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Whey Valorization

Innovations, Technological Advancements and Sustainable Exploitation

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This book is dedicated to ***Shri Kashi Vishwanath Ji*** *located on the Western bank of the holy river* ***Ganga****, Varanasi, Uttar Pradesh, India, and is one of the twelve* **Jyotirlingas**.

The main deity is known by the names Shri Vishwanath and Vishweshwara, literally meaning ***Lord of the Universe****. Varanasi city was called Kashi in ancient times, and hence the temple is popularly called Kashi Vishwanath Temple.*

and
***Bharat Ratna, Mahamana Pandit Madan Mohan Maiaviya* Ji**, Founder of Banaras Hindu University, the largest residential University in Asia and one of the largest in the World.

Preface

Whey is a yellowish or greenish opaque liquid obtained as a by-product of cheese, casein, and other dairy products. Whey represents 85–95% of the milk volume and retains about 55% of the milk nutrients. Approximately 20% of the total protein content of the milk is retained in the whey. The main constituents of both sweet and acid whey are water approximately 93% of the total whey volume, lactose, 70–72% of the total solids, whey proteins, 8–10% of the total solids, and minerals, 12–15% of the total solids. Whey also contains essential amino acids, vitamins, macrominerals like calcium, sodium, magnesium, and potassium and trace amounts of metals such as zinc and copper. Due to its nutritional profile, whey has a very high Biochemical Oxygen Demand (BOD) that can vary from 40,000 to 60,000 mg/L and a very high Chemical Oxygen Demand (COD) between 50,000 and 80,000 mg/L. The major problem associated with whey comes from its potential to damage the environment. The waste load of whey is equivalent to that of 100–175 times that of a similar volume of domestic wastewater. The growth of the modern dairy industry depends on analysis, and whey contains a huge number of various compounds at a wide range of concentrations, which renders the analysis of this complex matrix challenging. To overcome the challenge of uncovering whey complexity and meet the demands of the modern dairy industry, research has to move from conventional analytical methods to sensitive, high-throughput, green technologies, and sustainable analytical approaches.

Substantial effort was made in the last few years to find new methods of whey valorization and reduce the polluting effects of whey. Whey is rich in lactose that makes it more suitable for industrial production of various biotechnological products. There are many modern approaches to whey valorization, and sustainable solution for whey valorization can be provided by its bacterial conversion into low-cost fermentation medium to produce different biopolymers, bioethanol, bioplastic, and other bioactive compounds. Fermentative processes converting it into value-added products will allow both to reduce the environmental pollution potential and to valorize the whey. This book focuses on the microbial processes useful to promote the bioremediation of whey-derived products. Special attention

was paid to the production of biopolymers (i.e., poly-hydroxyalkanoates, PHAs; bacterial cellulose), natural pigments, edible coatings, exopolysaccharides, and health-promoting whey drinks (from lactic and acetic fermentations), and bioactive peptides which may be exploited as value-added products in various sectors of food and pharmaceutical industries (e.g., probiotics, functional beverages, and bio-packaging).

This book comprises of 17 chapters and focuses on exploitation of whey through the extensive analysis of its molecular composition. Whey can be exploited to have different valuable compounds such as lactose, proteins, and peptides. It also covers the biotechnological treatments of whey using biochemical and enzymatic treatment and microbial transformation. Various high value products like bioethanol, glycerol, Bioplastics (PLA), bacteriocins, exopolysaccharides, bacterial polysaccharides (PHA, PHB, Xanthan), single-cell proteins, probiotics, bioactive peptides, organic acids (lactic, butyric, acetic acid), enzymes, and biogas using microbial conversion of whey. The book also covers the use of whey for preparation of different food products such as whey powder, condensed whey, spreads, various whey-based beverages including fermented beverages, recent trends, opportunities, and challenges in functional carbonated whey-based beverages.

