pH 2.5–3 led to isoelectric precipitation of protein, which was very viscous with reduced solubility and increased setting to form a soft coagulum [126]. Several research also has been done on the unfolding of whey proteins following heat treatment. Investigations using differential scanning calorimetry (DSC) have shown the entire thermodynamics of the unfolding of whey proteins. During heating (20 °C to 110 °C) of α-lactalbumin and β-lactoglobulin at a concentration of 3–9% and pH 7, they are reported to have a transition temperature (Tt) of 65 °C and 73 °C at 10 °C per min; the Tt increased with the increase in the rate of heating. However, it was observed that increasing the pH from 6.4 to 7.3 increased the denaturation of the β-Lg and reduced the Tt from 79 °C to 74 °C [127]. This suggests the ability of the whey proteins to unfold at increased pH and electrostatic repulsive forces within the polypeptides. At a pH beyond 7, the thiol group of the whey proteins gets exposed and as a result increases the thiol-disulfide interchange reaction [124]. Hence, increasing the ionic strength conceal the exposed thiol groups, thereby increasing the hydrophobic interactions. Some of these protein fractions can reverse their denaturation (renaturation) depending on the pH. α-Lactalbumin, being a small protein with four disulfide bonds, can reverse their denaturation by
80–90% at 3–9% concentrations [127]. Kronman and coworkers reported that when the pH is lowered to 3.75 or below, aggregates of \( \alpha \)-lactalbumin are formed following heat treatment, and when the pH is adjusted to 5.2 or above, the protein aggregation is reversed [128]. An acidic pH leads to exposure of apolar amino residues that facilitate protein–protein aggregation, leading to the unfolding of \( \alpha \)-La. Protein aggregation is also dependent on bound calcium content and, hence, removal of calcium during acidification leads to protein coagulation. The subjection of \( \alpha \)-La to a reduced pH of 3 removes bound calcium from the molecule, making it more susceptible to irreversible heat-induced protein denaturation.

4.2. Hydration and Solubility of Whey Proteins

The property of proteins to interact with water in different systems describes the solubility or hydration properties of whey proteins. It is considered as one of the important factors in whey protein preparations. Several physical and chemical characteristics influence the hydration properties of whey proteins. Physical parameters include protein particle size, shape, agglomeration state, and nature of porosity, and the chemical parameters include protein surface net charge, hydrophobicity, and hydrophilicity [129]. Hydration properties play roles in optimizing both processing and storage conditions [130]. For example, whey protein powders obtained through controlled spray drying conditions to yield particle sizes of 150–200 \( \mu \)m is most effective in improving the hydration properties [131]. Determining the amino acid composition can help to estimate the water-binding properties of pure proteins. Besides, other factors like protein structural conformation, polarity, ionic strength, pH, and temperature also influence the water-binding capacity of the proteins. Determining the solubility of whey proteins can help to decide their specific applications to food products such as beverages. After precipitation of casein at isoelectric pH 4.6, the whey protein fraction remains soluble in the supernatant. Hence, reduced solubility at pH 4.6 is usually used to analyze the extent of the protein denaturation. Whey protein concentrates (WPCs) at concentrations from 5 to 100% display a wide range of solubilities, and this is due to their different production methods. To obtain an improved and consistent solubility, the whey proteins and derivatives must be produced under processing conditions that lead to minimal heat denaturation and aggregation of protein components. Currently, the spray drying technique is largely being used to produce different whey protein powder forms. Therefore, the conditions associated with the spray drying method can be controlled and optimized to produce powders with enhanced solubility. However, in some cases, the proteins get partially denatured before the spray drying step and this mostly affects the functional performance of the whey protein products. The effect of pH largely influences the hydration or solubility of the whey proteins by altering the net charge of the proteins. Proteins possessing a net positive or negative charge tend to dissolve in water as compared to the proteins having a minimal net charge (for example in the isoelectric point). However, under certain conditions, whey proteins remain in the soluble form at their isoelectric pH. Ionic strength also varies according to ion species and valency. In the presence of salts, \( \beta \)-lactoglobulin remains soluble even at pH 4–5. Heat and some processing treatments often lead to protein denaturation and aggregation, thereby reducing their solubility. However, the addition of salts like sodium chloride at a 0.01 M concentration has been shown to improve the solubility of \( \beta \)-lactoglobulin when heat-treated at 80 °C for 15 min. Increasing the salt concentration was effective in increasing the solubility of the whey protein fractions by reducing the aggregation, and at a 0.5 M concentration, the protein precipitation was completely inhibited [132].

