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Whey proteins processing and emergent derivatives: An insight perspective from constituents, bioactivities, functionalities to therapeutic applications

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ABSTRACT

The massive research interest in whey has strengthened its position among coagulated milk products. Previously conducted reviews demonstrate that whey-derived functional foods provide a cascade of beneficial applications that promote health and wellbeing, and in managing numerous chronic diseases. To improve the understanding about how whey protein processing brings about new products that help in tackling health challenges is what we have attempted in this review paper. Herein, we provide an insight perspective into whey proteins processing and its derivatives from constituents, bioactivities, functionalities to therapeutic applications, drawing from: (a) prime constituents of whey protein; (b) composition and production of sweet/acidic whey; (c) bioactive peptides aspects of whey and its health/wellbeing benefits; (d) whey processing techniques: improving whey proteins' functionalities; (e) whey and its derivatives-based products: generating new functional foods and beverages and (f) whey-derived products in health and wellbeing: some therapeutic applications.

1. Introduction

The demand for health-promoting dairy products, functional foods, and nutraceuticals has escalated over the past several decades. Besides either fortified or enriched foods that incorporate novel bioactive components, functional foods exhibit therapeutic potentials beyond basic nutritional needs, whether from plant and animal sources (Klopčič et al., 2020). Food and pharmaceutical industries are involved in developing functional foods, cosmetics, skincare products, anticancer and immune-modulating agents, probiotics/prebiotics/symbiotics for managing and preventing non-communicable diseases (NCDs) (Sharma, 2019). Novel biotechnology methods and sophisticated microbial manipulation techniques are key in developing high-value dairy products, infant formulas, and functional foods from milk, colostrum, and whey to meet consumer demands (Sharma, 2019). Among such developing high-value dairy products, whey is widely believed to have started around 5500 BCE with therapeutic potential, which was initially suggested by the "Father of Modern Medicine" – Hippocrates (Brandelli et al., 2015).

Whey's classification into two major types include sweet (produced by enzymatic coagulation frequently used chymosin) and acid (produced by the addition of mineral acids and organic acids, like tartaric,

Abbreviations: WP, Whey powder; WPs, Whey Proteins; WPP, Whey protein powder; WPI, Whey protein isolates; WPC, Whey protein concentrate; WPH, Whey protein hydrolysate; β-La, β-Lactoglobulin; α-La, α-Lactalbumin; Igs, Immunoglobulins; BSA, Bovine serum albumin; GMP, Glycomacropeptide; L_F, Lactoferrin; CVDs, Cardiovascular diseases; GSH, Glutathione; PKU, Phenylketonuria; CCP, Colloidal calcium phosphate.

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acetic, and fumaric) (Chandrapala et al., 2015). Sweet whey has titratable acidity and pH range between 0.10–0.2%LA, and 5.8–6.6 respectively, whereas acid whey has titratable acidity percentage > 0.40%LA, and pH > 5.0 (Ramos et al., 2016). Previously, 'whey' produced by cheese and casein manufacturing was deemed environmental pollutant with limited use at animal feed supplements. Howbeit, the remains were disposed off in streams or dumped on wasteland, which negatively impacted on the aquatic life and soil quality/productivity owed to the high organic load, and high oxygen demand for biodegradation, i.e., about 27–60 g/L and 50–102 g/L, respectively (Brandelli et al., 2015; Smithers, 2015; Yadav et al., 2015). Given the need to reduce waste, the effective utilization and conversion of liquid whey into a wide range of valuable human food supplements is on the increase (Deeth & Bansal, 2018). Other contributory factors, which have enhanced the utilization of whey products, include the abundant presence of health-promoting food constituents and the marketability of manufactured products considered 'generally recognized as safe' (GRAS) by the food/drug regulatory agencies (Brandelli et al., 2015). Recently, the increased consumer demand for whey-derived nutrient-rich components has made such cost-effective products available in the market like functional foods and nutraceuticals. For instance, whey-derived products exhibit a rich source of vitamins and minerals, the higher digestible proteins and essential amino acids, and excellent source of sulfur-linked amino acids, which would supply the energy to perform various metabolic body functions (Singh & Geetanjali, 2016).

Key selected reviews involving various aspects of whey protein conducted within the past two decades in terms of aims/objectives and key sections are shown in Table 1. Whereas Minj and Anand (2020) reviewed the bioactive properties, functional characteristics, associated processing limitations, and applications of different whey protein fractions and derivatives involved in the field of food formulations, encapsulation, and packaging, Doost et al. (2019) and Gilblin et al. (2019) respectively reviewed the principles of whey protein conjugation to carbohydrates, applicable in food products, as well as how whey proteins can modulate cellular redox pathways and conversely how whey proteins are oxidised during processing. Researches conducted worldwide with emphasis on the health benefits of the whey were reviewed by Kadam et al. (2018), and whey from different milk sources and whey proteins in the context of other known food antioxidant were compared by Corrochano et al. (2018). Other conducted reviews included selected uses of whey and whey preparations in the food industry (Królczyk et al., 2016), whey protein, its fractions, and the therapeutic effect, and its application in the food processing and pharmaceutical field (Kassem, 2015), most recent advances on the controlled modifications of whey protein structures for specific functionalities (Guyomarc'h et al., 2015), health benefits of whey proteins, especially research advances about their biological properties (Solak and Akin, 2012), as well as physiological properties of bioactive peptides obtained from whey proteins, including the stabilities of such peptides obtained during their gastrointestinal route (Madureira et al., 2010). Earlier workers like Madureira et al. (2007) and Jovanović, Barać, and Maćej (2005) respectively reviewed the biological properties of whey proteins, and whey protein properties and their possible use in dairy industry.

These (above-mentioned) previously conducted reviews demonstrate that whey-derived functional foods provide cascade of beneficial applications that promote health and wellbeing, and in managing numerous chronic diseases. However, continuous effort is required in understanding how whey protein processing brings about the (new) products that help in tackling health challenges, hence, the need for more literature synthesis regarding whey-derived functional foods and its processing to help supplement existing information. In this review, we provide an insight perspective into whey proteins processing and its derivatives from constituents, bioactivities, functionalities, to therapeutic applications, drawing from a) prime constituents of whey protein, b) composition and production of sweet/acidic whey, c) bioactive peptides aspects of whey and its health/wellbeing benefits, d) whey

Table 1
Summarized selection of reviews involving various aspects of whey protein conducted within the past two decades.

References	Aim/Objective of Review	Key Aspects of the Review
Minj and Anand (2020)	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.	Whey protein derivatives: concentrates, isolates, and hydrolysates; biological properties of whey proteins associated with bioactive peptides; functional properties of whey proteins; current applications of whey proteins and its derivatives
Doost et al. (2019)	This work reviewed basic principles of whey protein conjugation to carbohydrates, applicable in food products.	Whey proteins: practical limiting issues and potential solutions, Covalent conjugation via Maillard reaction, Preparation techniques
Gilblin et al. (2019)	This review summarises how whey proteins can modulate cellular redox pathways and conversely how whey proteins can be oxidised during processing.	Whey proteins and their ability to modify redox pathways – studies in animal models and humans, Bioavailable antioxidant whey peptides, Redox modification of whey during processing, as well as oxidation, glycation, and racemisation of whey proteins.
Kadam et al. (2018)	This review of the research conducted worldwide emphasizing the health benefits of the whey.	Commercial status, health benefits of the Whey that evolves from healthy aging, sarcopenia, bone health, physical performance, sports nutrition, weight management, infant nutrition, immunity, to gastrointestinal support.
Corrochano et al. (2018)	The review compares whey from different milk sources and puts whey proteins in the context of other known food antioxidants.	Do whey products show antioxidant activity in vitro? Can whey products boost intracellular antioxidant defenses in vitro? Do whey products act as antioxidant protector in vivo?
Królczyk et al. (2016)	This review discussed selected uses of whey and whey preparations in the food industry.	Meat and meat products, Products with reduced fat content, Bakery and confectionary products, Dairy products, Other uses of whey in the food industry,
Kassem (2015)	This review is focused on whey protein, its fractions, and the therapeutic effect, and its application in the food processing and pharmaceutical field.	Whey proteins, Whey protein products, Major whey protein fractions, Minor whey protein fractions, Health properties of whey protein fractions such as anti-microbial and anti-viral, anti-oxidant, Immune modulating and Wound healing
Guyomarc'h et al. (2015)	This work reviewed the most recent advances on the controlled modifications of whey protein structures for specific functionalities	Native whey proteins, Structural characteristics of whey protein assemblies and related functionalities, Surface charge and apparent isoelectric pH values, Surface hydrophobicity
Solak and Akin (2012)	This review described the health benefits of whey proteins, especially research advances about their biological properties	Antimicrobial and antiviral activities, immune modulating activity, anticancinogenic properties, cardiovascular health, physical performance, weight management, bone health, other health benefits
Madureira et al. (2010)	This work reviewed the physiological properties of bioactive peptides obtained from whey proteins, including the stabilities of	Production of bioactive peptides; peptides with antihypertensive and antithrombotic activities; peptides with opioid and ileum-

(continued on next page)

Table 1 (*continued*)

References	Aim/Objective of Review	Key Aspects of the Review
	such peptides during their gastrointestinal route.	contracting activities; peptides with antimicrobial and immunomodulatory activities; peptides with nutrition system activities; other peptides with bioactivities; stability of bioactive peptides; protein–peptide interactions
Madureira et al. (2007)	This work reviewed the most recent research advances about biological properties of whey proteins.	Whey protein system, Whey protein concentrates and whey protein isolates, β-Lactoglobulin, α-Lactalbumin, Bovine serum albumin, Immunoglobulins, Lactoferrin, Lactoperoxidase
Jovanović, Barać, and Maćej (2005).	This work reviewed the whey protein properties and their possible use in dairy industry	Whey proteins, The influence of high temperature on whey proteins, Thermally induced complex between whey proteins and casein, Whey protein-based products, The application of whey proteins and whey protein products in acid fermented products. The application of the whey proteins and whey protein products in cheese making

processing techniques: improving whey proteins' functionalities, e) whey and its derivatives-based products: generating new functional foods and beverages and f) whey-derived products in health and well-being: some therapeutic applications. We believe that this perspective review will help enhance the understanding regarding the biochemical and functional properties of whey-derived natural products, especially their usefulness in tackling health and disease.

2. Prime constituents of whey protein

Whey proteins and their derivative components play an imperative and revolutionary role in the pharmaceuticals, healthcare products, and formulations of numerous functional foods, besides this these WPs exhibit numerous therapeutic applications in the prevention of various diseases (Brandelli et al., 2015; Deeth & Bansal, 2018). Moreover, the physical and functional properties of WPs and their derivatives can be significantly affected by (a) extrinsic factors; temperature, oxidation-reduction potential, surfactants, environmental stress, surface charge, (b) intrinsic factors; composition, structure, charge, hydrophobicity, hydrophilicity, surface charge, bound ligands, microbial load, (c) processing conditions; homogenization, acidification, heating, freezing, drying, hydrolysis and (d) other factors; lipids, sugar/salt, rigidity/flexibility, pH (neutral, acidic and alkaline), genetics (Minj & Anand, 2020).