Varanasi, India — Amrita Poonia
Bitola, North Macedonia — Anka Trajkovska Petkoska

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About the Editors

Amrita Poonia is working as an Assistant Professor in the Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India. She has published one book with Springer, two laboratory manuals, more than 26 book chapters at National and International level. Dr. Poonia has conducted/offered a Massive Open Online Course (MOOC) under agMOOCs on Functional Foods: Concept, Technology, and Health Benefits from (8 October, 2018 to 19 November, 2018) and awarded with the Certificate of Appreciation for offering an MOOC under agMOOCs, by Commonwealth of Learning (COL) Canada. She has published more than 78 publications in esteemed National and International Journals. Dr. Poonia has supervised 2 PhD scholars and 40 MSc students. She has 12 years of teaching experience at UG, PG, and PhD levels. She has received Research Incentive Grant under Institutions of Eminence (IoE Scheme) of Banaras Hindu University. She has successfully completed a project on "Identification of Food borne pathogen (*Listeria monocytogenes*) in *Shrikhand* Using PCR (Polymerase Chain Reaction)" at BHU New Faculty Members XI-Plan Grant in 2012. She is a Life Member of Association of Food Scientists and Technologists, India, and Indian Dairy Association.

Anka Trajkovska Petkoska is a Full Professor at the University St. Kliment Ohridski-Bitola, N. R. Macedonia. She has been involved in the development of a number of novel products, proprietary material formulations, and cutting-edge technologies. Examples include a variety of micro- and nano-composites, multi-functional thin films and coatings for customized applications, food-contact materials, and antimicrobial formulations. Her interests also include sustainable food systems, healthy diets and lifestyles, circular economy, zero-waste, and no hunger in the world. She is the author and co-author of numerous peer-reviewed scientific articles, three books and seven book chapters, training materials and courses for undergraduate and graduate levels. She has been granted two US patents and is co-inventor of several patents pending.

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Abbreviations

AAB	Acetic acid bacteria
ABTS	2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
ACE	Angiotensin-converting enzyme
ALA	α-lactalbumin
BCAA	Branched chain amino acid
BLG	β-lactoglobulin
BOD	Biological oxygen demand
BSA	Bovine serum albumin
BW	Beeswax
CarW	Carnauba wax
CBs	Carbonated beverages
CFV	Cross-flow velocities
COD	Chemical oxygen demand
DIM	3,3′-Diindolylmethane
DLS	Dynamic light scattering
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimeter
DWPC	Demineralized whey protein concentrate
EAA	Essential amino acids
EPS	Extracellular polysaccharide
ESF	Electric field strength
FAO	Food and Agriculture Organization of the UN
FTIR	Fourier transform infrared spectroscopy
GMMs	Genetically modified microorganisms
GMO	Genetically modified organism
GMP	Glycomacropeptide
GTs	Glycosyltransferases
HB	Hydroxybutyrate
HePS	Heteropolysaccharides
HIV-1	Human immunodeficiency virus - type 1

HoPS	Homopolysaccharides
HPMC	Hydroxypropyl methylcellulose
HPP	High pressure processing
HV	Hydroxyvalerate
Ig	Immunoglobulin
La	Lactalbumin
LAB	Lactic acid bacteria
Lf	Lactoferrin
Lg	Lactoglobulin
LP	Lactoperoxidase
MF	Microfiltration
MMCs	Mixed microbial cultures
MWCO	Molecular weight cut-off
NF	Nano filtration
P	Purity
PEG	Polyethylene glycols
PES	Polyether sulfone
PHA	Polyhydroxyalkanoates
PHBs	Polyhydroxybutyrates
PLA	Polylactic acid
Pr	Pressure
PS	Polysulfone
PTFE	Poly-tetra-fluoroethylene
PVA	Poly vinyl alcohol
PVDF	Polyvinylidene difluoride
PWF	Pure water flux
R	Recovery
SCP	Single-cell protein
SDG	Sustainable development goals
SEM	Scanning electron microscopy
SWP	Sweet whey powder
TAG	Triacyl glycerides
TEM	Transmission electron microscopy
TG	Trans glutaminase
TMP	Transmembrane pressure
UF	Ultra filtration
US	Ultrasound
UV	Ultraviolet
WPC	Whey protein concentrate
WPI	Whey protein isolate
α-LA	α-lactalbumin
XRD	X-ray diffraction
β-LG	β-lactoglobulin

Chapter 1
Whey Production Status, Types, Characterization and Functional Properties

Amrita Poonia, Vasundhara Rao, and Bimlesh Mann

Abstract Finding innovative food items that improve customer's health has always piqued people's curiosity. The dairy sector is possibly the best illustration of how new products with alleged health benefits are introduced. The largest commercial byproduct of the dairy sector is whey, which means that over the past two centuries, a lot of study has been conducted to learn more about its chemical make-up and the biological functions of its constituent parts. Whey products now have a key place amid nutritional foods because of their nutritional content. Whey's prominence among coagulated dairy products has been reinforced by the enormous scientific interest in it. Past studies have shown that whey-derived functional products have a wide range of positive effects on wellness, health, and the treatment of many chronic illnesses. This chapter focuses on the production status, functional properties and characterization of the whey along with discussing the characteristics and different types of whey.

