Chapter 12
Whey Protein from Milk as a Source of Nutraceuticals

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12.1 Introduction

Whey protein comprise of protein mixture obtained from whey, the liquid milk component that separates during the production of cheese. During the production of cheese, there is coagulation of the fats in the milk, resulting in the separation of whey as by-product. Whey can also be considered as the liquid part of milk remaining after the curdling and straining of the milk. As a byproduct of cheese or casein production, whey along with whey proteins, has many commercial and nutraceutical applications. Sweet whey results from the production of rennet hard cheese, such as Swiss cheese, cheddar, etc. Sour whey (acid whey) is obtained during the production of dairy products' acid types, including strained yogurt, cottage cheese, etc. Whey proteins contain β-lactoglobulin (BLG), α-lactalbumin (LALBA), proteose peptones, immunoglobulins, lactoferrin, and serum albumin [1–3]. Whey protein is the globular proteins' collection from whey. Cow milk protein contains 80% casein protein and 20% whey protein, while human milk protein is 30% casein protein and 70% whey protein. The whey protein fraction constitutes around 10% of the whey's total dry solids. The protein is usually a mixture of immunoglobulins, bovine serum albumin (6%), alpha-lactalbumin (13–19%), beta-lactoglobulin (~48–58%), etc. [1–3]. They are soluble in nature, in spite of the pH. The common analytical techniques, including hyphenated techniques, used in biosciences can be used to quantify these whey protein components [4, 5]. Figure 12.1 shows the common proteins found in whey proteins.

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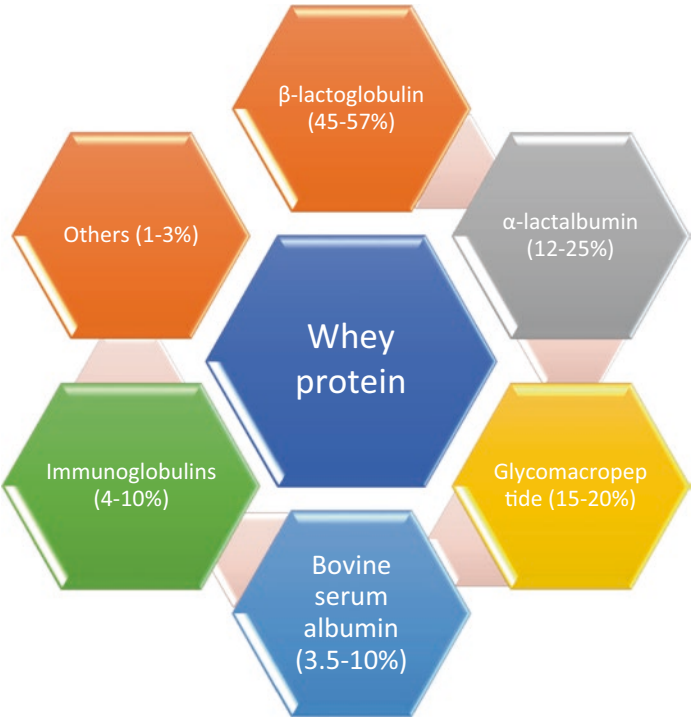

Fig. 12.1 % Protein contents of whey proteins

Cysteine, an amino acid, in whey protein is required for glutathione synthesis in the body; glutathione is a powerful cellular antioxidant in the body [6]. Whey protein may decrease cancer risk in animals. Whey protein has been recognized as potential source for formulating novel nutraceuticals due to its several health and therapeutic benefits. One of the underlying mechanisms through which whey protein components show their biological effects is possibly through intracellular translation of cysteine to glutathione, an important intracellular antioxidant [7]. Studies have shown that whey protein has great therapeutic properties for the treatment of cardiovascular diseases, AIDS, cancer, etc.

This chapter focuses on the therapeutic and health benefits of whey protein for the formulation of nutraceuticals. The chapter also explored the biological, biochemical, and physiological properties of whey protein and its components. The use of other food and agricultural byproducts in the formulation of nutraceuticals have been extensively discussed in other chapters. Whey protein is usually available as a supplement from dietary source, with several health and therapeutic claims attributed to it and its components [8, 9]. Whey is a major component of several protein-based powders primarily used by bodybuilders and athletes every day to obtain the required daily intake of protein. Whey protein contains high leucine level, one of the three amino acids with branched chain, which makes it suitable for the repair and growth of muscles.

12.2 Types of Whey Protein

There are several popular types of whey protein. Their main difference is in the way they have been processed. The three major types of whey protein include hydrolysate, concentrate, and isolate. Whey protein hydrolysates are partially hydrolyzed and predigested for easier metabolizing, although they are generally costly. Whey protein that is highly hydrolyzed might be less allergenic compared to other types of whey protein. Native whey proteins are obtained from skim milk directly without being byproduct of the production of cheese, and is made as whey protein isolate or whey protein concentrate. Whey protein concentrate contains 70–80% protein, with some lactose and fat; its flavor is the best [10–12]. Whey protein isolate contains at least 90% protein, less lactose and fat; it does not have many beneficial nutrients commonly seen in whey protein concentrates. Whey protein hydrolysate, also called hydrolysed whey, is pre-digested for faster absorption, and results in 28–43% more spike in the levels of insulin than whey protein isolate. Overall, the best option appears to be whey protein concentrate, since it is more cost effective and retains many beneficial nutrients naturally found in whey [12]. Additionally, many individuals prefer the taste, mainly because of the fat and lactose. For those who have challenges tolerating concentrate partly due to being lactose intolerant, or those who lay emphasis on protein while maintaining low carbohydrates and fat, whey protein isolate or whey protein hydrolysate can be the preferred option [12]. These are the main types of whey protein; isolate, hydrolysate, and concentrate. They vary in their protein content, digestibility, taste, and price.