Additionally, whey can be transformed into whey protein powder (WPP), whey protein concentrate (WPC), whey protein hydrolysate (WPH), whey protein isolates (WPI), and other metabolites by using sophisticated processing techniques including, chromatographic séparation, membrane séparation, ultrafiltration, fermentation and non-filtration techniques (Krunic et al., 2018; Sharma, 2019; Yadav et al., 2015). The WPP is produced from the whey obtained during cheese making by following the clarification, heat-treatment and drying to obtained fine powder. Moreover, these WPP are manufactured and commercialized in different segments, including demineralized, acid, sweet and other reduced forms, in which the protein and lactose content range from 11.0–14.5%, and 63–75%, respectively (Sharma, 2019; Yadav et al., 2015). The WPC is formulated from the WPs that comprises the lowest level of fats. Furthermore, the protein content in WPC ranges from 65 to 70%. The different variants of WPC are commercialized at a different level of protein (w/w) via 35, 50, 60 and 80% (Madureira et al., 2007). The WPC is also an adequate source of bioactive constituents, carbohydrates, and amino acids, as well as primary lysine and sulfur-containing AAs, which is an excellent alternative source for lysine-deficient diet (Krunic et al., 2018; Khaire & Gogate, 2019).

Besides, the WPH is among very digestible and less allergic proteins, due to their manufacturing method. In the production of WPH, the proteins are hydrolysed that ultimately results in protein breakdown into smaller peptides and polypeptides. The protein content in WPH range between 80 and 90%, which are frequently utilized in the production of infant's formula (Khaire & Gogate, 2019; Sharma, 2019; Krunic et al., 2018). The WPI is among the purest form of proteins, being produced by the additional purification steps to remove lactose (<1%) and fat. The WPI comprises of higher protein content, i.e., >90% which are the most preferred by sports persons (Khaire & Gogate, 2019; Yadav et al., 2015; Krunic et al., 2018; Sharma, 2019). The physico-chemical properties of (major and minor) whey proteins and their attributes in health and diseases are presented in Table 2. The whey proteins, that is, β-lactoglobulin (β-La), α-lactalbumin (α-La), Bovine serum albumin (BSA), Lactoferrin (L_F), Lactoperoxidase (LPO), Glycomacropeptide (GMP), Protease-peptone, can be seen to possess varying concentration (g/L), molecular weight (Kilodalton), isoelectric point (pI), whey protein %, amino acid residues, and health attributes. What is important to reiterate here is, all play different but yet vital role to human wellbeing, and are required in different quantities (Guo & Wang, 2019; Khaire & Gogate, 2019; Kilara & Vaghela, 2018; Minj & Anand, 2020; Ramos et al., 2016). We, in the subsequent sub-subsections, will provide more insights into these (above mentioned major and minor) whey proteins.

2.1. *β-Lactoglobulin*

β-Lactoglobulin (β-La) is the most abundant whey-derived protein that originates in bovine milk. These globular WP belong to the lipocalin family which, are synthesized in the mammary gland by epithelial cells (Deeth & Bansal, 2018). The structure of β-La contains two disulfide bonds at Cys66-Cys160 and Cys106-Cys119 and one free sulfhydryl group on Cys121 buried within the protein structure. β-La exhibits various functional properties including binding of fatty acids, vitamin A and D, metabolism of phosphates in the mammary gland (Fenelon et al., 2019), binding and transportation of retinol, synthesis of glutathione (GSH), the prebiotic effect on *Bifidobacterium* and *Lactobacillus* (Krunic et al., 2018), increase of pre-gastric esterase activity, angiotensin-converting enzyme (ACE) inhibitory activity (Guo & Wang, 2019). Besides these positive attributes, certain authors reported that the consumption of β-La might cause allergy (Allergen; Bos d 5) to infants (Villa et al., 2018).

2.2. *α-Lactalbumin*

α-Lactalbumin (α-La) second most abundant bovine-derived WP which contributes approximately 20–25% (1.5 g/L^{-1}) of the total WPs (Yadav et al., 2015). The conformation of α-La is highly heterogeneous i.e., composed of 26% α -helix and 14% β-sheet (significantly unfolded) and the stabilization of the molecular structure is due to the occurrence of strong hydrogen bonding between calcium ions and α-La. The composition and structural conformation of bovine α-La showed approximately 72% homology to human α-LA (Tavares & Malcata, 2016). α-La is an excellent source of essential amino acids (EAAs) including leucine, isoleucine, valine, aspartic acid, cysteine, and also recognized for its calcium-binding, heat stability, and non-gelling protein (Guo & Wang, 2019). α-LA plays a crucial role in milk production, these proteins are produced in epithelial cells of the mammary gland and associated with the enzyme β-1,4- galactosyltransferase to form lactose synthase, which converts glucose and galactose into lactose (Layman et al., 2018).

Purified α-La from bovine milk/whey can form complexes with oleic acid which exhibits almost similar biological activity to Human α-La

Table 2
Physicochemical properties of whey proteins and their attributes in health and diseases.

Protein	Concentration (g/L)	Molecular weight (Kilodalton)	Isoelectric point (pI)	Whey protein%	Amino acid residues	Health attributes
β-lactoglobulin (β-La)	3–4	18.4	5.2	50–55	162	Source of BCAAs including cysteine, act as a carrier molecule, modulate lymphatic response, possess excellent gelling properties, stabilizing agent, anti-hypertensive and hypo-cholesterolemic activity
α-lactalbumin (α-La)	1.5	14.2	4.7–5.1	20–25	123	Source of EAAs and BCAAs major tryptophan, anti-cancerous, anti-proliferative effects, positive effect on gastric mucosa, supplements for infant formulae, increase brain serotonin level
Immunoglobulins (Igs)	0.6–0.9	150–1000	5.5–8.3	10–15	*	Immunomodulating properties, opioid activity, treatment of HIV, passive immunity
Bovine serum albumin (BSA)	0.3–0.6	69	4.7–4.9	5–10	582	Source of EAAs, lipids synthesis, inhibits tumour growth, inhibit the growth of human breast cell line (MCF-7)
Lactoferrin (L_F)	0.05	78	8.0	1–2	700	The anti-oxidant, anti-bacterial, anti-viral, iron-binding glycoprotein
Lactoperoxidase (LPO)	0.006	89	9.6	0.5	612	Anti-bacterial, reduction of hydrogen peroxide, a non-immune biological defence mechanism
Glycomacropeptide (GMP)	1.2–1.5	8.6	4.0–4.8	10–15	102–169	Source of BCAAs and N-acetyl-necromatic acid, effective against phenylketonuria (PKU)
Proteose-peptone	0.5	4–20	*	20–25%	136	A mixture of proteins and peptides left in solution after heating/acidification (pH-4.7)

Data compiled from different sources; (Guo & Wang, 2019; Khaire & Gogate, 2019; Kilara & Vaghela, 2018; Minj & Anand, 2020; Ramos et al., 2016)

made lethal to tumor cells (HAMLET) (Tavares & Malcata, 2016). α-La exhibits various functional and therapeutic applications including; decrease the risk of cancer (breast and colon cancer) (Ramos et al., 2016), increases lean body mass (Yadav et al., 2015), immunomodulatory and antitumor activity, helps in the absorption of minerals, lactose biosynthesis, used as sports supplements (Tavares & Malcata, 2016), prevent from gastric mucosal injury caused by intake of nonsteroid anti-inflammatory drugs (NSAID) (Krunic et al., 2018), Moreover, α-La is rich in tryptophan (precursor of serotonin) and neutral amino acids which increases the serotonin level in the brain, this increased serotonin level ultimately results in the reduce stress and depression (Sharma, 2019).

2.3. Immunoglobulins

Whey protein, despite being a high-biological-value protein from milk, could be available with and without immunoglobulins (Igs) (Bell, 2000). As serum proteins produced by white blood cells (WBC), Igs are crucial in transferring passive immunity (Bell, 2000; Mehra, Singh, et al., 2021a; Mehra, Kumar, et al., 2021b). Igs in human and bovine milk/colostrum are categorized into different classes; IgG (IgG_1 and IgG_2), IgA, IgE, IgD and IgM, based on their mechanism action, charge and size (Atkinson et al., 2017). The concentration of Ig in whey can be brought about by the selectively removal of major whey proteins such as α-lactalbumin (αLA), β-lactoglobulin (βLG) and bovine serum albumin (BSA) (Xu et al., 2000). Additionally, camel colostrum comprises high content of whey protein mainly Ig able to provide the new-born with immunity (El-Hatmi et al., 2007). More so, some whey proteins comprise of bovine immunoglobulins, which are similar to human immunoglobulins (Bell, 2000). The understanding behind this is that IgGs possess immuno-modulatory properties, which are basic and crucial for the transfer of humoral immunity to the neonate (Kumar et al., 2014), which would be effective against the human herpesvirus and microbial infections (Masuzawa et al., 2016; Mehra, Singh, et al., 2021a; Mehra, Kumar, et al., 2021b). Also, colostrum would comprise both immunostimulatory and immunosuppressive immunoglobulins (Bell, 2000).

2.4. Lactoferrin

Lactoferrin (L_F) is considered an iron-binding glycoprotein, also commonly known as the red protein of colostrum, milk, and whey (Siqueiros-Cendón et al., 2014). This glycosylated protein exists as a single peptide chain of transferrin family, thus a polypeptide chain and composed of 689 amino acids (Mw of 80 kDa), which are folded in two homologous lobes (N- and C-lobes) (Guyomarc'h et al., 2015; Mehra, Singh, et al., 2021a; Mehra, Kumar, et al., 2021b). Essentially, each lobe comprises two domains (N_1, N_2 and C_1, C_2) that form in between it is a cleft, and with a binding site for one iron ion. The increase in iron goes with heat stability of L_F but opposite for flexibility, which reduces. Additionally, LF would be the main basic protein found in milk, even though the charge distribution on L_F surface remains highly uneven (Guyomarc'h et al., 2015). L_F exists as a natural iron scavenger, maintain signalling pathway, anti-oxidant, microbial, inflammation properties (Siqueiros-Cendón et al., 2014), inhibition of hepatic CYP1A2 enzyme (Mehra, Singh, et al., 2021a; Mehra, Kumar, et al., 2021b), prevention and treatment of brain tumours, chemo- and radiotherapy for treating cancer (Cutone et al., 2020).

2.5. Glycomacropeptide

Glycomacropeptide (GMP) or caseinomacropeptide (CM) is a casein-derived glycoprotein (amino acid residue 106–169) that is released into whey during cheese-making process by the action of coagulating enzyme i.e., chymosin on κ-casein (Deeth & Bansal, 2018; Sharma, 2019). GMP concentration was found to be 9–15 folds higher in sweet whey as compared to the GMP concentration in mature milk (Foisy Sauvé et al., 2021). Besides this GMP is an excellent source of N-acetylneuraminic acid which increases sialic acid connotation (Gupta & Prakash, 2017). Supplementation/consumption of GMP exert several health potentials viz act as an immunomodulator and anti-inflammatory protein (Foisy Sauvé et al., 2021), have a prebiotic effect on *Bifidobacterium* and *Lactobacillus* sp. and maintain bone health (Sawin, 2016), enhance calcium absorption (Krunic et al., 2018), inhibits adhesion of several cariogenic bacteria including *Sobrinus, Sanguis and Streptococcus mutans* (Gupta & Prakash, 2017).