4.3. Gelation Ability of Whey Proteins

Whey proteins have the capacity to form gels with different properties varying from soft, smooth curds to viscous, rubbery, and stiff gels. Their properties differ based on hardness, cohesiveness, color, stickiness, and mouthfeel [133]. Gels formed from a whey protein concentrate appears as stiff, transparent gels to curd-like opaque gels. Whey proteins with reduced protein concentrations and a low ionic strength form translucent grey, weak gels. Mild heating of whey proteins can lead to
specific protein–protein interactions, leading to the formation of gels, whereas extensive thermal processing can lead to the formation of coagulation or curd-like gels. During gelation, a structural network is formed within proteins in which the water is entrapped, leading to reduced syneresis. The shape of the gel formed, its color, mechanical strength, and elastic properties play an important role during food applications. Whey proteins undergo conformational changes when heat-treated beyond its critical temperature, leading to form aggregates. These aggregates when cooled set to form a soft to firm, clear to opaque viscous gels. The gel formation varies depending on the type of protein, concentration, temperature, pH, and calcium [134]. Heat-induced gels are formed in two steps, wherein the first step, the protein exposed to heat undergoes conformational changes through protein–protein interactions and unfolding of polypeptide segments, leading to the formation of a structural network [135]. However, a balance between the repulsive and the attractive forces are necessary to form a three-dimensional network. In certain cases, limited protein unfolding during heating leads to exposure of hydrophobic residues, which later associate with cooling. This forms a gel network based on the number of interactive sites, their reactivity, and the amount of the repulsive forces between the solute molecules. Hence, they are highly affected by pH and ionic strength. In protein gels containing serum albumin, approximately 1–2 molecules of protein arrange themselves to form a highly dense, viscous network depending on the degree of cross-linking [136]. In another study, gels obtained from the whey fraction β-Lg at high and low ionic strengths contained protein aggregates in the form of a continuous branching network [137], whereas whey proteins with lysozymes formed gels containing straight, rod-like protein molecules arranged in small, irregular clusters with a bead-like structural network [137].

4.4. Emulsification Property of Whey Proteins

Emulsions are referred to as heterogeneous systems formed by dispersing one or more phases in a continuous phase and they can be stabilized by incorporating surface-active agents that are amphiphilic in nature, i.e., they have an affinity for the dispersed and dispersing/continuous phases. The main intent to add an emulsifier is to reduce the interfacial tension and facilitate diffusion of the dispersed phase. Emulsion systems stabilized using a protein have an interfacial membrane formed around the oil droplet to avoid any formation of coalescence, flocculation, creaming, or oiling-off [138]. However, to be an effective emulsifier, the protein must be soluble and able to lower the interfacial tension at the oil/water interface. Hence, factors such as pH, salts, protein concentration, and temperature play an important role in protein adsorption onto the surface of the fat globule, influencing the emulsifying properties of the whey proteins [139]. Emulsions formed of whey protein–coconut oil displayed reduced creaming stability with enhanced viscosity and protein adsorption at pH 5. This suggests that the electrostatic nature of the proteins largely influences the emulsion stability [140]. Besides, the availability of the hydrophobic groups surrounding the fat globules also plays role in protein adsorption. Studies suggest that whey protein-based emulsions can be improved through the partial unfolding of proteins during emulsion formation, like while undergoing homogenization [141]. Whey protein concentrates (WPCs) enriched with α-La and β-Lg were analyzed for their emulsifying properties in an oil/water emulsion. WPC alone and β-Lg added WPC demonstrated similar emulsifying properties, which tell that origin and whey processing have little to no effect on the emulsifying properties. WPC with added α-La demonstrated a moderate emulsifying capacity with reduced stability, indicating β-Lg-enriched whey proteins to be more effective emulsifiers [142]. In another study, emulsions formed from whey protein–coconut oil contained more protein at pH 5 as compared to pH 7 at the interfacial surface. At pH 9, β-Lg was strongly adsorbed and found to predominating, whereas at pH 3, α-La-associated protein adsorption increased. This suggests that protein adsorption and their emulsifying properties are extensively affected by the pH of the medium. At an alkaline pH, molecular expansion of β-Lg led to more adsorption, whereas, at an acidic pH, α-La was found to adsorb more readily probably due to the reduced stabilizing effect of the bound calcium [139]. The stability of emulsions and the role of proteins as emulsifying agents were found to be correlated with the surface hydrophobicity when
examined through fluorescent probe cis-parinaric acid [143]. Reduction in the surface hydrophobicity significantly lowered the emulsifying capacity of the β-Lg and serum albumin (BSA), in proportion with the extent of thermal denaturation. This tells that, both being hydrophobic in nature, BSA and β-Lg underwent a conformational change during heating, leading to the reduced affinity of apolar residues to hydrophobic sites. Emulsions with added WPCs also vary with the composition of the lipid content, ash, and sulphydryl content, and is often used to predict the emulsifying capacity of various WPC samples in aerated emulsions.