12.3 Health and Nutritional Benefits of Whey Protein

Whey protein has very high protein efficiency ratio (PER) and very high biological value (BV). Biological value is a measure of amount of absorbed protein from a food which becomes incorporated into the body proteins of an organism. This incorporated protein is used for functions such as tissue formation in the body. Biological value is calculated as the percentage of protein efficiently utilized and PER measures the gain in weight per gram of consumed protein. Proteins with high biological value are very vital and essential to humans [13–16]. Whey protein contains all essential amino acids (EAAs) in sufficient amounts compared with protein from other sources, including corn, wheat gluten, soy, etc. The whey protein's amino acids are absorbed faster in comparison with free amino acid (AA) and proteins from other source [17]. As stated earlier, whey proteins are rich source of EAAs, including the branched chain AAs, such as isoleucine, valine, and leucine. The amino acid profile of whey protein is similar to the proteins of the muscle, with almost all the AAs in similar magnitudes [18]. The whey proteins branched-chain AAs play an important role in the repair and growth of tissue. Leucine is an important amino acid in protein metabolism during protein synthesis. In addition, whey

protein contains sulfur-containing amino acids, which is why whey protein amino acids such as methionine and cysteine boost the functions of the immune system by conversion to glutathione intracellular. Fluid whey from cattle milk has around 50% and 20% of the nutrients and milk protein contents, respectively, corresponding to an average protein content of 4–7 g/L. While the major whey protein components include α-lactalbumin, β-lactoglobulin, BSA, and the fraction of proteose-peptone, the minor whey protein components include immunoglobulins, lactoferrins, ceruloplasmins, as well as certain enzymes such as lipase, xanthine oxidase, and lysozyme (Table 12.1) [18, 19].

Whey proteins are found in milk and dairy products, with high calcium bioavailability linked to vitamin D activities, a major factor that increase calcium absorption in the intestine. Calcium is essential to bones [23]. The whey protein bioactive peptides contain approximately 3–20 AAs and might exhibit antihypertensive, citomodulatory, immunomodulatory, antithrombotic, antimicrobial, and opioid activities; they can modulate our mood and intestinal microbiota, and show action against allergies, infections, and atopic dermatitis in children and infants that consume infant formula [24–26]. Whey proteins derived bioactive peptides can also be generated during food processing. Enzymatic hydrolysis is a common method for the production of peptide fragments used in hypoallergenic infant formulas [24, 26].

12.4 Whey Protein Applications as Nutraceutical

The high digestibility of whey proteins is mainly because of improved plasma amino acids that initiates protein synthesis, making whey proteins quite attractive in clinical nutrition and therapeutical applications [21]. Whey proteins are administered in the patients' postoperative care and strongly recommended for growth and repair of body cells [17]. As excellent source of EAAs, if carefully and professionally administered, whey proteins help in developing nutraceuticals and functional formula for clinical applications [20]. The common nutraceutical and health benefits of whey proteins are shown in Table 12.2.

12.4.1 Muscle Synthesis and Resistance Exercise

Resistance exercise, concentric (shortening), isometric (non-lengthening), and eccentric (muscle lengthening) contractions result in damage to the skeletal muscle and also generate inflammatory markers [38]. Anabolic interventions using amino acid supplements and protein hydrolysates expedite repair. The ingestion of leucine and its metabolic derivative, β-hydroxy-β-methylbutyrate, is beneficial to soreness recovery. Resistance exercise such as weight-lifting raises products of oxidation in plasma and disturbs leukocyte functionality and redistribution [39]. Nanoparticles of whey protein isolate were formulated using ethanol desolvation, and their $ZnCl_2$

Table 12.1 Molecular weight and percent of whey protein fraction

Whey protein	% Whey protein component	Nutraceutical functions	Number of AA residues	Molecular weight (Da)	Concentration (g/L)	References
β-Lactoglobulin	45 to 57	BLG contains high levels of branched chain AAs (25.1%), and binds hydrophobic molecules, contributing in reducing intestinal lipid absorption	162	1.8×10^4	1.3	[12]
α-Lactalbumin	12 to 25	α-Lactalbumin has the highest content of tryptophan (6%) of all the proteins from dietary sources. It is a rich source of cysteine, threonine, leucine, and lysine. It can bind to minerals, including calcium and zinc, and improve their absorption	123	1.42×10^4	1.2	[20]
Bovine serum albumin (BSA)	3.5 to 10	BSA has good lipid binding properties and good amino acid profile	582	6.9×10^4	0.4	[21]
Immunoglobulins	4 to 10	Four classes of immunoglobulins present in whey include IgE, IgM, IgA, and IgG. Their functions include improved immunity and antioxidant protection	–	1.5×10^5 to 9×10^5	0.7	[12]
Glycomacropeptide	15 to 20	Glycomacropeptide is formed from κ-casein digestion during cheese production, and has high levels of EAAs that improve the absorption of minerals	64	7×10^2	1.2	[21]
Lactoferrin	3	Lactoferrin inhibits proinflammatory cytokines production and protects against hepatitis.	700	7.7×10^4	0.1	[20]
Lactoperoxidase	0.4	It has strong antimicrobial properties and forms part of the innate immune system	612	7.8×10^4	0.03	[22]
Lysozyme	1	It has antimicrobial properties and forms part of the innate immune system	129	1.4×10^4		[22]