2.6. Other minor proteins

In addition to the above mentioned (major whey) protein types, the whey has been shown to exhibit several minor proteins including bovine serum albumin (BSA), folate binding protein (FBP), proteose peptone 3 (PP3) and osteopontin that are released from the κ-casein during the initial step of enzymatic coagulation. These minor whey proteins possess unique functional properties according to their nature of action (Guo & Wang, 2019; Khaire & Gogate, 2019). The BSA is the prime component of blood serum, which enters the milk through the secretory cells and shared about 1.2–1.5% of total milk protein. This multifunctional protein is known for its nutrient-binder property primarily fatty acids (C16 and C18) moreover, it also binds the metal ions and flavours compounds (Deeth & Bansal, 2018). Some of the recent studies suggest that the physicochemical and immunological properties of BSA found in milk are identical to those found in blood (Deeth & Bansal, 2018; Kilara & Vaghela, 2018; Sharma, 2019). The FBP is a folate-trapping protein being composed of around 220–237 AAs residue with a molecular weight of 26.5 kDa. This protein exists in both particulate and soluble forms. The FBP significantly enchase the folate bioavailability in neonates, which can be utilized as an alternative of folic acid and in the preparation of infant's formula (Fenelon et al., 2019; Nygren-Babol & Jägerstad, 2012).

Proteose peptone 3 (PP3) is a phosphorylated glycoprotein, that represents the major factor of proteose peptone. These are the low-molecular-weight fraction which makes them more surface-active (Kilara & Vaghela, 2018; Sharma, 2019). The PP3 offers good emulsifying properties in soybean oil and ice cream. This protein act as an immunomodulator, anti-bacterial and also inhibit the activity of lipase (Deeth & Bansal, 2018). Osteopontin is a glycosylated, phosphorylated protein that exists in two isoforms and its concentration in whey ranges from 18 to 22 mg/L (Deeth & Bansal, 2018). This protein exhibits numerous therapeutic applications including wound healing, angiogenesis, cognitive development, and immuno-modulation. Some of the recent studies suggest that this protein shows good affinity for lactoferrin and might be synergistic with lactoferrin. The osteopontin can be isolated from whey via anion-exchange chromatography (Christensen & Sørensen, 2016). The presence of different classes of enzymes viz lysozyme, lactoperoxidase, isomerase, ligases, transferase, cathepsin D, ribonuclease and hydrolases were also reported in whey (Deeth & Bansal, 2018; Fenelon et al., 2019; Madureira et al., 2007).

3. Composition and production of sweet/acidic whey

Basically, whey (also termed as lactoserum) would refer to turbid light yellow-green thin liquid obtained after the casein coagulation in milk, either through the action of protease enzyme involving rennet or by the acid treatment (Brandelli et al., 2015). As a liquid fraction, whey accounts for about 20% of the total protein content of bovine milk, different from casein that accounts for about 80%. Generally, the whey proteins comprise four major whey protein fractions, namely: β-lactoglobulin (50–55%), α-lactalbumin (20–25%), immunoglobulins (IgG, IgM, IgA) (10–15%), and bovine serum albumin (5–10%). Some authors consider lactoferrin, osteopontin, glycomacropeptide, proteose peptone 3 to be minor WPs (<300 mg/L) viz (Barone et al., 2020; Sharma, 2019; Smithers, 2015). The high organic load of whey is due to the presence of milk nutrient residues such as lactose, proteins, lipids, and vitamins (Smithers, 2015). Lactose, as main constituent of whey, comprises nearly 75% of dry matter or 50 g/L (Ramos et al., 2016). Besides, the removal of excess water to obtain the dehydrated product or whey powder is a challenging and expensive process. Thus, the effective utilization of whey is inextricably linked to the effective utilization of lactose. Unfortunately, lactose has limited marketable demand, and not often used as a sugar source in food/drinks, including lactose-intolerance in some humans (Ramos et al., 2016; Smithers, 2015).

Fig. 1 shows a pictorial description of the process of milk to whey, where Fig. 1(a) shows complex colloidal emulsion system of milk, Fig. 1(b) shows a schematic representation of making sweet whey by enzymatic coagulation; and Fig. 1(c) shows a schematic representation of making sour whey by acidic coagulation (Guo & Wang, 2019; Kelly, 2019; Kilara & Vaghela, 2018; Sharma, 2019). Essentially, milk is a heterogeneous lipid-protein-carbohydrate and water emulsion, which can be defined as a complex of the colloidal system as shown in Fig. 1(a), wherein fat is emulsified as globules and casein micelles (protein) are in a continuous phase of water together with lactose, minerals and WPs (Pereira, 2014). Whey (lactoserum) is ether produced by coagulation

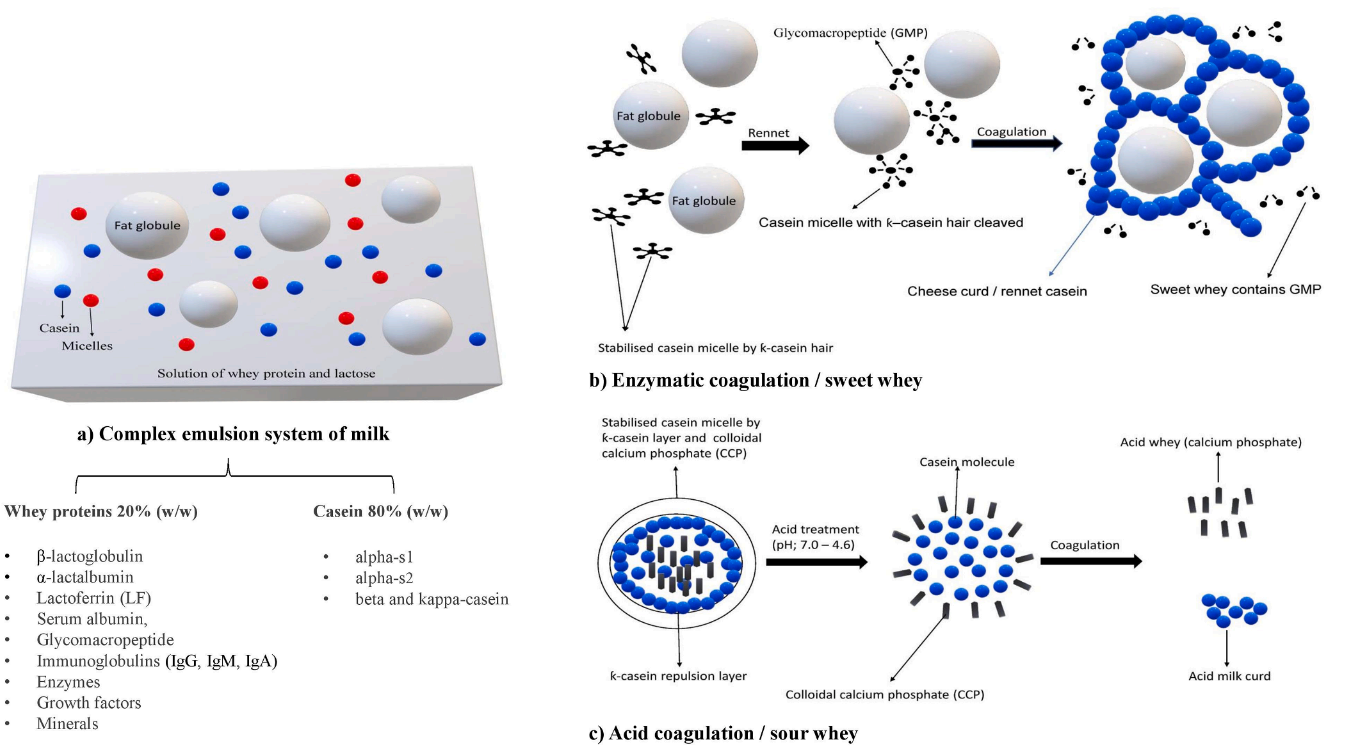

Fig. 1. Pictorial description of the process of milk to whey: (a) shows complex colloidal emulsion system of milk; (b) schematic representation of making sweet whey by enzymatic coagulation; and (c) schematic representation of making sour whey by acidic coagulation. Data compiled from different sources; (Guo & Wang, 2019; Kelly, 2019; Kilara & Vaghela, 2018; Sharma, 2019).

described previously and separation of curd (casein). Historically speaking, about 3000 years ago the first coagulation of milk was seen in the ruminant stomach, which was used for transporting and storing milk products. Based on scientific discoveries, we can now say that milk coagulation occurred while storing or transporting due to the presence of the naturally occurring enzyme "rennet" in the stomach of calves (Corredig & Salvatore, 2016).

The mechanism behind the rennet coagulation is illustrated in Fig. 1 (b). In sweet whey production, the proteolytic action of enzyme "rennet" cleaves the casein micelle hair i.e., κ-casein hair (casein protein which stabilizes the micelle structure), results in the crumpling of the micelle structure which leads to the curdling of milk. After the completion of the curdling process, fat globules are still emulsified by the casein curd while the leftover liquid (serum phase) can be strained out by cutting followed by pressing (Guo & Wang, 2019; Pereira, 2014). Fig. 1(c) represents the mechanism behind acid whey production. In this process, milk is treated with minerals / organic acid (tartaric, acetic and fumaric) at different pH conditions (4.6–5.2). In normal pH conditions (neutral), the casein micelle is stabilized by κ-casein hair via the electrostatic repulsion of colloidal calcium phosphate (CCP). The addition of acid in milk, results in shrinkage of the micelle hair layer due to the neutralization in κ-casein electrostatic repulsion, while the CCP binds casein molecules is solubilized into the serum phase. The casein micelle loses its stability and coagulated into milk curd. The whey strained from the acid coagulated milk curd is called "acid whey" (Guo & Wang, 2019). The acid whey is an adequate source of nitrogen, calcium, magnesium, phosphorus, potassium which can be an alternative source of inorganic fertilizer alone or in a mixture (Ketterings et al., 2017).

Nonetheless, the commercial use of acid whey is limited due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD) values were 31 and 35–50 respectively (Smithers, 2015). The coagulation induced by the different mechanisms i.e., acid/rennet results in the difference in their physicochemical properties. The concertation of lactose in acid whey is low i.e., 44.0–46.0 g/L as compared to sweet whey (46.0–52.0 g/L), this might be due to some of the lactose being converted into lactic acid (Ramos et al., 2016). Sweet whey has higher fat content (5.0 g/L) as compared to acidic whey (0.4 g/L). Moreover, the concentration of minerals in both acidic and sweet whey ranges from 4.3 to 7.2 g/L and 2.5–4.7 g/L, respectively. The sweet whey exhibits an adequate amount of glycomacropeptide (GMP) due to lack of rennet κ-casein cleavage, where the ash content in acid whey is approximately 8.0 g/L which is higher than that of sweet whey i.e., 5.0 g/L, this is due to calcium released from micelle into serum phase during acid coagulation (Ketterings et al., 2017; Smithers, 2015).