4.5. Improvement in the Functionality through Conjugation

Whey proteins are one of the nutritionally beneficial systems enriched with amino acids and bioactive peptides and, hence, popularly used in developing functional foods. Whey protein products are greatly in demand for developing nutraceutical foods due to their several health benefits associated with their bioactive properties, such as being antimicrobial, antioxidant, antihypertensive, opioid, and promoting mineral binding [144]. However, the incorporation of WPH into food formulations is not a trivial task because of their heat stability issues [145]. To ensure product safety and shelf stability, most liquid foods and medical beverages undergo thermal treatments, and this can cause whey proteins to denature irreversibly to form gels and aggregates. Besides, due to the poor emulsification properties, the use of hydrolyzed proteins in developing formulated powders can be significantly affected, because of the increased stickiness of the powder particles during the spray drying process. Moreover, the use of whey proteins with high protein levels can lead to extensive fouling and blockage of the equipment and pipelines. Sedimentation and gelation of denatured proteins, particularly during long-term storage, show signs of unwanted sensory attributes in the final product. Developing value-added products using whey proteins can create a lot of technical limitations, leading some producers to switch to some other protein types. However, such proteins may differ in terms of their amino acid profile and digestion benefits of whey proteins.

Recently, the stability of whey proteins has been addressed by several researchers, primarily by conjugating them with carbohydrates. Heating of whey proteins or peptides with reducing carbohydrates leads to a series of chemical reactions, referred to as the Maillard reaction, and it is during the early stages of heating where a covalent bridge is formed between the protein and carbohydrate molecules, and a conjugated protein is formed [146]. Conjugation of whey proteins with reducing sugars via the Maillard reaction process (i.e., glycation) is an advancing area of interest, with some previous reports showing improvement in the physiological, nutritional, and functional properties, including thermal stability, solubility, emulsification capacity, water binding, and antioxidant activity of the whey protein/peptide-based ingredients [147].

However, the main challenge with conjugation is that it can occur naturally or can be initiated, which significantly influences the characteristics of the food products in terms of physical, chemical, biological, and organoleptic properties. Furthermore, conjugation of whey proteins with reducing sugars via the Maillard reaction is a very complex reaction process involving a series of processing parameters, including pH, temperature, and time. Hence, scaling up of the process present serious challenges as an insufficient binding of the proteins to carbohydrates can lead to coagulation of proteins and an uncontrolled Maillard reaction can lead to the formation of unwanted or adverse effects, such as the formation of products that leads to the generation of off-flavors and toxic compounds [148]. Therefore, for wider applications in developing value-added health ingredients, the effect of the different process parameters on the Maillard reaction, and their impact on the functional and health properties of the proteins, need to be studied.
5. Current Applications of Whey Proteins and Its Derivatives