Table 12.2 Nutraceutical and health benefits of whey protein

Nutraceutical and health benefits	Study evidence	References
Anticarcinogenic properties	A study reported that when whey protein hydrolysate was fed to rat models with induced colon cancer, they significantly developed less microscopic and macroscopic tumours in comparison with the group that consumed untreated whey protein	[27]
Anti-diabetic properties	Whey protein reduces blood glucose level in healthy people, reduces ghrelin (hunger hormone) secretion, boosts satiety hormones release (glucagon like-peptide 1 [GLP-1], leptin, and cholecystokinin), and maintains muscle mass. A study showed that that the amino acid cysteine, which is abundant in whey protein, can be applied as auxiliary therapeutic measure in the control of vascular and glycaemia inflammation in diabetic individuals	[28, 29]
Antihypertensive effects	Bovine whey proteins' beta-lactoglobulin peptide sequence has high inhibitory actions against Angiotensin I converting enzyme (ACE), which catalyzes Angiotensin I translation into Angiotensin II, a potential vasoconstrictor. It reduces the risks of hypertension	[7, 21]
Antioxidant properties	Antioxidant properties of whey proteins have been reported, with potential benefits against oxidative stress, commonly reported in chronic non-communicable diseases (CNCDs) and in preterm neonates	[20]
Cardioprotective and hypotensive effects	Whey protein can reduce the risk of cardiovascular diseases. The risks of cardiovascular diseases can be ameliorated with quick-absorbable whey protein extracts	[30]
Immune improvement in HIV patients	Whey protein isolates are used in the treatment HIV patients and are widely recommended by health and nutrition experts. Whey protein consumption can significantly increase glutathione status. A study concluded that supplementation with whey protein increased CD4 cell counts in HIV patients	[31]
Immunomodulatory properties	Whey protein improves innate mucosal immunity in early stages of life and has a protective effect in certain immune conditions	[32]
Infants' immunity	As a result of its high beneficial immune properties, whey proteins are among the most suitable protein choices for mothers to feed their infants. The antimicrobial and immunomodulatory properties of whey proteins are associated with the stimulation of immune cells and protective microflora proliferation in the GI tract of humans and animals, indicating the possible whey protein role in strengthening immune system in infants and children	[26]

(continued)

Table 12.2 (continued)

Nutraceutical and health benefits	Study evidence	References
Muscle synthesis and resistance exercise	Whey protein hydrolysates were studied for their capability of translocating and accumulating GLUT 4 in membrane, consequently augmenting skeletal muscle glucose trapping. L-leucyl-L-isoleucine (a peptide) and L-isoleucine (an amino acid) in the whey protein hydrolysate made the most contribution	[33]
Phenylketonuria treatment	A study reported that glycomacropeptide of whey protein with low content of phenylalanine is suitable as the major source of protein in the diet of PKU patients. Glycomacropeptide in such diet can significantly ameliorate the effects associated with phenylketonuria	[34]
Postoperative care	Whey proteins are administered in the patients' postoperative care and strongly recommended for growth and repair of body cells	[17]
Prebiotic and gut functions	Fortification with whey protein may ameliorate tolerance to enteric nutrition and have influence on inflammation. Yeast and lactic acid bacteria (LAB) require to remain viable to exercise their therapeutic properties. Whey protein gels protect the microorganisms such as probiotics from adverse conditions	[35]
Skin protective effects	A group of researchers studied the effects of whey peptides consumption at 200 and 400 mg/kg two times a day on skin alterations (wrinkle formation, suppleness, and thickness) induced by chronic UV-B radiation in mice. The whey peptides ameliorated dermatoheliosis through preventing increase in melanin granule formation, wrinkling, and dermal stiffness. The whey peptides reduced matrix metalloproteinase (pro-MMP-9 and MMP-2) expression and vascular endothelial growth factor (VEGF) expression	[36]
Treatment of obesity and overweight	A study done for 12 weeks indicated that preloads of whey protein concentrate done 30 min before the main meal of ad libitum has more health benefits than the one of soy protein isolates on anthropometry, appetite, body composition, and calorie intake of obese men	[37]

incorporation capacity analyzed. The level of zinc loaded into the suspensions of the nanoparticles was within zinc requirements per day for a healthy adult. The nanoparticles were stable after storage at 22°C for one month [40]. Glucose transporter 4 (GLUT 4) of cell surface is the main glucose transporter isoform which is expressed in the skeletal muscles that determine the muscle glucose transport rates in cell membranes, in responding to muscle contraction and insulin. Whey protein hydrolysates were studied for their capability of translocating and accumulating GLUT 4 in membrane, consequently augmenting skeletal muscle glucose trapping. L-leucyl-L-isoleucine (a peptide) and L-isoleucine (an amino acid) in the whey protein hydrolysate made the most contribution [33]. Whey protein supplementation effects compared with casein diet effects on the muscle functional recoveries,