4. Bioactive peptides aspects of whey and its health/wellbeing benefits

Recently, bioactive peptides or cryptides has become the theme of scientific research due to their multifunctional properties (Krunic et al., 2018; Madureira et al., 2010; Mann et al., 2019). These peptides are the isolated protein fragments that are synthesised from the hydrolysis of whey proteins, which are mostly composed of 2–20 AAs residue per molecule (Brandelli et al., 2015; Minj & Anand, 2020). The bioactivity of peptides remain inactive while encoded in a sequence of the native protein, which further can be released by the hydrolysis by proteolytic enzymes (plant, microbial or by digestive origin) and fermentation with proteolytic stater cultures (Brandelli et al., 2015). Obtained bioactive peptides display a wide range of physiological actions on the cardiovascular, immune, nervous and gastrointestinal systems (Brandelli et al., 2015; Krunic et al., 2018; Madureira et al., 2010). A schematic overview of bioactivities of whey-derived bioactive peptide production with their physiological actions, in the context of major and minor whey proteins is presented in Fig. 2. On one hand, we show how whey proteins and its components through the various strategies employed to release and synthesise bioactive peptides. On the other hand, we show the various bioactivities involved within the immune, nervous, cardiovascular, gastrointestinal and metabolic systems.

Bioactive peptides were categorised based on the protein from which they are derived or by their physiological effects. Some of the different known bioactive peptides were cytomodulatory, opioid, immunomodulator, anti-hypertensive-oxidant-thrombotic and miscellaneous

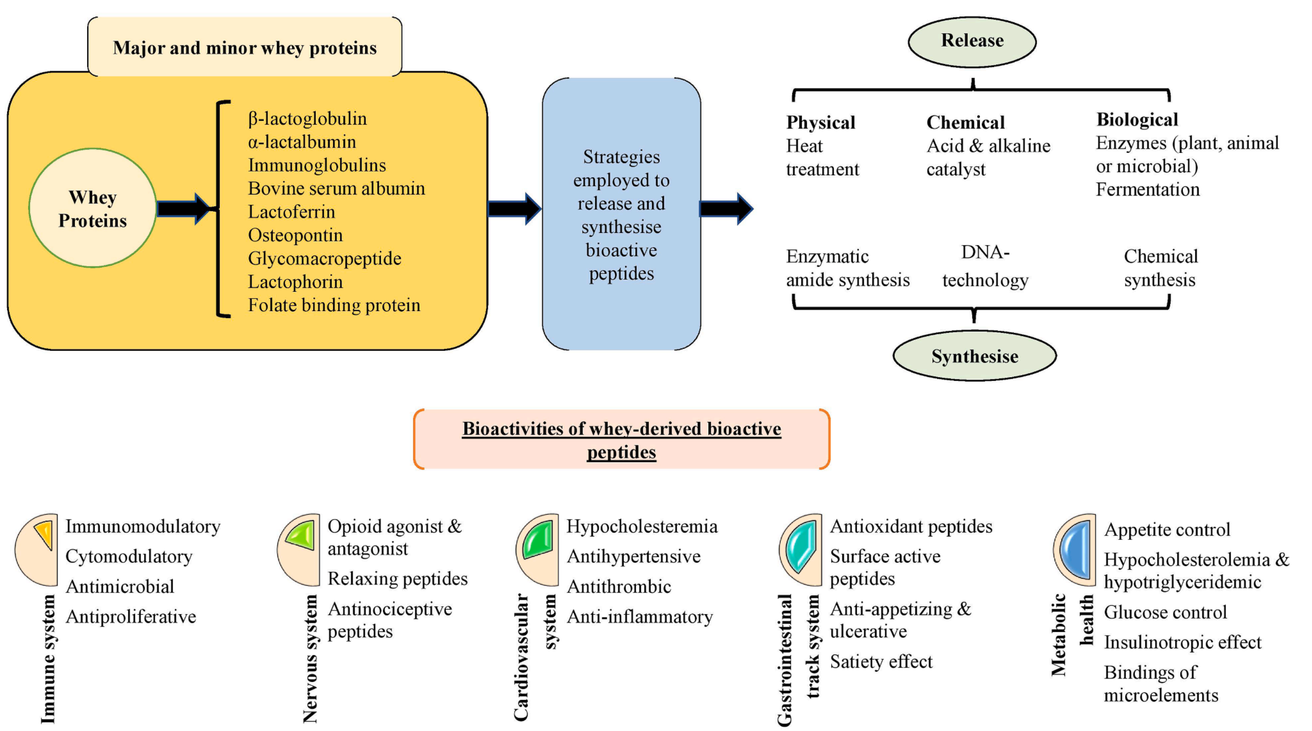

Fig. 2. A schematic overview of bioactivities of whey-derived bioactive peptide production with their physiological actions, in the context of major and minor whey proteins.

peptides (Brandelli et al., 2015; Mann et al., 2019; Minj & Anand, 2020). The ACE inhibitors peptides are the most extensively studied group of peptides, which are isolated from the milk protein hydrolysates (Krunic et al., 2018). The fermentation of milk by lactic acid bacteria or by proteinases is the most favoured and frequently used strategy to release ACE-inhibitor peptides from milk protein (Hayes et al., 2007). Furthermore, the hydrolysis of whey proteins with trypsin produces hydrolysates exhibiting good ACE-inhibitory activity. These ACE inhibitors regulate the peripheral blood pressure which ultimately reduces the oxygen demand from the human heart (Mann et al., 2019).

Recently the use of whey-based antioxidants attracts food manufactures and researchers due to their availability and safety as compared to synthetic antioxidants including propyl gallate, butylated hydroxyanisole (Brandelli et al., 2015). The mechanism action of these antioxidant peptides was investigated by different researchers involves in the chelation of transition metal ions, free-radical scavenging and the inhibition of lipid peroxidation (Mann et al., 2019). Peng et al. (2010) reported that the hydrolysis of WPI by alcalase results in good antioxidant activity in a liposome-oxidizing system which can be used in the food sector as a replacer of synthetic antioxidants. The whey proteins processed at low temperature exhibits a good number of dipeptides or glutamylcysteine which encourage the synthesis of glutathione (Park & Nam, 2015). The whey protein concentrate hydrolysed by thermolysin could be used as food additive which shows good anti-oxidant activity to prevent lipid oxidation (Contreras et al., 2011). It is well known that whey protein exhibits good anti-microbial properties due to the presence of lactoferrin, immunoglobins, lysozyme and lactoperoxidase which can act as a natural food bio-preservative (Mann et al., 2019). Lactoferrin is a multifunctional protein and its peptide i.e., lactoferricin is released by the action of proteolysis under acidic conditions, a reaction that occurs in the human body naturally. These peptides exhibit strong anti-microbial, anti-tumour and immunomodulatory properties (Gifford et al., 2005).

The major whey protein fraction i.e., β-La produce β-Lactorphin; Amino acid sequence f(102–105) and β-Lactotensin; amino acid sequence f(146–149) peptides when hydrolysed with pepsin, trypsin and chymotrypsin. These peptides represent good ACE-inhibitory, opioid agonist, ileum contracting and antinociception properties. Similarly, when α-La is hydrolysed with pepsin it generates α-Lactorphin with f (50–53) amino acid sequence (Madureira et al., 2010; Mann et al., 2019). Shin et al. (2007), reported that trypsin and papain can be used at the ratio of 1:1 for the removal of β-La and α-La, and to produce low antigenic hydrolysate. Furthermore, the authors also reported additional ultrafiltration is needed before using it in the preparation of an infant's formula. Similarly Guadix et al. (2006), reported that it is possible to produce WPH with reduced allergenicity up to 99.97% by using *Bacillus licheniformis* Protex 6L- a commercially available alkaline subtilisin in a continuous stirred tank membrane reactor. Jeewanthi et al. (2017), experimented to identify the appropriate food grade enzyme to hydrolyse WPC, which gives good bioactivity. For that reason, authors hydrolysed WPC-35 with different enzymes viz alcalase, protease S and M, α-chymotrypsin, trypsin and pepsin at different intervals of hydrolysis time. Based on the obtained results from the findings authors reported that the Protease-S represent the highest proteolytic and angiotensin converting enzyme inhibitory activity.

The hydrolysis of BSA with trypsin results in the generation of Albutensin A and Serophin that represent the amino acid sequence of f (208–216) and f(399–404) respectively. Both peptides showed a wide range of bioactivity viz Heum contracting and opioid activity (Madureira et al., 2010). Opioid peptides are those types of peptides that are comprised of Tyr-Gly-Gly-Phe present at the N-terminal side. These peptides show similar properties to opium or act like morphine in the brain (Mann et al., 2019). β-casomorphins are the prime peptides of opioids. These peptides are also found within the encrypted primary sequence of lactoferrin, BSA and β-La (Brandelli et al., 2015). The cytomodulatory peptides are isolated from the different dairy-product including whey that stimulates the immune function and inhibits cancer cell growth. These peptides are also known for their anti-carcinogen properties (Hayes et al., 2007; Mann et al., 2019). Minerals binding peptides of whey are generally released from the proteolytic digestion that has the potential to bind with cation including zinc, iron and calcium (Zhao et al., 2014). The anti-appetizing peptides are those types of peptides that suppress the appetite and prevent obesity. The consumption of whey proteins is associated with the release of cholecystokinin-an appetite-suppressing hormone (Park & Nam, 2015). Similarly Jakubowicz and Froy (2013) mentioned that the oral supplementation of WPH positively affects the insulinotropic response.

Overall, the bioactive aspects of whey-derived products and WPs makes them packaged with innumerable beneficial effects, and that is why they continue to provide a wide range of applications to the food and pharmaceutical industries, such as replacements for egg proteins, emulsifiers, making confectionery and bakery products, manufacture of dairy products (ice-cream, yogurt, probiotics), salad dressings, gel formation, recombined products, sports supplements, infant formulas and dietary supplements (Fenelon et al., 2019; Singh & Geetanjali, 2016). Further, the WPs and isolates can be utilized as functional foods which exhibit many positive attributes towards human health and prevention of non-communicable diseases like cancer, cardiovascular diseases, diabetes mellitus, gut function disturbances, obesity management, and improvement of muscle synthesis (Brandelli et al., 2015; Gerez et al., 2012; Khaire & Gogate, 2019; Layman et al., 2018; Singh & Geetanjali, 2016; Tavares & Malcata, 2016).