5.1. Role of Whey Proteins and Derivatives as Food Ingredients

In food applications, whey proteins and derivatives are gaining attention due to their immense benefits owing to several functionalities, including gelation, foaming, emulsification, solubility, and thermal properties. The addition of the whey proteins is known to improve the food sensory quality and enhance the texture. For example, whey proteins have been previously added to foods such as yogurt, bakery foods, energy bars, pasta, and beverages to influence the overall quality and nutrition of the foods. A study reported the effect of adding a complex of non-heat-treated whey protein and high methoxyl pectin in low-fat yogurt [149]. The whey protein acted as a good fat-replacer and texturing agent for the yogurt. Another study showed the ability of the whey proteins to stabilize emulsions and improve the overall texture when added into whole-fat yogurt prepared from skim milk powder. When the droplets merger was used, it yielded whey protein agglomerates with a high molecular weight and reduced emulsifying capacity; however, when passed through a high-pressure homogenizer at 20–100 MPa, it yielded a more stable emulsion [150]. In a study, the effect of the addition of the milk-protein ingredients on the microstructure of probiotic yogurt (prepared with a combination of commercial starter culture and Bifidobacterium lactis Bb12) was analyzed during a 28-day-period refrigerated storage [151]. One sample was added with sodium caseinate at the level of 2% and the other was added with a whey protein concentrate at 2%. It was reported that the addition of sodium caseinate transformed the firmness, adhesiveness, and the overall viscosity of the product, whereas the product added with whey protein demonstrated an improved water holding capacity, viscous texture, and low syneresis as compared to the caseinate. Whey protein in combination with a plant protein was added into a date bar and the nutritional profile was optimized applying a response surface method (RSM) targeting the school children [152]. An addition of 6.05% of whey protein concentrate (WPC) was found to be ideal. Several research studies are still ongoing to utilize whey proteins and their derivatives to develop nutraceutical and functional foods.

5.2. Benefits of Combination of Whey Proteins and Derivatives with Other Supplements

Extensive hydrolysis of whey proteins using enzymes can lead to the formation of bitter peptides, reducing their acceptability in food applications. Enzymatic hydrolysis breaks down the protein fractions like α-lactalbumin, β-lactoglobulin, and serum albumin to generate whey protein hydrolysates containing bitter peptides. This bitter taste of the peptides are often masked using various inhibitors and some of these inhibitory compounds include sucralose, fructose, adenosine 5’ monophosphate, sucrose, adenosine 5’ monophosphate disodium, monosodium glutamate, sodium chloride, sodium gluconate, and sodium acetate [153]. Several techniques involve identifying the bitter peptides and removing them to improve their sensory properties. Liu and coworkers identified four peptides contributing to bitterness in a whey protein hydrolysate. Fractionation techniques (ultra-filtration and chromatography) were used followed by LC-TOF-MS/MS (Liquid chromatography-time of flight-mass spectrometry) to identify the peptides and the constituent amino acids [83]. Gad and his team reported an improvement in the antioxidant and metal chelating activities of the whey protein concentrate (WPC) when supplemented with freshwater algae, spirulina, in both in vitro and in vivo subjects using rat models [154].

Application of whey proteins can also be limited as some of its components like α-lactalbumin and β-lactoglobulin are associated with causing allergenicity, particularly in children. Some children are found to develop gastrointestinal problems [155], atopic dermatitis [156], respiratory allergies [157], or anaphylactic reactions [158] after ingestion of cow milk protein. Hence, it is important to assess the allergenicity risks before the administration of whey protein diets. One of the effective methods that were reported to reduce the allergenicity in whey proteins is heat treatment. Bu and coworkers analyzed the antigenicity of α-lactalbumin and β-lactoglobulin in whey protein isolates through competitive ELISA (enzyme-linked immunosorbent assay) after exposure to heat treatment [159].
It was observed that, above 90 °C, the antigenicity in the protein fraction decreased significantly. Treating whey proteins with enzymes is also known to reduce the allergenicity [104]. Whey protein concentrates were hydrolyzed with trypsin and fed to mice subjects. An increase in the secretion of IFN-γ was observed in the subjects, which suggests the ability of the hydrolysates to lower the allergenicity of the whey proteins [160].