including excitability, elasticity, extensibility, and contractility, have been studied using rat models. The whey protein supplementation caused a quicker recovery from sustained injury due to concentric and isometric exercise compared with the casein diet [41]. Churchward-Venne et al. [42] studied the effects of beverage supplementation with varied leucine doses or mixture of branched chain AAs on myofibrillar protein synthesis following resistance exercise. Results indicated that beverage with low protein content (6.25 g) could be equally effective as high protein counterpart (25 g) at promoting the rates of myofibrillar protein synthesis when supplemented with high levels of leucine (5 g) [42]. Leucine constitutes 10% of the amino acids in whey protein and is vital in improving muscle hypertrophy. Lollo et al. [43] compared the effects of health parameters, body composition, and performance produced after intake of whey protein hydrolysate for 12 weeks in players. The whey protein hydrolysate intervention caused significant decrease in lactate dehydrogenase and creatine kinase (muscle damage markers) [43]. In a randomized study, Volek et al. [44] compared the effects of daily consumption of whey protein and soy protein on the growth of muscle mass using subjects under resistance exercise. The gains in lean body mass were significantly higher for whey protein consumption group than the group that consumed soy protein, and the considerable response was associated with increased leucine levels and quicker absorption [44].

12.4.2 Management of Phenylketonuria

Phenylketonuria (PKU) is an inborn metabolic error and genetic disorder characterized by mutation in phenylalanine hydroxylase gene and reduced metabolism of phenylalanine (an amino acid), resulting in excess phenylalanine accumulation [45]. Untreated PKU or failure in phenylalanine usage results in several health complications, including seizures, osteopenia, mental disorders, intellectual disability, and behavioral problems. The common strategy for managing PKU is by consuming diet low in phenylalanine. Van Calcar and Ney [34] reported that glycomacropeptide of whey protein with low content of phenylalanine is suitable as the major source of protein in the diet of PKU patients. Glycomacropeptide in such diet can significantly ameliorate the effects associated with PKU. Phenylalanine plasma level, systemic inflammation markers, food intake, and energy expenditure can be efficiently lowered in comparison with casein diet, which has high level of phenylalanine. Solverson et al. [46] reported significantly lower respiratory exchange ratio and the total fat mass in PKU mice that consumed glycomacropeptide. Osteopenia characterized by skeletal fragility because of decreased minerals in bones is among the common complications associated with PKU [47]. Demirdas et al. [47] and Ney et al. [48] reported that osteopenia was ameliorated after consumption of glycomacropeptide diet. There was improvement in bone health and a higher radial bone growth [48]. Strisciuglio and Concolino [49] concluded that glycomacropeptide diet is healthier alternative over regular synthetic AAs, as it enhances satiety and taste of diets resulting in improved patient compliance.

12.4.3 Anticancer Effects

Many studies suggest that whey protein can have therapeutic effects on patients with cancer. Whey protein hydrolysis may enhance the efficacy of the anticancer activities. Attaallah et al. [27] reported that when whey protein hydrolysate was fed to rat models with induced colon cancer, they significantly developed less microscopic and macroscopic tumours in comparison with the group that consumed untreated whey protein. Castro et al. [50] studied whey protein anticancer effect using model of melanoma B16F10 cells, and reported significant increase in caspase-3 expression in the media that contained whey protein isolate. The Caspase-3 role in mediating cell death via apoptosis is common [51]. A Caucasian female aged 48 years with recurring cervical cancer was given whey protein (10 g three times in a day) and a weekly testosterone enanthate intramuscular injection prior to and during standard-of-care chemotherapy. Improvements in physical activity, lean body mass, and general quality of life were observed due to the combination therapy [52]. Zhang et al. [53] studied whey protein hydrolysate's protective effect against oxidative damage on pheochromocytoma PC12 cells of rat. At 100–400 μg/ml dose of hydrolysate, there was 20–30% increase in the viable cells in comparison with those incubated in hydrogen peroxide, suggesting whey protein hydrolysate may have antioxidant potentials [53].

12.4.4 Skin Protection

Dermatoheliosis, also known as photoaging, is the characteristic changes to skin induced by chronic exposure to UV radiations. Kimura et al. [36] studied the effects of whey peptides consumption at 200 and 400 mg/kg two times a day on skin alterations (wrinkle formation, suppleness, and thickness) induced by chronic UV-B radiation in mice. The whey peptides ameliorated dermatoheliosis through preventing increase in melanin granule formation, wrinkling, and dermal stiffness. The whey peptides reduced matrix metalloproteinase (pro-MMP-9 and MMP-2) expression and vascular endothelial growth factor (VEGF) expression. In addition, they prevented increase in number of apoptotic, 8-hydroxy-2′-deoxyguanosine (8-OHdG)-positive and Ki-67-positive cells caused by chronic UV-B radiation [36]. The whey peptides inhibited type IV collagen degradation, proliferation, DNA damage, and angiogenesis induced by the radiation [36].

12.4.5 Infant Foods/Formula

Bovine milk is a rich source of protein, with whey protein constitute 20% of the total protein. Formulating physiologically suitable foods/formula for infants is required to meet up with their nutritional requirements [20, 21]. Lactose performs many biological functions in infants, and are found in whey protein concentrate, isolate, and hydrolysate; although in low levels, but beneficial to infants [20]. The monosaccharide units in lactose are glucose and galactose, which perform many metabolically related developments in babies and children, including energy provision and protein sparing actions [54]. As a result of its high beneficial immune properties, whey proteins are among the most suitable protein choices for mothers to feed their infants.