Keywords Whey · Functional products · Characterization · Production status · Types of whey · Nutritional content

1.1 Introduction

The dairy business is organized into numerous segments, all of which produce polluted effluent. These effluents have varying properties depending on the product produced. Furthermore, wastewater management, operational circumstances, climate and cleaning-in-place methods, all have an impact on the characterization of dairy effluents. Among some of the essential criteria that distinguish these wastes,

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dairy discharges have a comparatively high amount of organic pollutant, as determined by biological oxygen demand (Prazeres et al., 2012). Whey has a biological oxygen demand of 27–60 g/L as well as a chemical oxygen demand of 50–102 g/L (Carvalho et al., 2013).

Whey is the liquid part that is generated as a byproduct during the cheese making operation or during the aggregation of casein milk process (Mollea et al., 2013). Cheese whey is utilized as animal feed and also in culinary applications. Whey dumping into public sewers is prohibited by several municipal councils since it disrupts the biological mechanism of wastewater treatment facilities. When whey is dumped on land, the physicochemical properties of the soil are altered, which lowers agricultural yields and poses serious environmental contamination problems. It also hinders biodegradability, lowers dissolved oxygen levels, and poses a serious threat to aquatic life, the atmosphere, and public health when released into water bodies (Yadav et al., 2015). Whey constituents are challenging to break down and provide a significant challenge to any treatment facility for wastewater that also handles other effluents. Hence, before it is disposed of, whey must be properly managed (treated as well as reused), which would be both cost-effective as well as environmentally benign (Carvalho et al., 2013). The presence of remaining milk nutrients such as lactose, lipids, vitamins etc., are the main contributing factors for the increased organic load (Acunha et al., 2016). In quite a while, the existence of whey nutrients, both organic as well as inorganic is viewed as an intriguing resource for the creation of several products with additional values (Mishra et al., 2021). To address this whey management problem, a number of cutting-edge technologies are being used, and today a significant portion of whey is used and converted into valuable goods. Until now, a sizable portion of whey is still going to waste. Thus, further processing of whey is crucial to optimize advantages and reduce environmental contamination (Yadav et al., 2015). Whey, a byproduct of cheese production, is becoming known as an essential functional food. By a number of interconnected mechanisms, including an increase in the release of the hormones insulin as well as incretin, a slowing of stomach evacuation, decrease in hunger and energy intake, whey protein has been shown to lower postprandial glycaemia. It's interesting to note that for the past few decades, the dairy industry has used a variety of methods to separate the main components of whey, which include fractions that are richer in proteins, lactose, as well as minerals. A wide range of novel products based on whey are now possible thanks to the development of innovative separation technologies including ion exchange, electrodialysis, as well as membrane filtration. Incorporating whey into food and various applications has the potential to enhance worldwide competitiveness, foster sustainable economic expansion, and create job opportunities. (Poonia, 2020). These products include functional food components with added value and commodities for the pharmaceutical, medical sectors as well as food sector (Tsakali et al., 2010; Kumar et al., 2013; Persico & Bazinet, 2018). Thirty percent of whey continues to be employed for pig feeding, spreading as fertilizers on farmland, and sometimes thrown into rivers or even the ocean. Approximately 70% of whey is processed into various products. Whey was once thought to have medicinal value in the seventeenth and eighteenth centuries, but it eventually lost its use as a cheese

production byproduct (Jelen, 2011). Whey was traditionally disposed of on farming fields or in waterways and oceans, but by the mid-twentieth century, statutory rules were created by many governments throughout the world restricting its discharge in the ecosystem in the nations that produced cheese (Božanić et al., 2014).