5. Whey processing techniques: Improving whey proteins' functionalities

Smithers (2008) considered the transformation of whey into vital derivates termed as "from the gutter to gold". The rapid expansion of novel processing technologies, scientific knowledge and sophisticated analytical procedures have given a better insight into the biological properties of whey and its derivatives towards human health and disease. This advancement in technology brings more curiosity to researchers to identify and to isolate novel constituents from whey. The techniques used for whey processing and isolation of different ingredients are shown in Fig. 3 (Kelly, 2019; Kilara & Vaghela, 2018; Krunic et al., 2018; Minj & Anand, 2020; Ramos et al., 2016; Singh & Geetanjali, 2016; Bylund, 2003). At present, whey was processed by adopting different advanced techniques including reverse osmosis, evaporation/drying, ultrafiltration (UF), non-filtration, chromatographic methods, ion exchange, electrodialysis, fermentation, chemical, as well as other treatments that are able to secure transformed products (Kelly, 2019; Kilara & Vaghela, 2018; Ramos et al., 2016). Whey derived isolates obtained by different techniques continue to remain crucial for food and pharmaceuticals (manufacturing) industries (Bylund, 2003). This is because these isolates are utilized as a functional ingredient for making health care supplements, confectionery and baking products, an alternative of skim milk powder, infant's formula, which serves as vehicles of bioactive compounds in foods including nano (emulsions, nanotubes), micro (emulsions, core–shell microstructures) and macro (bulk liquids, gels, films, particles and beads) formulas, coating material, nano-hydrogels and so on (Kilara & Vaghela, 2018; Minj & Anand, 2020).

The ultrafiltration and diafiltration membrane processing are the most favorable and routinely practiced techniques to process the whey with consequent low levels of non-proteinaceous substances and enhanced functional properties of resultant WPCs (Mann et al., 2019). The ultrafiltration techniques permit the manufacturers to produce WPC with the desired concentration of protein and the selective permeation of minerals, lactose, and other low-molecular-weight fractions compounds, where the diafiltration is employed to produce WPC with purified and high protein content (Mollea et al., 2013). The function of the proteins is associated with their natural structures, which significantly

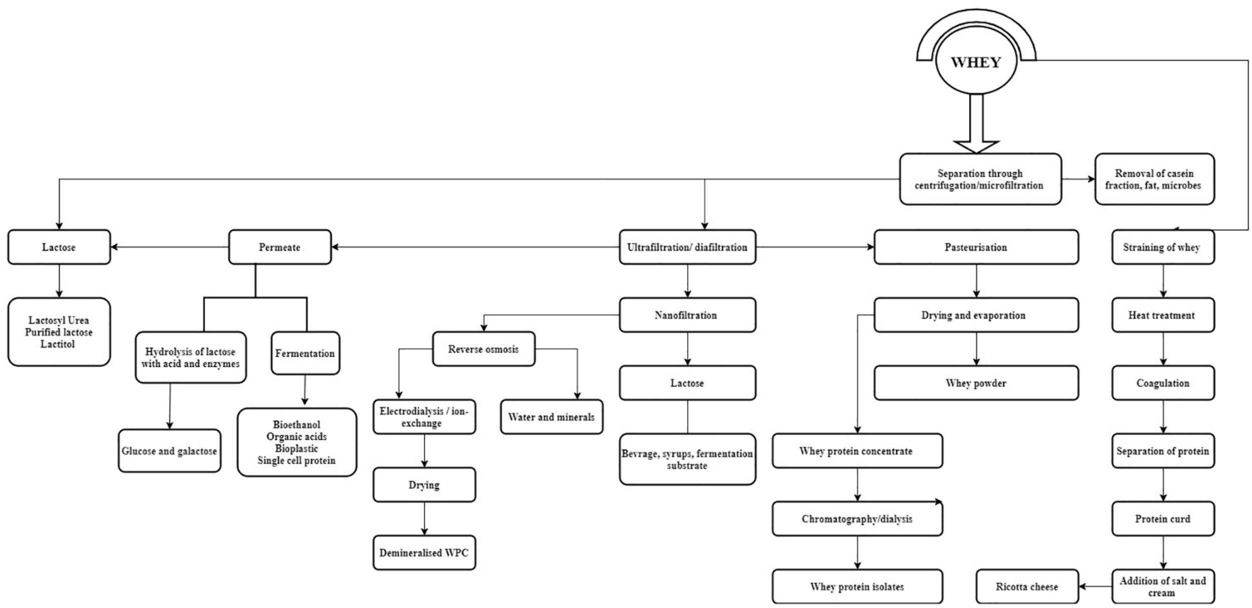

Fig. 3. Strategies and techniques used for whey processing to secure different ingredients (Bylund, 2003; Jelen, 2011; Kelly, 2019; Khaire & Gogate, 2019; Onwulata & Huth, 2008; Ramos et al., 2016).

depend upon the internal and external factors viz., temperature, pH, solvent effects. Alteration in the natural structures of proteins affects their functional properties. The fractionation of whey proteins accentuates the nutritional and functional properties of the distinct protein (Mollea et al., 2013; Khaire & Gogate, 2019).

The effect of ohmic heating versus standard heating in the processing of sweet whey was studied by Costa et al. (2018). These workers subjected sweet whey to ohmic heating at different levels of 2, 4, 5, 7 and 9 V.cm^{-1} at a frequency range at 60 Hz and heating 72–75 °C/15 s. The samples were analyzed for scavenging activity, particle size distribution, viable cell count. While concluding the results, the authors suggested that ohmic heating as an alternative for whey processing given the positive effects exhibited on the microscopic, rheological and sensory parameters of tested products. The authors understood that ohmic heating between 4 and 5 V would enhance the bioactive peptide release. Besides, heat treatment of whey below 75 °C would preserve the native proteins and other bioactive components, with simultaneous inactivation of pathogens (Xiong et al., 2019). This preservation can be applied while the processing of whey into different food products. The purification of lactoperoxidase (LPO) from the whey is successfully done by using the dye-affinity chromatography technique (Urtasun et al., 2017).

The pre-treatment of proteins with a pulsed electric field (PEF) could improve the degree of succinylation (Xu et al., 2021). Similarly, Shi et al. (2021) reported that high-pressure homogenization pre-treatment had a beneficial impact on the surface hydrophobicity, emulsifying activity, gel hardness, free-sulfhydryl groups, and cross-linking efficiency of WPI pretreated with citric acid. Meng et al. (2021) reported that sonication can be employed to modify the functional properties of whey proteins. This technique markedly enhanced the antioxidant, foaming, and emulsifying properties of WPI, without affecting their molar masses. Moreover, the authors reported that the use of such a sonication process decreased the size of protein aggregates and led to conformational changes (secondary and tertiary structures) in the WPI. Nonetheless, the manufacturing of demineralized WPC through a combined nano-filtration technique is proposed by Marx et al. (2019). For their experiment, these workers showed whey optimized with heat treatment (80 °C, 30 sec), membrane filtration (1.4 μm), diafiltration (0.75, 1.5) and the demineralization and concentration conducted solely by nano-filtration or combined (nano-filtration and diafiltration). This technique to produce de-mineralized WPC with minimal loss of bioactive components and extended shelf life is promising. Besides, other procedures commonly employed at the industrial level for the production of whey protein fractions include selective elution, precipitation, adsorption, and membrane filtration (Mollea et al., 2013).

The effect of high hydrostatic pressure on the structural and functional properties of aqueous WPI (dispersed, 5% w/v) over storage time was studied by Carullo et al. (2021). Aqueous dispersed phase was treated with 100–600 MPa pressure at different times i.e., 15–30 min. Further, the high hydrostatic pressure-assisted hydrolysis of aqueous WPI was performed by bromelain and α-chymotrypsin or a combination of both at 1:1 w/w. From the results, it was noted that the treatment of 400 MPa for 15 min results in the maximum degree of unfolding which ultimately enhances their interfacial properties. Furthermore, the combined use of enzymes at 1:1 showed synergistic effects on the hydrolysis of WPI. The aptness of supercritical CO_2 continuous reactor for the continuous fractionation of WPI was studied by Lima et al. (2021). The continuous reactor having dimensions (12 m length, 10 mm diameter, 97 mL volume) in which the samples were treated at different pressures (i.e., 8–24 MPa) and temperatures (i.e., 50–60 ℃). The highest fractionation i.e., α-La (47.5%) and β-La (11.2%) was observed at 16 MPa and 60 °C. The purposed technique can be employed at the industrial level for the large-scale continuous production of α-La and β-La streams. It is possible to recover protein fractions (β-La, α-La, BSA) and lactose from cheese whey by using an aqueous two-phase extraction system (González-Amado et al., 2021). For the experiment the authors had designed the extraction system made from polyethylene glycol (PEG), $(NH_4)_2SO_4$ and Na_2SO_4 in which (a) PEG −1500 with $(NH_4)_2SO_4$ for the separation of lactose and PEG-300 with Na_2SO_4 for fractionation of proteins. The system, that is (a) PEG −1500 as mentioned above, showed the recovery of 95% of proteins and 80% of lactose.

6. Whey and its derivatives-based products: Generating new functional foods and beverages

Consumers nowadays are more inclined to take control of their health and prefer to use foods of natural origin that offer potential health benefits beyond nutritional needs. To meet the consumers' demands, food manufacturers and researchers are continuously working to develop and formulate novel products with new technologies to gain more visibility in the food markets. The popularity of whey and its derivatives-based functional foods like probiotics, essential amino acid-enriched products, protein-containing sports supplements, and beverages are on the upswing. Such products include probiotic drinks, thirst-quenching drinks, and ready to serve drinks, flavoured drinks, smoothies, supplemented drinks, electrolyte drinks and all show new revolutionary trends in the foods and beverages industry.

Liu et al. (2018) reported the utilization of whey protein isolate (WPI) and whey protein concentrate (WPC) at different ratios to establish the protein-based 3-D printing food simulant. These workers showed that the mixture of WPI + WPC at the ratio of 5:2 was favourable material for extrusion-based 3-D printing. Moreover, the authors also suggest that this technique could be beneficial for the food sector in 3-D printing for the customization of food products. The tolerance and safety of partially hydrolysed whey protein-based infants' formula (PHWP-IF) versus intact cow milk protein formula (IPF) in a double-blind, randomized trial was studied by Picaud et al. (2020). The diet of infants from different countries were supplemented with 2.3 g/100 kcal, WPH-IF and 2.0 g/100 Kcal standard -IF as a placebo for 14 days −17 weeks. At the end of the study, the authors reported that PHWP-IF is well tolerable and safe for infants, moreover it supports the growth of infants. Rathod and Amamcharla (2021) used the fibrilization technique to convert the whey protein globular structures to fibrils, which ultimately enhanced the water holding capacity and viscosity as compared to the native protein structure. The WPI and micellar casein concentrate were combined at a ratio of 80:20 followed by heating at low pH. Further, the obtained fibrillated milk whey protein isolate product showed increased viscosity and coagulation time as compared to non-fibrillated WPI, which can be used further as a functional ingredient in dairy-based foods.