12.4.6 Gut Prebiotics and Functions

Prebiotics are food compounds that induce the activities and growth of probiotics (beneficial microorganisms), including fungi and bacteria [55]. Gut dysfunctions, such as compromised intestinal barrier, abnormal patterns of motility, and delay in gastric emptying, are severe conditions in patients that are critically ill. Fortification with whey protein may ameliorate tolerance to enteric nutrition and have influence on inflammation [35]. Yeast and lactic acid bacteria (LAB) require to remain viable to exercise their therapeutic properties. Gels of whey protein protect the microorganisms from adverse conditions. Gerez et al. [56] studied the encapsulation efficacy of pectin- and whey protein-loaded *Lactobacillus rhamnosus* CRL 1505 for improved rate of survivability in bile milieu and low pH. The results showed that whey protein layer with pectin beads can be applied as carrier for probiotics in acidic functional food. Zhao et al. [57] isolated the dipeptide glycine-L-tyrosine (Gly-Tyr) that has specific binding capacity to calcium from whey protein hydrolysates. The peptide chelating mechanism was studied and the main binding sites were the amino/imino group's nitrogen and the carbonyl group's oxygen atom [57]. The study concluded that the peptide may boost the absorption of calcium in GI tract; the development of the calcium-peptide chelate supplement for improved bioavailability of calcium has promising potentials. Walsh et al. [58] studied the "effects of carbonation on probiotic survivability, physicochemical, and sensory properties of milk-based symbiotic beverages". *Bifidobacterium* and *Lactobacillus acidophilus* remained viable in yogurt stabilized with whey protein concentrate and high-methoxyl pectin [58]. Microparticles of alginate and whey protein isolate showed suitability for *Saccharomyces boulardii* (probiotic yeast) oral delivery systems. A study done with the simulation of intestinal and gastric fluid showed 40% survival rate for encapsulated yeast in comparison with 10% survival rate for free yeast [59].

12.4.7 Management of Obesity and Overweight

Obesity is a health condition characterized by excessive accumulation of body fat to an extent that may have negative effects on health, usually defined by body mass index (BMI) of 30 and above; on the other hand, overweight is characterized by more body fat than is optimal for health, usually defined by BMI within 25–29.9; normal weight and underweight are defined by BMI of 18–24.9 and below 18 respectively. Change of diet helps to combat obesity and overweight; whey protein is beneficial for this purpose [54]. In as study, ad libitum was prepared using high-fat diets that contain adequate whey protein levels or leucine supplement and fed to mice for 1 week. The study concluded that the diet rich in protein has ameliorating effects on metabolic disorders that are surely because of liver lipogenesis attenuation-mediated satiety modulation [60]. A study done for 12 weeks indicated that preloads of whey protein concentrate done 30 min before the main meal of ad libitum has more health benefits than the one of soy protein isolates on anthropometry, appetite, body composition, and calorie intake of obese men [37].

12.4.8 Anti-Diabetic Effects

Diabetes mellitus (DM), also called diabetes, is various metabolic disorders that manifest as a high level of blood sugar for a long period of time [61, 62]. Different types of DM include type 1 DM (T1DM), type 2 DM (T2DM), gestational diabetes, and other minor types [63, 64]. T2DM constitute about 90–95% of all cases of DM [63, 64]. DM is one of the major public health issues that has several complications, including angiopathy, loss of vision, decreased blood flow resulting in tissue hypoxia, diabetic ketoacidosis, foot ulcers, nerve damage, difficult wound healing, etc. [65]. T2DM can be treated using hypoglycemic drugs, exercise, and diet control/restrictions [66]. Whey protein reduces blood glucose level in healthy people, reduces ghrelin (hunger hormone) secretion, boosts satiety hormones release (glucagon like-peptide 1 [GLP-1], leptin, and cholecystokinin), and maintains muscle mass [28]. Jain [29] showed that that the amino acid cysteine, which is abundant in whey protein, can be applied as auxiliary therapeutic measure in the control of vascular and glycaemia inflammation in diabetic individuals. Badr et al. [67] studied whey protein effects on diabetic wounds recuperation on a mouse model induced with T2DM. In comparison to untreated T2DM mice, whey protein supplementation significantly enhanced diabetic lesions closure through restricting the inflammatory cytokines access via the maintenance of normal levels of IL-6, IL-1β, TNF-α, and IL-10. The supplementation with whey protein modulated the chemokines TGF-β, CX3CL1, KC, MIP-2, and MIP-1α expression in wound tissues in comparison to mice with untreated T2DM. Salehi et al. [68] studied the whey protein's insulin secreting effects and reported that increased serum levels of threonine, lysine, valine, isoleucine, and leucine was the major mechanism of action for this