Whey is a significant dairy by-product that is getting more attention from researchers throughout the world for its correct use. It is a by-product from cheese, paneer, chhana and casein industry and has nutritional importance as human food (Poonia & Kumari, 2018). Many physiologically active substances found in milk, including immunoglobulins, bioactive substances such as peptides and proteins, and fats/lipids, offer ongoing protection against infections and diseases (Minj and Anand, 2020). For young animals, milk proteins are indeed a crucial supply of amino acids because they help with nutrient as well as trace element absorption and are a source of naturally occurring peptides with a multitude of physiological roles. Cow's milk has roughly 3.5 g of protein/100 mL and about 20% of which is whey (Bendtsen et al., 2013). Beta-lactoglobulin, alpha-lactalbumin, proteose peptone, immunoglobulins, as well as bovine serum albumin, among others, make up the diverse array of proteins that make up whey (Mignone et al., 2015). Lactoferrin and lactoperoxidase are examples of "low abundance" proteins that account for up to 1% of the overall protein content of whey. It has been suggested that each of these proteins has dietary as well as physiological purposes (Mortensen et al., 2012). Acidic whey or sweet whey is formed based upon the casein coagulation process. Typically, 80–90 L of whey is generated from every 100 L of milk which was used to make cheese. The typical yield of cheese, which varies depending on the kind produced (such as hard or semi-hard), is 1 kg from 10 L of milk, with the remaining 9 L being whey (Guimarães et al., 2010). Whey protein are small, round molecules that can be categorized as secondary, tertiary, or quaternary structures. They have various combinations of cross-sulfur bonds and are sensitive to heat, lose their phosphate groups, and are less affected by calcium. On the other hand, monomeric β-lactoglobulin (β-Lg) is a compact, small globular protein that contains 162 amino acids, including five cysteine residues. Four of the cysteine residues form two S-S bonds, while the remaining cysteine residue (Cys 121) has a free thiol group that is concealed in the center of the β-Lg structure. This free thiol group plays a vital role in forming films (Schmid & Müller., 2019).

1.2 Production Status of Whey

About 50% of the milk produced in Europe in 2020 was used to make cheese, where 80–90% of the milk's volume is lost as whey and produces 54.8 million tonnes of whey (Eurostat, 2020), making sweet whey the greatest volume production in the dairy sector. Whey is valued for its nutritional and technical features, which include lipids, minerals, proteins, as well as lactose. New technologies have helped to increase the value of sweet whey. More precisely, the production of whey-derived components and the transformation of whey from an underused waste stream into a

lucrative dairy byproduct may be attributed to the advent of membrane filtering technology in the 1960s (O'Mahony & Fox, 2014). Just 50% of the estimated 180–-190 × 106 tonnes of whey produced annually in the world is thought to be processed (Mollea et al., 2013). With an estimated record production level of 198.4 million tonnes in 2019–2020, India has established itself as the greatest milk manufacturer worldwide. Almost 50% of the cheese whey produced globally is processed and converted into different food and feed items. A little over half of this quantity is employed in liquid form, 30% is used in powdered form and 15% is used as lactose as well as its byproducts. The remaining portion is utilized as whey protein concentrates (Spalatelu, 2012). At a pH of about 6.5, casein begins to coagulate due to rennet; this form of whey is known as sweet whey. The production of fresh cheese or the majority of industrial casein results in the production of acid whey (pH less than 5), which is produced by techniques that use fermentation or even the application of organic as well as mineral acids to coagulate the casein (Silviya et al., 2016).

1.3 Different Components of Whey

There are five basic components which can be found in whey namely β-lactoglobulin, α-lactalbumin, Glycomacropeptide, Immunoglobulins and Bovine Serum Albumin. Components of whey are discussed in brief in the following sections.