A wide variety of recently developed novel food/beverage products from whey and its derivatives has been summarized in Table 3**.** Based on whey based novel products, Zhou et al. (2019) studied the utilization of cheese whey in the production of bioethanol and galactonic acid, and Christensen et al., (2011) studied the utilization of organic whey in the bioethanol production using *Kluyveromyces marxianus,* Bosco & Chiampo, (2010) investigated the milk whey when utilized for the production of biodegradable plastic. Other studies include the utilization of whey permeates and retentate for the microencapsulation of *Lactobacillus plantarum* ATCC 8014 by spray drying (Eckert et al., 2017), the utilization of whey permeates medium in the production of xanthan gum by *Xanthomonas campestris* (Savvides et al., 2012), as well as whey based sports drink (Abella et al., 2016). Seyhan et al. (2016) investigated whey-based beverage supplemented with phytosterols and soy isoflavones wherein the beverages were prepared by adding either soy isoflavones or phytosterols as functional compounds (at levels of 0.25%, 0.50% or 1.0% w/v) with probiotic bacteria (*Lactobacillus acidophilus* LA-5 or *Lactobacillus casei* LBC-81). Additionally, the production of caproic acid from acid whey by open culture fermentation by Chwialkowska et al. (2019), the preparation of strawberry flavoured whey beverage with xylooligosaccharides (XOS); XOS is added in whey at level of 1.25 g/100 mL by Souza et al. (2019), the utilization of whey permeate as a feedstock for the biomass and lipid production of the microalgae *Chlorella protothecoide* by Espinosa-Gonzalez et al. (2014), utilization of whey for the production of liquid fertilizer (Akib & Setiawati, 2017), and the utilisation of whey permeate in wheat fermentation for the ethanol production by *Saccharomyces cerevisiae* by Parashar et al. (2016) are also shown in Table 3.

Based on whey-based probiotic beverage/product, Sasi (2015) investigated beverage formulation with whey and aloe vera juice with probiotic organism *Bifidobacterium bifidum* where there was blend of whey and aloe vera incorporated at the ratio of 70:30 and fermented for 9 h by using 1% *Bifidobacterium bifidum* inoculums. Sabokbar and Khodaiyan (2015) investigated the probiotic beverage prepared with whey and pomegranate juice fermented with kefir grains, which involved the fermentation at different temperature (19 °C and 25 °C) and of kefir grains inoculum (5% and 8% w/v). Additionally, Faisal et al. (2017) investigated the probiotic whey beverage with the incorporation of orange powder and flavours using fuzzy logic.

Based on fruits and vegetables-based whey beverages, the preparation of guava flavoured whey beverage by using cold plasma technology (Silveira et al., 2019), a beverage made using guava juice and sweet whey; the different combination of sweet whey and guava juice (85:15, 80:20, 75:25 and 70:30) (Yonis et al., 2014), and the utilized guava pulp in the preparation of whey-based beverage; whey and guava pulp was added 67.5 and 20% respectively. Formulation processed at different time/temperature combinations (60 °C, 65 °C, and 70 °C) (Singh et al., 2014), were shown in Table 3. Others include Whey raspberry flavoured beverage prepared by ohmic heating; different ohmic heating conditions (10, 100, and 1000 Hz at 25 V; 45, 60, and 80 V at 60 Hz) (Ferreira et al., 2019), RTS drink with papaya and whey (at different concentration 25, 50, and 100) (Panghal et al., 2017), RTS drink with tomato and whey at different ratios (Bangaraiah et al., 2014), A beverage made from sweet whey doum fruit; by soaking doum fruit in sweet whey in a ratio of 1:5 (w/v) (Tawfeuk & Khalil, 2019), Whey carrot beverage (WCB); ingredients are incorporated in different ratio carrot juice (25%); sugar (12%) and cheese whey (63–62%) (Abd El Raoaf et al., 2014), Mulberry-whey beverage; sweet whey (SW) and black mulberry (BM) juice were blended in different levels; 25%, 50%, and 100% (AbdulAlim et al., 2018).

Based on fermented beverage, studies investigated include those of Aly et al. (2019) who prepared sweet whey-based fruits beverages fermented with *Lactobacillus Plantarum*; sweet whey with cactus pear juice at the different percentage (10, 20, 30%) and kaki juice (10 and 20%), Kaur et al., (2019) who prepared carbonated whey beverage with the addition of strawberry and pineapple juice; fermentation is carried out at 35 ± 1 °C for 36 h with yeast culture (*Clavispora lucitaniae* @ 0.5% v/v) and the juice is added at a different percentage (15, 20, 25 and 30 per cent), and Nursiwi et al., (2017) who fermented whey beverage produced with the incorporation tomato juice; tomato juice was added at different concentration (5, 10, 15%), with probiotic bacteria *Lactobacillus acidophilus* and *Lactobacillus plantarum*. Additionally, Sohrabi et al. (2016) prepared unfermented and ferment whey-based beverage fortified with vitamin E, whereas (Skryplonek, 2018) utilised acid whey in the preparation of fermented yogurt type beverage.

7. Whey-derived products in health and wellbeing: Some therapeutic applications

Each isolate of whey i.e., β-La, α-la, α-La, BSA, Igs, L_F, L_P, GMP and other minor components exhibits its unique function and mechanism of action. For instance, BSA exhibits the binding property with fatty acids and immunoglobins, which remains a crucial role in the development of passive immunity (Ulfman et al., 2018). The iron-binding protein "lactoferrin" enhances the absorption of iron in the digestive tract, which ultimately inhibits the growth of enteric microorganisms. Lactoferrin as shown by recent studies could possess anti-bacterial properties and at the same time, serve as a natural preservative particularly during milk storage where it helps to control the pH level (Minj & Anand, 2020). Glutathione, in whey, would act as a natural anti-oxidant, which regulates the various cellular process (Minj & Anand, 2020). GMP depicts various functional properties (emulsifying and foaming abilities) and major therapeutic applications in persons afflicted with the treatment of phenylketonuria (PKU) (Zaki et al., 2016). Further, these WPs serve as a great source of free cysteine that stimulates the production of glutathione (GSH) (Foisy Sauvé et al., 2021), which can be utilized as a cheaper medium for the production of bacteriocin by *Bacillus sp.* P11 (Leães et al., 2011).

The efficacy of WP and its derivatives in the prevention and treatment of various diseases have required deliberations evidenced in the published meta-analysis, as well as double-blinded, placebo-controlled, animals and human trials. Obtained results from the different scientific results suggest that whey protein (WP) and its derivatives are effective in

Table 3
Summary of whey-derived novel foods and beverage products.

Product	Study aim	Key findings	Reference
Whey based novel products			
Organic acids	The utilization of cheese whey in the production of bioethanol and galactonic acid	*Saccharomyces cerevisiae* and *Gluconobacter oxydans* are utilized to produce ethanol and galactonic acid. Finally, 110 g ethanol, 320 g galactonate, and other protein 150 g was produced from 1 kg cheese whey protein	(Zhou et al., 2019)
Bioethanol	Utilization of organic whey in the bioethanol production using *Kluyveromyces marxianus*	Ethanol production is carried out in batch and continuous fermentation. The study reveals that freezing and pasteurization was not essential, and *K. marxianus* was able to utilize lactose for the production of ethanol. High ethanol (2.5–4.5 g/l/h) productivity was achieved in continuous fermentation using Ca-alginate-immobilized *K. marxianus*	(Christensen et al., 2011)
Bio-plastic	Milk whey was utilized for the production of biodegradable plastic	This study suggests that whey can be utilized for the preparation of biodegradable plastic without any aseptic conditions. Obtained results showed polyhydroxyalkanoates at the carbon to nitrogen ratio (C/N) = 50	(Bosco & Chiampo, 2010)
Encapsulation	Utilized whey permeates and retentate for the microencapsulation of *Lactobacillus plantarum* ATCC 8014 by spray drying	Results of the study reveal that WP and WR are suitable for the preparation of wall material to protect bacterial cell at high temperature	(Eckert et al., 2017)
Growth Media	The utilization of whey permeates medium in the production of xanthan gum by *Xanthomonas campestris*	Whey permeates (de-proteinated, partially hydrolysed by *β*-lactamase and partially hydrolysed and de-proteinated) utilized as culture media. Hydrolysis of *β-lactamase* produced 28 g/l xanthan gum. This study reveals that WP can be utilized as low-cost media for the production of xanthan gum	(Savvides et al., 2012)
Drink	Whey based sports drink	For the preparation of sports drink; acid whey (3.32% lactose) was fermented with the culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Obtained fermented whey (2.84%) with stabilizer added at different percentage (T1 = 0%, T2 = 0.1%, T3 = 0.125%, and T4 = 0.15%). Among all combinations, T3 was found to be best as a hypertonic sports drink	(Abella et al., 2016)
Probiotic drink	Whey-based beverage supplemented with phytosterols and soy isoflavones; beverage prepared by adding either soy isoflavones or phytosterols as functional compounds (at levels of 0.25%, 0.50% or 1.0% w/v) with probiotic bacteria (*Lactobacillus acidophilus* LA-5 or *Lactobacillus casei* LBC-81)	Prepared functional beverage with phytosterols is more prefer with sensory panellist as compare to the incorporation of isoflavones	(Seyhan et al., 2016)
Acid whey	Production of caproic acid from acid whey by open culture fermentation	An economically effective method for the production of caproic acid	(Chwialkowska et al., 2019)
Functional beverage	Preparation of strawberry flavoured whey beverage with xylooligosaccharides (XOS); XOS is added in whey 1.25 g/ 100 mL	The utilization of XOS in the preparation of whey-based beverages is an interesting approach	(Souza et al., 2019)
Biomass	Utilize whey permeate as a feedstock for the biomass and lipid production of the microalgae *Chlorella protothecoide*	WP can be utilized as a carbon source for the heterotrophic growth and lipid accumulation of *Chlorella protothecoide*	(Espinosa-Gonzalez et al., 2014)
Fertiliser	Utilization of whey for the production of liquid fertilizer	This study suggests that whey can be used in collaboration with other liquid waste for the production of liquid fertilizer	(Akib & Setiawati, 2017)
Ethanol	Utilized whey permeate in wheat fermentation for the ethanol production by *Saccharomyces cerevisiae*	WP was hydrolysed to release fermentable sugar and integrated into the wheat substrate as a co-substrate (alternative of water). The result of the study suggests that incorporation of whey doesn't affect the fermentation process and can be utilized to design various value-added products including ethanol	(Parashar et al., 2016)
Whey-based probiotic beverage/product			
Fermented drink	Beverage formulate with whey and aloe vera juice with probiotic organism *Bifidobacterium bifidum*; blend of whey and aloe vera were incorporated at the ratio of 70:30 and ferment for 9 h by using 1% *Bifidobacterium bifidum* inoculums	Prepared beverage with 1% *Bifidobacterium bifidum* achieved highest sensory score. This probiotic beverage could be an interesting approach to develop functional beverages	(Sasi, 2015)
	Probiotic beverage prepared with whey and pomegranate juice fermented with kefir grains; fermentation was carried out at different temperature (19 °C and 25 °C) and of kefir grains inoculum (5% and 8% w/v)	Study revealed that pomegranate juice and whey with kefir grains is an interesting approach to produce a probiotic beverage	(Sabokbar & Khodaiyan, 2015)
Flavoured drink	Probiotic whey beverage with the incorporation of orange powder and flavours using fuzzy logic	A new approach to utilize orange powder with whey to provide a new variant of whey-based functional probiotic drink	(Faisal et al., 2017)
Fruits and vegetables-based whey beverages			
Guava	Preparation of guava flavoured whey beverage using cold plasma technology	Cold plasma is a promising technology for the preparation of whey-based beverages (with minimum deterioration of bioactive components)	(Silveira et al., 2019)
	A beverage made using guava and sweet whey; the different combination of sweet whey and guava juice (85:15, 80:20, 75:25 and 70:30)	Beverage made in the ratio of whey (75%); guava (25%) has good sensory properties	(Yonis et al., 2014)
	Utilized guava pulp in the preparation of whey-based beverage; whey and guava pulp were added at levels of 67.5 and 20% respectively. Formulation processed at different time/ temperature combinations (60 °C, 65 °C, and 70 °C)	Different time/ temperature combinations significantly effect on compositional parameter	(D. Singh et al., 2014)
Raspberry	Whey raspberry flavoured beverage prepared by ohmic heating; different ohmic heating conditions (10, 100, and 1000 Hz at 25 V; 45, 60, and 80 V at 60 Hz)	Ohmic heating treatment reduced anthocyanins content besides this (1000 Hz-25 V and 80 V-60 Hz) can be used for processing pigment fruits for making beverages	(Ferreira et al., 2019)