effect. Mortensen et al. [69] studied the effects of various whey protein fractions on postprandial hormone and lipid responses in T2DM. The whey protein hydrolysate and whey protein isolate addition to fat-rich diet reduced the responses of postprandial triglyceride in subjects with T2DM. The whey protein fractions induced very high insulin responses [69]. Toedebusch et al. [70] carried out a study titled "postprandial leucine and insulin responses and toxicological effects of a novel whey protein hydrolysate-based supplement in rats". They formulated and fed supplement of whey protein hydrolysate to rats in a period of 30 days, and reported a high level of leucine followed by an increase in the level of insulin [70]. Whey proteins are metabolized as amino acids and peptides in the gut which stimulate hormones in the gut (peptide tyrosine tyrosine (PYY), cholecystokinin) and incretin hormones (GLP-1 and gastric inhibitory peptide) that additionally induce the secretion of insulin from pancreatic β-cells. The peptides may inhibit dipeptidyl peptidase-4 (DPP-4) (incretins inhibitor) in proximal gut, and also inhibit the degradation of incretin [71]. Akhavan et al. [72] studied the whey protein action mechanism on reducing postprandial spikes in levels of glucose in a randomized study over 230 min. Whey protein caused reduced plasma insulin, C-peptide, and glucose level, and increased PYY and GLP-1 levels than preloads of glucose. The authors suggested that pre-meal whey protein consumption reduces post-meal glycaemia through insulin-independent and insulin-dependent action mechanisms [72]. Supplementation with whey protein hydrolysate and its leucine supplements can enhance insulin resistance [73].

12.4.9 Immunomodulation Properties

Whey protein improves innate mucosal immunity in early stages of life and has a protective effect in certain immune conditions [32]. Occurrence of a chronic skin disease known as atopic dermatitis characterized by scaly, itchy, and swollen rashes is growing worldwide, with infants mostly affected. Alexander et al. [74] conducted a systematic review and reported that atopic dermatitis incidence was significantly reduced in infants partially fed with whey protein hydrolysate formula in comparison with the infants fed with bovine milk. The study concluded that whey protein-based infant formula has protective effects against atopic dermatitis [74]. Badr et al. [75] studied the whey protein concentrate effects on plasma cytokine profiles, blood parameters, and proliferation/migration of immune cells in mice. The TNF-α, IL-10, IL-1β, and IL-1α plasma levels, as well as the cholesterol levels and ROS levels were significantly lesser in the group that received whey protein treatment in comparison with the control [75]. In the group that received whey protein, the IL-8, glutathione, IL-7, IL-4, and IL-2 levels improved significantly. In addition, the proliferation capability of monocytes, macrophages, and lymphocytes in response to different antigens stimulation increased [75]. The cytokines CXCL12 (CXC chemokine ligand-12) and CCL21 (CC chemokine ligand-21) attract and tether immune cells to their direction [75]. Badr et al. [75] reported that in vitro

migration of T cells, dendritic cells, and B cells towards CXCL12 and CCL21 increased significantly in the patients that received whey protein in comparison with the control group. In a different study, it was concluded that consuming 20 g of whey protein isolate per day enhanced and ameliorated the conditions of psoriasis patients [76].

12.4.10 Hypotensive and Cardioprotective Properties

Whey protein consumption can reduce the risk of cardiovascular diseases (such as ischemic stroke), although the exact role of whey protein peptides in regulating vascular endothelial functions still need more studies to sufficiently be conclusive. Ballard et al. [30] carried out a study and reported that the ingestion of whey protein-derived extract (NOP-47) results in improved endothelial functions and can increase the AAs level of postprandial plasma. Arterial dilation improvement was independent of the vasoactive compounds' circulation, including prostacyclin, nitric oxide, and hyperpolarizing factor derived from endothelium [30]. The study concluded that the risks of cardiovascular diseases can be ameliorated with quick-absorbable whey protein extracts [30]. Sheikholeslami and Ahmadi [77] reported whey protein supplementation effects and the effects of resistance training on risk factors of cardiovascular diseases and antioxidant status in young men with overweight BMI. The results suggest that whey protein consumption and resistance training have synergistic effects, which were demonstrated by very high total antioxidant property, high density lipoprotein (HDL) level, and glutathione level [77].

12.4.11 Immune-Boosting for HIV Patients

Whey protein isolates are used in the treatment HIV patients and are widely recommended by health and nutrition experts. Glutathione deficiency is one of the major problems associated with HIV. People infected with HIV have low concentrations of glutathione in the lymphocytes of their blood. Introducing whey protein to their diets elevates glutathione level. As a strong antioxidant, glutathione helps maintain muscular tissue functional and structural integrity [7, 21]. Low cell levels of glutathione encourage the growth of HIV, while high glutathione greatly decreases the replication capacity of the virus. Whey protein consumption can significantly increase glutathione status. Sattler et al. [31] concluded that supplementation with whey protein did not increase lean body mass or weight in HIV patients who ate adequately, but it increased their CD4 cell counts. This shows that whey protein can boost immune cells in HIV patients, while helping them maintain normal weight.