1.3.1 α-Lactalbumin

According to reports, alpha-lactalbumin is the second-most significant protein in whey numerically, making up around 20% of the total protein in whey. In the mammary gland, it is exclusively produced. In adult adenocarcinoma cell lines including Caco-2 and HT-29, it exerts notable anti-proliferative properties. In addition, it destroys tumor cells (Brück et al., 2014). It also exerts bactericidal effects on the respiratory system especially the upper one, and safeguards the stomach mucosa. Due to its restriction on cell proliferation, alpha-lactalbumin is crucial in lowering the risk of various malignancies. It was also discovered to be successful in treating cognitive decline in a different investigation. The elevated tryptophan concentration of alpha-lactalbumin, which raises the plasma tryptophan (large neutral amino acid) ratio, is responsible for this (Gupta & Prakash, 2017). As a milk protein, alpha-lactalbumin is a promising option for vitamin encapsulation. By using calorimetry, spectroscopy, as well as molecular docking, the binding characteristics and systemic transformation of cattle apo alpha-lactalbumin after contact with vitamin D3 were examined by Delavari et al. 2015. When vitamin D3 was present, tryptophan fluorescence quenching demonstrated that the protein structure changes. Furthermore, data from far UV CD revealed that vitamin D3 affected the protein's

conformations. According to molecular modelling, vitamin D3 binds strongly to the hydrophobic pocket of alpha-lactalbumin by hydrogen bonds, Van der Waals contacts as well as hydrophobic interactions.

1.3.2 β-Lactoglobulin

Beta-lactoglobulin accounts for around 50% of the overall protein content in cattle's whey but is missing in human milk. It contains mainly essential as well as amino acids (branched chain). Inside the beta-lactoglobulin structure is a retinol binding protein (a transporter of tiny hydrophobic molecules such as retinoic acid). This protein is capable of modulating lymphatic responses. It can also attach to hydrophobic ligands, like fatty acids. Le Maux et al. 2012 has established that beta-lactoglobulin serves as a carrier molecule, altering the bioavailability of linoleate as well as linoleic acid (Gupta & Prakash, 2017). The physicochemical and functional features of 69 amyloid aggregates produced from whey protein beta-lactoglobulin have recently piqued researchers' curiosity. Seventy Besides from common culinary applications as foaming, gelation, and emulsification, 71 beta-lactoglobulin fibrils were used in biomaterials like biosensors, hybrids, as well as nanocomposites (Heyn et al., 2019).

1.3.3 Bovine Serum Albumin

Bovine serum albumin is not generated in the udder, and yet is released in milk following passive escape from the bloodstream. The capacity of bovine serum albumin to bind to diverse ligands reversibly is its most important trait (It is the primary fatty acid carrier and may attach to free fatty acids, other lipids, as well as flavoring chemicals. Its characteristic, however, is compromised by denaturation during heating (Ezzat Alnakip et al., 2014). Bovine serum albumin suppresses tumor development by modulating the activity of independent process growth regulating factors. It also clings to fatty acids deposited in the human body, hence it contributes to lipid production. Moreover, Bovine serum albumin has antioxidant properties. Bovine serum albumin is comprised of one polypeptide chain of 583 amino acid residues. The structure is maintained by the cross-linking of 17 disulfide bridges (cysteine amino) acid residues. It has a molecular mass of around 66.8 kDa with three homologous domains I-III, and each of them includes two subdomains, A as well as B, with distinct binding characteristic. Bovine serum albumin has two Tryptophan amino acid residues, Trp-134 as well as Trp212, in subdomains IB and IIA, respectively (Rombouts et al., 2016).

1.3.4 Immunoglobulins

It belongs to the diverse family of glycoproteins, which also includes IgG1, IgG2, IgGa, as well as IgM. These are the main bioactive ingredients in both human and cow whey. Igs make approximately 70–80% of the protein in bovine colostrum, whereas they only make up 1–2% of the protein in mature milk. Immunoglobulins are antibodies and biologically gamma-globulins. The whey component of milk includes a significant quantity of immunoglobulins, accounting for 10–15% of overall whey proteins (Kumar et al., 2018). Many research have demonstrated immunoglobulins' therapeutic potential. They are recognized to have important biological features. In an in vitro investigation, it has been demonstrated that bovine milk-derived Immunoglobulin G reduces the proliferative response of human lymphocytes to T cells at levels as small as 0.3 mg/mL of IgG. It was also established that the IgG content in bovine milk ranges between 0.6 mg/mL and 0.9 mg/mL and hence confers immunity that is passed to people (Ulfman et al., 2018).