5.3. Role of Whey Proteins and Derivatives as Encapsulating Agents and Coating Materials

As consumers become more health-conscious, they are looking for natural ingredients rich in nutrients inside their foods and beverages [161]. Hence, processors are responding to this trend by continually incorporating healthy ingredients in foods or as supplements. Recently, bioactive compounds (e.g., vitamins, antioxidants, minerals and ions, flavor, aroma compounds, lycopene, fats or enzymes or bacterial cells like probiotic microorganisms) have emerged as functional ingredients, leading to the production of novel formulations and value-added foods [162]. However, there are several challenges faced during the application of these bioactive molecules [163]. As a result, to overcome these challenges and considering the increasing demand for value-added novel ingredients in food, food manufacturers started implementing the process of encapsulation [164]. These wide ranges of active compounds can be encapsulated or packaged in a carrier material composed of whey protein. The process of encapsulation involves the incorporation of any solid, liquid, or gaseous materials, including ingredients, enzymes, cells, or other molecules in different carrier materials to produce capsules of varying sizes [161]. This facilitates transporting the agents at the delivery site and based on the strength of the carrier material, the core agents get released at various intervals.

Besides, entrapping in a whey protein gel is known to reduce rancidity issues and augment stability. For instance, fortifying foods with iron presents numerous difficulties, and to address this problem, whey protein isolate was used, by utilizing its gelling properties. The isolate was exposed to cold-set gelation to form a matrix, and subsequently iron was entrapped in it in the presence of ascorbate [165]. This led to improving the encapsulation efficiency of the whey protein to recover more iron and improve the in vitro bio-accessibility from 10% to 80%. The use of ascorbate contributed to strengthening the whey protein gel, which led to increased recovery of iron and improved its release characteristics. Similarly, a whey protein concentrate was used as an encapsulant to entrap folic acid. A favorable interaction between the folic acid and the protein matrix was observed, making it a suitable matrix for incorporating vitamins. When compared with a polymer (commercial resistant starch), the WPC capsules imparted a higher stability to folic acid [166]. Whey protein encapsulants can also be formed in combination with other carrier materials, such as carbohydrates and fats. A study demonstrated the efficiency of the whey protein isolate nanoparticle when combined with and without methoxyl pectin [167]. The results showed improved resistance to homogenization and overall stability of the encapsulants formed with pectin. Even during storage at pH 3, the nanoparticle suspension displayed higher interfacial pressures as compared to encapsulants without pectin. Such encapsulants can be potentially used as effective surfactants. An important benefit of the encapsulation process is to prevent the reaction of the core ingredient with other food components, like in the case of essential oils [168]. Besides containing several compounds like phenols, alcohols, esters, ketones, and aldehydes, essential oils exhibit a wide spectrum of antimicrobial activity against bacteria, yeasts, and fungi. Hence, to confer stability inside a food matrix, such oils can be microencapsulated using whey protein derivatives as the carrier material. For example, WPI was used to encapsulate cardamom essential oil [169]. It was found that the WPI microcapsules obtained had a spherical, regular, and smooth texture and, during storage, it was able to retain the oil at a 30% concentration.

In a study, a whey protein isolate was transformed into an edible film with ascorbic acid impregnated into it [166]. The film was then assessed for the oxygen-scavenging property. It was observed that the tensile strength of the film improved with reduced oxygen permeability. This suggests the ability of the WPI films to prevent oxygen diffusion and eliminate oxygen in food systems, thereby enhancing the storage stability of the oxygen-sensitive products [170]. Edible layering using whey
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proteins is also used to coat nuts to improve its shelf life by retarding the formation of rancidity in them. A study showed the efficacy of a whey protein isolate to delay the oxidation and rancidity in walnuts and pine nuts. The nuts coated with WPI generated improved sensory characteristics throughout storage at 25 °C for 12 days, as compared to uncoated nuts [169].