(continued on next page)

Table 3 (*continued*)

Product	Study aim	Key findings	Reference
Papaya	RTS drink with papaya and whey (at different level 25, 50, and 100)	Prepared RTS drink with 25% whey was found to be acceptable by sensory panellists, which bring insight that whey can be used for making RTS beverages	(Panghal et al., 2017)
Tomato	RTS drink with tomato and whey at different ratios	RTS drink made using whey: tomato (65:35) found to be best and this formulation can be exploited for commercial use	(Bangaraiah et al., 2014)
Doum	A beverage made from sweet whey and doum fruit; by soaking doum fruit in sweet whey in a ratio of 1:5 (w/v)	The prepared beverage contains an adequate amount of nutrients and was found acceptable by 15 trained sensory panellists	(Tawfeuk & Khalil, 2019)
Carrot	Whey carrot beverage (WCB); ingredients are incorporated in different ratio carrot juice (25%); sugar (12%) and cheese whey (63–62%)	Carrot juice with whey could be an interesting and nutritious product in the developing functional beverage	(Abd El Raoaf et al., 2014)
Mulberry	Mulberry-whey beverage; sweet whey (SW) and black mulberry (BM) juice were blended in different levels; 25%, 50%, and 100%	The prepared beverage made with 25% whey and 75% BM juice achieved the highest organoleptic scores by the trained panellists.	(AbdulAlim et al., 2018)
Fermented beverage			
Lactobacillus Plantarum	Prepared sweet whey-based fruits beverages fermented with *Lactobacillus Plantarum*; sweet whey with cactus pear juice at the different percentage (10, 20, 30%) and kaki juice (10 and 20%)	Kaki juice and cactus pear can be used in collaboration with whey to improve the quality characteristics of the beverage	(Aly et al., 2019)
Clavispora lucitaniae	Preparation of carbonated whey beverage with the addition of strawberry and pineapple juice; fermentation is carried out at 35 ± 1 °C for 36 h with yeast culture (*Clavispora lucitaniae* @ 0.5% v/v) and the juice is added at a different percentage (15, 20, 25 and 30 per cent)	Prepared beverage contained naturally produced CO_2, low alcohol level, effervescence with a tangy taste	(Kaur et al., 2019)
Lactobacillus acidophilus	Fermented whey beverage produced with the incorporation tomato juice; tomato juice was added at different concentration (5, 10, 15%), with probiotic bacteria *Lactobacillus acidophilus* and *Lactobacillus plantarum*	Prepared beverages with 5% tomato juice scored highest in organoleptic scores. The lactic acid (LA) and pH in prepared beverage ranged from 0.326 to 0.437% and 4.13 to 4.64, respectively	(Nursiwi et al., 2017)
Fortified	Preparation of unfermented and fermented whey-based beverage fortified with vitamin E	Both beverages were made by adding WPC (8.5%), WP (1.4%), mint for flavour (0.01%), vitamin E (0.18%). Obtained results showed that both the beverages were significantly different in their pH values. Sensory acceptability of unfermented beverage was lower as compared to product fermented by sensory panellists	(Sohrabi et al., 2016)
Yogurt	Utilization of acid whey in the preparation of fermented yogurt type beverage	Beverage prepared with pasteurized acid whey (WPC35), skim milk, condensed milk with bacteria cultures *Streptococcus thermophilus* and *Lactobacillus delbruecki ssp. bulgaricus*. Obtained results from the sensory analysis showed that beverage prepared with whey and condensed milk showed maximum sensory acceptability	(Skryplonek, 2018)

the suppression of tumour development (Deeth & Bansal, 2018), reduce the risk of pulmonary infection by the pathogen of Pseudomonas aeruginosa (Kishta et al., 2013) α-La exhibits anti-proliferative effects (Brück et al., 2014), insulinogenic effect (production of insulin), the existence of sphingomyelin in WP poses therapeutic properties to inhibit colon cancer (Anto et al., 2020), scavenge free radicals (Gad et al., 2011) WPH has the potential in the treatment of type 2 diabetes (Morato et al., 2013). A clinical study conducted by Kume et al. (2006) suggests that supplementation of WP could be effective in the presentation of portal fibrosis and hepatitis, supplementation of whey peptides with antioxidants was found to be effective against hepatitis (Takayanagi et al., 2011). de Moura et al. (2013) reported that consumption of WPH enhances induced heat shock protein HSP70 expression. The effectiveness of WPI in the treatment of postmenopausal women and cardiovascular diseases (CVD) was studied by (Pal et al., 2010).

Therapeutic applications of whey-derived products in the prevention, treatment of diseases: evidence obtained from animal models and clinical observations are presented in Table 4. Studies on therapeutic applications of whey-derived products have been directed to achieve diverse purposes, from radioprotective properties (Kimura et al., 2014), encapsulation (Gerez et al., 2012), osteoprotection (Kruger et al., 2005), phenylketonuria (PKU) (Solverson et al., 2012), oxidative stress (Flaim et al., 2017; Nabuco et al., 2019), blood pressure (Pal & Ellis, 2010), anti-diabetic (Mortensen et al., 2012), glutathione (Micke et al., 2002), glucose-lowering effect (Jakubowicz et al., 2014), common cold (Vitetta et al., 2013), inflammation (Ahmadi-Kani Golzar et al., 2017). Akhavan et al. (2014), conducted a trial on the human volunteers to investigate the mechanism action of pre-meal consumption of WP in glycaemic control. Specifically, the volunteers were divided into sub-groups and supplemented with control (water), glucose and whey protein. The consumption of WP (pre-meal) decreases post-meal glycemia by both insulin-dependent and insulin-independent mechanisms. Indeed, the supplementation of whey protein and leucine could show positive results in reducing anti-oxidant stress and insulin resistance in non-obese rats without any alteration in body weight (Tong et al., 2014). Bjørnshave et al.(2018) investigated the supplementation of whey protein in response to postprandial lipemiain. Therein, the participants (with and without type-2 diabetes) (n = 26) received 20 g WP and placebo (water) before a fat-rich meal (15 min) for 1 week. The supplementation of WP before the fat-rich meal had a differential effect on lipid response and hormones, which included glucagon, insulin and glucose-dependent insulinotropic peptide in both type-2 diabetes participants and non-diseased. Moreover, this specific supplementation in that study also assisted in lowering the gastric emptying in both groups.

Micke et al. (2002) investigated the effects of whey supplementation on plasma GSH levels. Therein, 30 HIV-infected patients were supplemented with whey protein products of two European manufacturers with dose 45 g/d for 2 weeks, and the trial subsequently continued 6 months with 18 patients having one of the products. Two weeks plasma significantly increased the total GSH level in one test product over the other one. Despite that all the patients continued the test trial, post-6 month appeared to significantly keep the range elevation of se-GSH over the initial values. Vitetta et al.(2013) studied the effect of bovine lactoferrin (Lf)/whey protein Ig-rich fraction for the common cold. In this specific trial, 90 participants were daily administered 600 mg of Lf/IGF supplementation for about 3 months. Over the study period, the total number of recorded colds were noticeably less compared to the treatment group (48), and in the placebo group (1 1 2). Total days sick with cold and cold severity reduced throughout the treatment period. Çakır et al. (2021) conducted an in-silico analysis to demonstrate the effect of β-La derived peptides against SARS-CoV-2. The liquid chromatography-quadrupole-time of flight mass spectroscopy

Table 4
Therapeutic applications of whey-derived products in the prevention, treatment of diseases: evidence obtained from animal models and clinical observations.