1.3.5 Glycomacropeptide

Glycomacropeptides, commonly referred to as casein macropeptides (CMP), make up 10–15% of the protein in whey. It is formed in whey as a result of the chymosin enzyme's breakdown of casein during the cheese-making process. It lacks aromatic amino acids like phenylalanine, tryptophan, as well as tyrosine but has a lot of amino acids with branched chains. The Glycomacropeptides alters the blood levels of regulating digestive peptides and reduces gastric acid output. It causes satiety because it causes the production of cholecystokinin, however human-fed GMP did not show the same effects (Karimidastjerd & Gulsunoglu Konuskan, 2021). The other function of Glycomacropeptides is to prevent cariogenic bacteria from sticking to oral surfaces, including *Streptococcus mutans*, *Sanguis*, as well as *Sobrinus.* As a result, Glycomacropeptides can alter the makeup of plaque bacteria to regulate their acid production, which in turn lessens enamel demineralization and encourages re-mineralization. The Glycomacropeptides is a provider of N-acetyl-necromatic acid as well, and sialic acid, which affects saliva's viscosity and protective role, can be added to food through diet (Huppertz et al., 2018).

1.4 Different Types of Whey

Whey is generally understood to be the serum or watery portion of milk that is left over after the curd has been separated. This is the consequence of milk proteins being coagulated by an acid or proteolytic enzyme. The methods used to separate casein from liquid milk determine the kind and content of whey in dairy establishments.

The type of whey that is most frequently encountered comes from the production of cheese or specific casein products, where manufacturing is centered on coagulating the casein using rennet, a commercial anticipation for clotting casein that contains chymosin or even other caseincoagulating enzymes (Macwan et al., 2016). The by-product "whey" is categorized into two type's namely sweet whey as well as acid whey, depending on the processing procedure employed. Sweet whey is produced from the creation of most varieties of cheese or certain casein products and has a pH of roughly 6–7. Other components are protein 6–10 g, fat (5–6 g), lactose (46–52 g), and minerals (2.5–4 g). The application of rennet into milk is the very first step in the processing of sweet whey. Rennet functions by coagulating the casein in milk, leads to the creation of curd. The curd is again separated from the liquid that is sweet whey. Casein coagulation is caused by rennet at pH of 6.0–6.5. Since it is developed during the acid coagulation (milk proteins), that is when making cottage cheese, "Acid whey" has a lower pH and, as a result, a longer shelf life than "sweet whey," which is created during the enzymatic coagulation (milk protein), that is when making cheddar and other types of cheese (Carvalho et al., 2013). Acid whey contains a pH range of 4.5–5.7. Other components of acid whey are protein (6–8 g), fat (5–6 g), lactose (44–46 g) and minerals (4.3– 7.2 g). In order to cuddle the casein for the production of the majority of industrial caseins, lactobacilli, lactic acid as well as mineral acids are often used to produce acid whey. It also happens as a result of the manufacturing of acid-curdled cheeses like cottage cheese. Although the mineral concentration of acid whey typically surpasses that of sweet whey, the lactose level is typically lower (Kotoulas et al., 2019).

1.5 Nutritional and Chemical Composition of Whey

Whey, the milk's yellow-green fluid component also known as cheese serum, is produced during the coagulation of milk with proteolytic enzymes as well as acids after the curd has been separated. Because to the dumping difficulties caused by its significant biological oxygen requirement and high organic content, it was regarded as a significant dairy waste for many years (Esfanjani et al., 2015). Whey proteins are currently used for their bioactive components and are acknowledged as a possible nutritional source. It has a substantial connection to the dairy sector and is employed in a number of different commercial food uses due to its high nutritious makeup. Whey proteins have gained immense popularity due to their exceptional nutritional value and significance in human nourishment. Numerous studies conducted in the past decade have underscored their indispensable role in the human diet. According to a joint technical report published by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) in 1985, whey proteins possess high digestibility and comprise a complete protein composition. The superior nutritional quality of whey proteins can be attributed to their elevated concentration of crucial amino acids such as lysine, tryptophan, isoleucine, and threonine. This results in a greater Protein Efficiency Ratio (PER) of whey proteins that is 3.2 in comparison

to casein that is 2.6 (Kumar et al., 2018). Whey's chemical make-up changes depending on the process used to produce it (Minj and Anand, 2020). Whey typically comprises of around 50% of the components found in milk, including minerals, some fat, and lactose (up to 70%). The key distinctions are the greater calcium, lactic acid, phosphate as well as lactate levels of acid whey compared to sweet whey. Colloidal calcium is much more soluble in an acidic medium, thus when casein is acidically coagulated, some of the calcium releases and is transferred to the whey. In contrary, sweet whey includes glycomacropeptides created by the enzyme hydrolysis of κ-casein, with the exception of whey proteins (Lisak et al., 2013).