12.4.12 Food and Nutraceutical Additives

Whey protein is widely applied in food production mainly due to its functional properties such as emulsification properties, foaming capacity, thermal stability, gelation capacity, etc., and its nutritional and nutraceutical properties. Whey protein improves food quality, including nutritional value, texture enhancement, and improvement in sensory properties. Many studies have shown the nutritional and structural effects of whey protein in foods, including functional foods, bakery preparation, energy bars, pasta, beverage, yoghurt, infant formula, etc. [78, 79]. Krzeminski et al. [78] in their study showed the capability of non-heated complex of high methoxyl pectin and whey protein as texturing agent and fat replacer in a reduced-fat yoghurt. Formulations of the skim milk with adjusted composition conferred a texture similar as that of whole-fat yoghurt. Kuhn and Cunha [80] carried out a study on the effects of high-pressure homogenization on emulsions of whey protein isolate and flaxseed oil and reported good emulsion stability induced by whey protein. Merging of the droplets led to the generation of protein aggregates with high molecular weight, which resulted to reduction in the emulsification capacity (EC) [80]. High-pressure homogenization (20–100 MPa) and higher passes number led to emulsions of more stability [80]. Akalın et al. [81] reported milk/whey protein-based ingredients' effects on probiotic yogurt microstructure in refrigeration period of 28 days. A 2% sodium calcium caseinate fortification improved yoghurt viscosity, adhesiveness, and firmness [81], while 2% whey protein concentrate improved water binding capacity more than sodium calcium caseinate. Yoghurt fortified with whey protein concentrate had better texture and lesser whey collection at the yoghurt surface. Nadeem et al. [82] formulated a whey protein-fortified date bar and a plant protein for school children. The nutrients were optimized using response surface methodology, with whey protein concentrate (6.05%) shown ideal for this application [82]. In another study, Yadav et al. [83] used pearl millet, whey protein concentrate, water, barley flour, and carboxy methyl cellulose to formulate pasta, and reported promising results.

12.4.13 Encapsulation, Delivery Systems, Active Packaging, Edible Coating

Several foods, essential oils, vitamins, and additives can be packaged or encapsulated in whey protein gels for stability and reduction of rancidity. Sustaining iron accessibility in foods fortified with iron is usually challenging. Whey protein isolate cold-set gelling capability was evaluated to address this [84]. A study entrapped iron in the ascorbate presence with cold-set gelation, and studied TNO Intestinal Model (TIM) for the optimization of the iron-ascorbate ratio [84]. The whey protein's cold-set gelation done for ascorbate and iron showed suitable in improving the iron in vitro bioaccessibility and recovery, which, from 10%, improved to 80%. The

ascorbate gel strengthening effect was associated with better controlled release and encapsulation efficiency of iron. Mehyar et al. [85] evaluated the possibility of whey protein isolate-loaded cardamom essential oil microencapsulation. The microcapsules of whey protein isolate at 30% concentration optimally entrapped the essential oil during storage at known temperature conditions. The microcapsules of whey protein isolate had spherical shapes, regular and even contours, and smooth texture [85]. Pérez-Masiá et al. [86] achieved significant folic acid encapsulation with matrix of whey protein concentrate, which was associated with the interactions between folic acid and whey protein. The result indicated that whey protein can be better for this purpose than resistant starch commercially used for the stability of folic acid [86]. Janjarasskul et al. [87] studied edible ascorbic acid-impregnated film of whey protein isolate and its oxygen-scavenging potentials. The whey protein isolate film had proper tensile strength and decreased the permeability of oxygen, showing suitability for food products with oxygen sensitivity [87]. Gülseren et al. [40] made a comparison between encapsulation competency of nanoparticles of whey protein isolate desolvated suspension with methoxyl pectin and another without methoxyl pectin. The methoxyl pectin-containing suspension showed resistance to homogenization, with improved stability. The complex also showed better interfacial pressures under pH 3 storage, in comparison with the suspension of whey protein isolate nanoparticles without methoxyl pectin; this suggests their potential use as surfactants.

12.4.14 Antihypertensive Properties

Whey proteins have been studied for use as dietary supplements for the reduction of blood pressure. Bovine whey proteins' beta-lactoglobulin peptide sequence has high inhibitory actions against Angiotensin I converting enzyme (ACE), which catalyzes Angiotensin I translation into Angiotensin II, a potential vasoconstrictor [7, 21]. It reduces the risks of hypertension.

12.5 Applications of Whey Protein for Functional Foods and Nutraceutical Development

Functional foods are foods that have additional function usually associated with disease prevention and/or health promotion by the addition of new ingredients or more of the existing ingredients; they improve overall health and well-being, as well as reduce disease risks [20, 88]. Whey protein plays immunomodulatory roles due to its high concentrations of cysteine and IgA/IgG immunoglobulins. Cysteine is an amino acid that increase glutathione production. Glutathione is the main antioxidant system in the body, and can prevent cellular aging and oxidative stress. Consequently,

whey protein has immune properties via the stimulation of antibodies production by the lymphocyte [88]. The antimicrobial and immunomodulatory properties of whey proteins are associated with the stimulation of immune cells and protective microflora proliferation in the GI tract of humans and animals, indicating the possible whey protein role in strengthening immune system in children and adults [26]. Bioactive peptides of whey protein have mineral binding capacity, and can go along with the fact that intestinal microbiota regulation improves absorption of mineral in the digestive and gastrointestinal tracts [26]. Peptides counteracts calcium inhibitory effects on the absorption of iron and also act in synergy with ascorbic acid (vitamin C) to promote iron absorption and bioavailability [89]; it is fairly scientific to state that whey protein peptides improve iron absorption and bioavailability. Opioid peptides are small molecules synthesized in vivo that act as hormones and neurotransmitters in the nervous system. Consuming dairy products, with whey proteins and low in fat can inhibit insulin resistance. Studies have also associated such products with blood pressure control and healthy weight because of high stimulus of satiety, consequently decreasing the occurrence of dental caries and coronary diseases [88]. Table 12.3 shows various studies that investigated different applications of whey protein fractions as nutraceuticals and other food applications.