Purpose of the study	Formulation	Description of the animal or human model	Results obtained	References
Radioprotective properties	Chronic ultraviolet protective effect of whey peptide	Mouse model; Hairless mice were treated with UVB (36–180 mJ/cm^2) and supplemented with the doses of whey peptides (200–400 mg/kg, twice/day) for 17 weeks to determine the changes; skin thickness, wrinkle, elasticity and melanin	Obtained results suggest that whey peptides inhibit the melanin granules, skin thickness, wrinkle formation, skin elasticity (photoaging). Further WPs also prevents type IV collagen degradation, DNA damage, angiogenesis, proliferation, and expression of matrix metalloproteinase (MMP)	(Kimura et al., 2014)
Encapsulation	Utilization of whey protein in encapsulation for the endurance of probiotic organism to pH	Probiotic organism; *Lactobacillus rhamnosus* CRL 1505	Whey protein with pectin could be a promising carrier for low pH functional food. This encapsulation made on *Lactobacillus rhamnosus* CRL 1505 survive under stressed conditions in the gastric tract	(Gerez et al., 2012)
Osteoprotection	Determine the effect of acid whey fraction on bone loss	Ovariectomised mice; OVX rat divided into four groups, 1 group as control and rest 3 groups supplemented with acid whey 3 g/kg diet for 4 months	Results of the study showed that the density (organic matter) of femoral bone was significantly increasing in OVX rat supplemented whey fraction after 4 months of supplementation. The study also reveals that acid fraction contains cysteine proteinase inhibitor and osteopontin which is crucial for the maintenance of bones	(Kruger et al., 2005)
Phenylketonuria (PKU)	Isolate and utilize glycomacropeptide (GMP) from cheese whey to demonstrate the effeteness in a murine model of phenylketonuria (PUK)	Animal trial (murine model of PUK); authors compare; phenylaniline, gross energy, growth, composition (lean mass and fat) in PUK murine and wild mice (low and high feed low phenylaniline (Phe)- GMP and casein) for 23 weeks	An increase in lean mass and growth observed in PUK mise supplemented with GMP. Further a reduced food intake, plasma Phe content was observed in mice feed with GMP as compared to casein. Overall GMP is a good source of protein with low Phe that attenuates the metabolic stress induced by high phenylaniline	(Solverson et al., 2012)
Oxidative stress	Effect of the supplementation of whey proteins (pre-and post-exercise) on antioxidant enzymes and oxidative stress in old age women	Randomized, double-blind, and placebo-controlled study; A total of 70 women aged $\geq$ 60 years were divided into three groups supplemented with 35 g of; whey protein-placebo (n = 24), placebo-whey protein (n = 23) and placebo-placebo (n = 23) for 12 weeks. Blood marker and oxidative stress was observed before and after the trial	A significant difference was observed in both oxidative stress and antioxidant enzyme among the groups. Reduction of uric acid in whey protein-placebo, and whey protein-placebo as compared to placebo-placebo. Overall supplementations of whey protein reduce the concentration of plasma uric acid in older women	(Nabuco et al., 2019)
Blood pressure	Evaluated the effect of supplementation whey proteins (WP) on blood pressure in overweight individuals	Human trial; 70 overweight men and women supplemented with glucose (control), whey protein, casein for 12 weeks. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measure at baseline and end of the study	Obtained results show that SBP and DBP are significantly decreased at 6 weeks as compared to the initial phase in volunteers supplemented with whey and casein	(Pal & Ellis, 2010)
Antidiabetic	Determination of the effect of whey protein fractions on hormones response and postprandial lipid response in type 2 diabetes	Subjects were subjected to isocaloric meals incorporated with butter (100 g) and carbohydrates (45 g). Isocaloric meals were; whey isolate (WI), whey hydrolysate (WH), enhanced α-lactalbumin whey and enhanced caseinoglycomacropeptide (CGMP)	The higher insulin response was observed in whey isolate and whey hydrolysate as compare to enhanced α-lactalbumin whey and enhance caseinoglycomacropeptide. Further, WP and WH caused an increased insulin response	(Mortensen et al., 2012)
Oxidative stress	Testing the product on markers of antioxidant status and oxidative damage, glucose metabolism, BMI, body composition, glycosylation, blood pressure, muscular performance among overweight people affected by T2DM or IFG.	Supplement (WPI) with 90% of protein, 2x20g dose per day, 30′ before lunch and dinner (total 40 g/d) for 12 weeks.	Significant changes: an increase in glutathione peroxidase, decrease in uric acid markers in blood samples. Improvement in anthropometric parameters and fat mass. No change in other measured parameters. Two-third of subjects judged the supplement positively.	(Flaim et al., 2017)
Glucose-lowering effect	Study the incretin, insulinotropic and glucose-lowering effect of whey protein pre-load in T2DM.	15 individuals with well-controlled T2DM, 50 g whey in 250 mL water followed by standardized high-glycemic-index breakfast. 30 min. following meal ingestion plasma-cc. of glucose, GLP-1 and insulin.	Consumption of whey protein shortly before a high-glycemic-index breakfast reduced by 28% glucose levels and increased insulin and C-peptide response by 105% and 43%, respectively.	(Jakubowicz et al., 2014)
Inflammation	Endurance training and Supplementation effect of whey proteins on insulin resistance and inflammation	Animal trial; Wistar rats (n = 40) studies under two-phase (a) rat feed with standard chow and high fat (b) supplemented with control (1), whey supplement (WS) (2), endurance training (ET) (3), and WS with ET	From the study, it was observed that WP supplementation is effective in high-fat diet-induced insulin resistance and decreased inflammation	(Ahmadi-Kani Golzar et al., 2017)

(LC-Q-TOF-MS) was used to hydrolyse the goat milk whey with trypsin and their characterization. Their results showed the peptides ALMPHIR and IPAVFK could serve as useful candidate in treating SARS-CoV-2, and this has to be after the validation of In-Vivo- Vitro studies. Serrano et al. (2020) studied the oral supplementation of nutritional syrup prepared by a combination of liposomal bovine L_f (32 mg/ 10 mL) and vitamin C (12 mg) 4–6 doses/day for 10 days were useful pre-treatment, able to tackle emergent COVID-19 infection. Oshiro et al. (2021) showed that the bovine- IgG fraction would bind specifically to the receptor-binding domain of SARS-CoV-2 protein, which suggests the IgG-enrich fraction very promising to neutralize the SARS-CoV-2.

Additionally, whey-derived products are much favoured and consumed by athletes and sportspersons. Whey protein (WP) and its derivatives involving athletic performance or bodybuilding clinical trials (randomized, double-blind, and placebo-controlled) suggest WP consumption improves body composition and physical function in older adults (Liao et al., 2017), muscle protein synthesis (Tang et al., 2009). Morton et al. (2009) reported that WPI is effective on rehydration after exercise, Tipton et al. (2007) determined the efficacy of WP supplemented before and after exercise and the obtained results showed that the consumption of whey protein before exercise is more effective in the synthesis of muscle protein. Wilborn et al. (2013) compared the WP and casein supplementation in body composition and performance in female athletes. These workers suggested WP more effective than casein supplementation. MacKenzie-Shalders et al.(2015) also showed WP supplementations would be effective in food intake and satiety in athletes. Moro et al. (2019) demonstrated the effectiveness of WPH on muscle protein synthesis. For the study, healthy individuals (n = 10) were supplemented with 0.08 g of WPH or whole whey protein /kg of body weight. These workers showed that the WPH contained bioactive peptides that could be easily digested compared to whole protein.

Clinical and nonclinical studies involving athletic performance/body composition concerning whey protein derived products are summarized in Table 5. Huang et al. (2017) investigated the positive effect of whey proteins on the injury-induced due to marathon and exercise in track runner using 12 healthy male track runner supplemented with whey (n = 6) and placebo (n = 6) after the daily training protocol for 5 consecutive weeks. These workers showed WP as potential source for nutrient supplement, and aerobic exercise for better physiological adaptation. Lollo et al. (2011) demonstrate the physiological and physical effects of whey protein (WP), hydrolysed whey protein (HWP) and casein (CAS) supplemented to elite soccer players. These workers found players treated with (CAS) resulted in muscle mass increase, where the WP and WPH improve the physical performance and muscle mass. Other studies that aimed to diagnose athletic performance/body composition include those of Martin et al.(2013) that compared whey proteins and casein in the recovery of functional properties of muscle, Miller et al. (2014) that compared whey protein and other protein in resistance exercise (with or without) on body composition, as well as Volek et al. (2013) who targeted the effectiveness of whey protein, carbohydrates, and soy protein supplementation in the growth of lean muscle mass.

8. Concluding remarks

In this insight perspective, the biochemical and functional properties of whey-derived natural products have been demonstrated. The usefulness of whey-derived natural products in health and disease has been summarised. We have shown how sophisticated analytical techniques remain crucial in realising the valuable whey components, and consequently transforming whey from "from gutter to gold" into cost-effective functional foods and nutraceuticals. Whey-derived products are used for making pharmaceuticals, cosmetics and skincare products, WP, WPI, WPC, WPH and a wide array of novel beverages. Whey proteins and isolates are often used by athletes and sports persons because they are effective for protein synthesis in muscles, supply rich energy sources,

Table 5
Clinical and nonclinical results with respect to whey protein derived products.

Clinical diagnosis	Aim of study	Type / subject	Key findings	References
Athletic performance / body composition	Investigate the positive effect of whey proteins on the injury-induced due to marathon and exercise in track runner	Randomized, double-blind, placebo; A total of 12 healthy male track runner supplemented with whey (n = 6) and placebo (n = 6) after the daily training protocol for 5 consecutive weeks. Three (pre-test, post-test, and end-test) assessment test was done to evaluate (exercise performance, biochemistry, and the body composition)	Obtained results from the study showed that the endurance performance of track runner supplemented with WP was significantly higher ($p < 0.012$) as of placebo, this increase might be due to muscle mass and amelioration of exercise injuries. Further, the WP can be utilized as a potential source for nutrient supplement, and aerobic exercise for better physiological adaptation	(Huang et al., 2017)
	Demonstrate the physiological and physical effects of whey protein (WP), hydrolysed whey protein (HWP) and casein (CAS) supplemented to elite soccer players	Human trial; 24 players were divided into three groups supplemented with; 8 = WP, 8 = HWP, 8 = CAS after daily training. Before and after the experiment subjects were analysed for body biochemical parameters (uric acid, glucose, total and HDL cholesterol)	The players treated with (CAS) resulted in muscle mass increase, where the WP and WPH improve the physical performance and muscle mass. Further, no abnormality found in body biochemical parameter after the 8-week intervention when no supplementation was made	(Lollo et al., 2011)
	Comparison of whey proteins and casein in the recovery of functional properties of muscle	Animal trial; Ankle of 20 rats was immobilized by casting for 8 days and feed with 13% casein. After the removal of cast rats supplemented with the same 13% casein and 13% whey protein	Whey proteins are more efficient in the recovery of isometric forces as compared to casein	(Martin et al., 2013)
	Comparison of whey protein and other protein in resistance exercise (with or without) on body composition	A meta-analysis of randomized controlled trials; Healthy volunteers (n = 626) was under observation to investigate effeteness is determined under two conditions by comparing mean difference (a) supplementation of WP versus another protein source (b) relationship between whey protein and body composition during resistance exercise	(a) WP found to be more efficient as compared to other protein, (b) increased lean body mass observed in supplementation of WP during resistance exercise	(Miller et al., 2014)
	Effectiveness of whey protein, carbohydrates, and soy protein supplementation in the growth of lean muscle mass	Human trial; A total of 63 healthy men and women were supplemented with; whey; n = 19, soy; n = 22, and carbohydrate; n = 22 respectively for 9 months. Daily protein intake subjected to volunteers (whey 1.4), (carbohydrates 1.1) and (soy 1.4) kg body mass^{-1}. The body composition was estimated at the initial phase and after 3, 6, and 9 months	The lean body mass was found to be significantly higher in volunteer supplements with whey protein i.e., 3.3 ± 1.5 kg followed by carbohydrates (2.3 ± 1.7 kg) and soy (1.8 ± 1.6 kg). Further, it can be concluded that WP was more effective than soy and carbohydrates in terms of promoting / build-up of lean muscle mass	(Volek et al., 2013)

cause rehydration of the body, and endurance athletic performance. Additionally, whey-derived bioactive components like essential amino acids, micronutrients, β-La, α-La, Igs, BSA, GMP, L_F and L_P possess many bio-medical, pharmaceutical and therapeutic applications.

Indeed, numerous randomized, double-blind, and placebo-controlled clinical trials have suggested the promising therapeutic applications of whey-derived products in the prevention of type 2 diabetes, obesity, CVDs, phenylketonuria, scavenging of excessive free radicals produced by oxidative stress, suppression of tumour development, anti-proliferative effects and treatment of metastatic carcinoma. Future reviews should seek to establish relevant synthesis on the quality impact of whey protein when incorporated into other products. Future studies (analytical as well as review synthesis) need to look whey protein product development, especially on optimisation studies, as well as developing strategies that will enhance short- and long-term shelf-life stability. Good manufacturing practices and quality control should be used in the manufacture of whey-based food products. Post-marketing surveillance should be conducted diligently for the tolerability of components of whey and their interactions with synthetic drugs and herbal remedies.

9. Ethics statement

This article does not contain any studies with human participants or animals performed by any of the authors

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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