1.6 Whey and Its Derivatives

Due to the increasing the prevalence of nutritious food, there is a growing global need for dietary items with high amounts of protein content. It is recommended that sedentary individuals consume an average of 0.8 g of protein per kilogram of body weight per day in order to maintain a healthy nitrogen balance and metabolic function. Various types of supplemental protein are available, including soy, whey, hemp, egg and casein. Among these options, milk whey has the highest concentration of readily available amino acids and is easily digestible, making it an efficient addition to the bodies cells (Layman et al., 2018). Milk whey proteins are considered to be healthy ingredients due to their various benefits such as controlling appetite, aiding exercise recovery, and promoting satiety. With the help of membrane filtration techniques, different components of whey protein can be used as food additives. The whey protein can be extracted in two main forms—whey protein concentrates and whey protein isolates. These forms have different protein concentrations and are processed using various treatments to produce whey products with different qualitative and quantitative profiles of proteins, minerals, lipids, and sugars (Sharma et al., 2013). Ultrafiltration as well as diafiltration methods are used to concentrate proteins and exclude molecular compounds such as minerals, lactose, and other low-weight components, resulting in the production of whey protein concentrate. Whey protein concentrate is the most concentrated form of protein supplement that contains all the macro- and micro-nutrients derived from the manufacturing process. However, it can be of different types based on protein concentration. Whey protein isolate is whey that has undergone another purification step to remove or reduce extraneous carbs and lipids and to reach a protein requirement of 90%. The disadvantage of whey protein isolate is that important micronutrients as well as the protein fractions such as lactoferrins, immune-globulins as well as β-lactoglobulins, are eliminated during the purification process despite being a high-quality protein (Minj & Anand, 2020).

1.7 Functional Properties of Whey

In addition to the nutritional and medicinal benefits they provide, whey proteins possess exceptional functional characteristics such as their ability to dissolve, generate foam, and create emulsions, form gels, and bind to water. Therefore, whey protein concentrate is considered to be a highly valuable ingredient and plays a crucial role in the creation of novel food items. The distinct structure and intermolecular interactions of protein molecules ultimately determine the functional properties of protein ingredients (Amid et al., 2013). Different functional property of the whey and its derivatives are explained in brief in the below paragraphs.

1.7.1 Emulsification

An emulsion is a mixture of two or more immiscible substances, where one or more phases are dispersed throughout a continuous phase. In the case of a protein-stabilized emulsion, proteins play a crucial role in creating an interfacial barrier around the lipid globule, which prevents undesired phenomena such as creaming, flocculation, oiling off as well as coalescence. The incorporation of whey protein concentrate in the batter of minced meat can serve as a successful binder and emulsifier. Furthermore, purified β-Lactoglobulin has demonstrated superior efficacy in facilitating emulsion formation when compared to other whey proteins (Scharfe & Flöter, 2020).

1.7.2 Water Binding Capacity and Viscosity

Proteins that have undergone denaturation possess considerable water-binding capacity despite being largely insoluble. These proteins display the lowest viscosity at their isoelectric pH (approximately 4.5) and their viscosity decreases within a temperature range of 30–65 °C. Thus, the water holding capacity and viscosity of whey protein concentrate are crucial functional properties that can be more effectively utilized in the production of an array of products including meat, fish, dairy, bakery, soups, and gravies, where the rheological properties are of significant importance (Fox et al., 2015).

1.7.3 Foaming

Foams are a direct outcome of the intricate interplay between proteins and air-water interfaces. The role of proteins in foam film formation is paramount, as they

significantly reduce interfacial tension. These proteins tend to accumulate at the surface of foam cells, where they undergo a process of partial unfolding, followed by intermolecular bonding, which results in a cohesive film, thus effectively stabilizing the foam cells (Albano et al., 2019). The foaming ability of whey protein can be enhanced by reducing the fat content. The utilization of whey protein in the production of frozen yogurt, with partial replacement of skim milk solids, has shown a marked improvement in body, texture, and consistency. Therefore, owing to its neutral taste and pH stability, whey protein can be considered as an excellent choice for a foaming agent (Sert & Mercan, 2022).