Whey protein-based packaging materials show great potential in replacing plastics and is one of the most promising biopolymers. Recently, whey proteins have been widely used in the field of active packaging to exploit their antimicrobial properties. Antimicrobial packaging refers to a form of active packaging with antimicrobial compounds infused in it. These compounds get released when used in edible films to impart an improved shelf life of the product. Whey protein isolate (WPI) edible films with antimicrobial properties have been developed with infused essential oils (extracted from spices such as rosemary, oregano, and garlic). Previous studies show the efficiency of whey proteins to improve the oxygen barrier properties and increase biodegradation when added in a compostable plastic film [171]. Due to their high emulsification properties, whey proteins are also used in forming stable emulsions. Cheese is often fortified with vitamins to enhance its nutritional value. However, during the ripening period, the vitamins tend to degrade. Hence, to improve the retention of vitamins, Tippetts and team studied the role of whey proteins to incorporate vitamin D3 in Cheddar cheese [172]. They formed an oil-in-water emulsion by adding sodium caseinate, calcium caseinate, whey protein, and vitamin D3 to obtain the final dose of 280 IU/serving. The nano emulsions were stable and about 74–78% of vitamin D3 was retained in the product. In another study, the ability of the whey protein isolates to generate stable nano emulsions under various thermal processes and ionic strengths was shown [173]. These emulsions were found to be stable under storage conditions even at higher protein concentrations without the addition of any polymers like gums and polysaccharides as a secondary layer. These results suggest the potentiality of the whey protein-based emulsions in the food and pharmaceutical industries.

6. Conclusions

From the findings above, it can be suggested that whey proteins and derivatives are functionally significant and have great potential in food applications. Whey proteins and peptides are now increasingly endorsed by nutritionists as an excellent source of nutrition. Peptides generated from whey are being incorporated in the form of ingredients in functional and fresh foods, dietary supplements, and even pharmaceuticals to deliver specific health benefits. Whey protein products are currently the subject of investigation for formulating new drugs and functional food ingredients for gut health and modulating the intestinal absorption of nutrients because of their biofunctional properties. Most of the bioactive peptides isolated and purified from whey proteins have good antioxidant, antihypertensive, anticancer, antidiabetic, and hypocholesterolemic activity. After absorption, these peptides exert their action on specific target organs. Such peptides, when enriched in diets, can be consumed by infants, geriatrics, diabetics, cardio-risk groups, and athletes. Commercial interest in the production and use of bioactive peptides has been increasing recently but industrial-scale production of such peptides is still not well established. Some commercial products have been launched in the market, claiming a specific biological activity and therapeutic effect. Besides, the functional characteristics of the whey proteins also play an important role during applications in food systems. However, the data available does not truly reflect the functional behavior of the whey proteins in food systems, which tells that in an actual food condition, the components sometimes extensively interact to change the functional characteristics of the proteins.

With the continuous expansion of the market for functional proteins, there is a need to develop simple cost-effective methods for the production, isolation, purification, and scalability of the whey proteins and peptides in huge amounts for the market. The whey processors must determine useful functional properties and demonstrate their effectiveness to be used as a functional ingredient so that the whey products can be promoted and marketed based on their performance. Recently, to meet the increasing demand from health-conscious consumers, food industries have started to explore protein blends (a mixture of proteins derived from various sources like casein, whey, plants, microbial sources,
etc.) for developing protein-rich foods and beverages. However, to make the concept feasible, there are several technical and marketing challenges that are reported during the development process. Besides, such products have been reviewed as less palatable. Hence, these preparations should be tested in a simulated food system followed by an actual food condition to validate the protein behavior and performance in commercial foods. Specific protocols should be designed regarding ingredient addition, temperature, pH, and other processing parameters. Such information is necessary to facilitate the appropriate processing methods during manufacturing to prevent compositional variation, the extent of the protein denaturation, and other conformational changes. Routine tests for assuring the food quality also should be of great value in providing information concerning the functional applications.

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