Raikos and Dassios [26] reported that infant formulas formulated with whey protein hydrolysates have higher ACE inhibitory effect compared with infant formulas formulated without whey protein hydrolysates. Peptides with low molecular weight, usually less than 3000 Da, are mostly responsible for this action [20]. Consequently, the hydrolysed fragment sizes can be crucial for antihypertensive properties, since tripeptides and dipeptides can be absorbed readily from intestine and quickly transported to the target sites, including the blood [26, 94, 95]. Other foods have been formulated, studied, or proposed without whey protein [96–100]. However, the antioxidant properties of whey proteins have been reported, with potential benefits against oxidative stress, commonly reported in chronic noncommunicable diseases (CNCDs) and in preterm neonates [26, 96]. This property is as a result of the high levels of sulfur-containing AAs, including methionine and cysteine that are integral part of enzymes and organisms' antioxidants systems [88].

12.6 Conclusion

Whey protein is the globular proteins' collection from whey, the liquid milk component remaining after milk curdling and straining. While the major whey protein components include α-lactalbumin, β-lactoglobulin, BSA, and the fraction of proteose-peptone, the minor whey protein components include immunoglobulins, lactoferrins, ceruloplasmins, as well as certain enzymes such as lipase, xanthine oxidase, and lysozyme. Three types of whey proteins include whey protein concentrate, whey protein isolate, and whey protein hydrolysate, with various compositions. Whey protein contains sulfur-containing amino acids, e.g., methionine and cysteine that boost immune functions of glutathione. Whey proteins are suitable for

Table 12.3 Studies that highlight the applications of whey proteins in nutraceuticals and functional foods

Title of study	Aim	Type of study	Conclusion	References
"Assessing Whey Protein Sources, Dispersion Preparation Method and Enrichment of Thermomechanically Stabilized Whey Protein Pectin Complexes for Technical Scale Production"	The study aimed at optimizing the production of whey protein pectin complexes with thermal stability using lab scale scraped-surface heat exchanger before technical scale upscaling	Research	Dispersions of whey protein powder-based complexes had bigger particles that have lower particle volume within submicron ranges, compared with the complexes formulated with whey protein concentrates. The authors concluded that various sources of whey proteins are suitable for producing pectin–whey protein complexes for applications in food and nutraceutical industries	[90]
"Direct ethanolic extraction of polar lipids and fractional crystallization from whey protein phospholipid concentrate"	Extraction of dairy lipid from whey protein phospholipid concentrate using exposure directly to ethanol, food-grade solvent	Research	The study proved that fractional crystallization and ethanol extraction are suited for whey protein phospholipid extraction and sphingomyelin enrichment	[91]
"Emerging trends in nutraceutical applications of whey protein and its derivatives"	This study aimed at providing invaluable insights into functional foods fortified with whey protein, and their relative technological challenges and improvement opportunities.	Review	The study acknowledged whey protein as a significant source of important nutrients. The authors concluded that several food formulas enriched with whey protein can be formulated for target human populations, including infants, athletes, phenylketonuria patients, diabetics, geriatrics, cardiac-risk group, etc.	[17]

(continued)

Table 12.3 (continued)

Title of study	Aim	Type of study	Conclusion	References
"Preparation and Characterization of Whey Protein-Based Polymers Produced from Residual Dairy Streams"	The study investigated alternative ways to formulate polymers from milk proteins, a renewable source, aimed at polyethylene replacement	Research	The study carried out protein-based thermoset elastomers synthesis with whey protein concentrate/ isolate. The whey protein concentrates and isolate showed significant potentials for application as polymers via copolymer free-radical polymerization. Such polymers can be used in functional food applications	[92]
"Whey and protein derivatives: Applications in food products development, technological properties and functional effects on child health"	The study aim was to demonstrate use of whey and whey protein in foods and their influence on infants and children health	Review	This study concluded that whey protein has several nutritional benefits and food technological applications for improvement in early stages of life; although further studies are required	[20]
"Whey Proteins and Its Derivatives: Bioactivity, Functionality, and Current Applications"	The study aimed at showing the current uses, functionality, bioactivity, and of whey protein and its derivatives	Review	It highlights the functional characteristics, bioactive properties, applications, and processing limitations of various fractions of whey protein and its derivatives in food formulations, packaging, and encapsulation	[22]
"Whey proteins characterization, free amino acids profile and antimicrobial study of some dairy drinks" based on Milk Serum	To characterize certain dairy drinks using milk serum in regards with free amino acids and whey proteins using reversed phase HPLC	Preprint (Research)	The results obtained proved that dairy drinks formulated from milk serum are important sources of bioactive components for human health	[93]

(continued)

Table 12.3 (continued)

Title of study	Aim	Type of study	Conclusion	References
"Whey Proteins—A Potential Nutraceutical"	This study aimed at exploring the potentials of whey proteins as nutraceuticals	Review	Many whey protein enriched food formulas can be formulated for target groups, including infants, athletes, phenylketonuria patients, diabetics, geriatrics, cardiac-risk group, etc.	[21]

nutraceuticals and functional food formulations, and have been shown to have cardioprotective and hypotensive effects, immune improvement in HIV patients, immunomodulatory properties, anticarcinogenic properties, anti-diabetic properties, antihypertensive effects, antioxidant properties, infants' immunity, muscle synthesis and resistance exercise, phenylketonuria treatment, postoperative care, prebiotic and gut functions, skin protective effects, and treatment of obesity and overweight. Whey proteins are suitable as the basis of various nutraceutical formulations; however, more control studies are required to explore their applications for other targeted therapies.

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