1.7.4 Gelation

Whey proteins exhibit remarkable gelling attributes, particularly at pH levels exceeding 7.0. The gelation characteristics of whey protein are closely associated with its protein, mineral, lipid composition, and pH, as reported by de Castro et al. 2017. The gelation process is a two-stage mechanism that involves the initial unfolding of a protein molecule, followed by subsequent aggregation. Researches and reviews has shown gelling properties of whey proteins and concluded that it possesses the ability to form gels, which can be combined with fruits to produce various types of gels that enhance the nutritional value of food products (Ni et al., 2015).

1.8 Characterization of Whey and Nanoparticle Synthesis

Due to its high protein content and excellent emulsification as well as gelling capabilities, whey protein isolate, a key by-product of whey proteins, has been employed extensively as a carrier material for encapsulation (Abbasi et al., 2014). Whey protein isolate is also readily bioavailable, has a substantial concentration of vital amino acids, and has a superior nutritional quality (Shen et al., 2018). The two primary substances that make up whey protein isolate is namely, α-lactalbumin & β-lactoglobulin, are capable of producing protective films (Thongkaew et al., 2014). Nanoparticles made from whey protein isolate are also feasible. In order to micro-encapsulate several bioactive as well as hydrophobic substances, like vitamins, taste compounds, carotenoids etc. with better stability and physicochemical qualities, a nanoparticles based delivery mechanism has been explored as a potential material (Ma et al., 2017). Thermal processing such as heating over 60 °C, that has been developed to generate encapsulating materials with enhanced physicochemical qualities, may increase the emulsifying abilities of whey protein isolates (Khan et al., 2019). Characterization of the whey products as well the nano particle synthesis of certain whey constituents is shown in Table 1.1.

Table 1.1 Characterization and nanoparticle synthesis of whey

Compounds	Instruments used and methods	Key points	References
TiO_2 nanoparticles	SEM + FTIR + XRD	Bio nanocomposite film based on whey protein isolate	Alizadeh Sani et al. (2018)
ZnO nanoparticles	XRD + Raman spectroscopy + TEM + UV-Vis absorption spectroscopy	Increase in calcination temperature, whey-assisted sol gel method was highly efficient	Soares et al. (2020)
Zein nanoparticles	FTIR + DSC + TEM + zeta potential+	Whey protein nanofibrils stabilized zein nanoparticles	Liu et al. (2021)
TiO_2 nanoparticles	GC-MS + zeta potential	Using whey protein-coated thyme essential oil as a supplement to form nanoparticles.	Abdel-Wahhab et al. (2021)
Whey protein isolate or nanoparticles (short linear glucan core–shell)	DPPH + TEM + zeta potential + FTIR	Nanocomplex with a large capacity for holding a high amount	Li et al. (2023)
Whey protein isolate–phytosterols nanoparticles	Encapsulation efficiency + loading capacity + zeta potential	It possesses excellent storage stability and resistance to oxidation, while also reducing lipid digestion	Zhou et al. (2022)
Whey protein Isolate–DIM nanoparticles	DSC + TEM + FTIR	Potential approach For encapsulating DIM	Khan et al. (2019)
Silver nanoparticle-deposited whey protein isolate	UV-Vis spectroscopy + DSC+	Application of nanoparticle amyloid fibril composite materials for catalysis	Lai et al. (2022)
Soy-whey based bioactive material for atorvastatin loaded nanoparticles	X-ray diffractometry spectra + FTIR + zeta potential	Potential biomaterial for encapsulation + therapeutic efficiency	Kanoujia et al. (2016)
Cellulose–whey Protein concentrate bio-nanocomposite films	DLS + zeta potential + SEM + DSC	Development of substitute petrol-based materials for health as well as ecological purpose	Shojaei et al. (2019)

1.9 Biological Functions of Whey

It is well known that whey proteins have biological characteristics and numerous organizations have progressively used these features in culinary purposes as well as scientific and technical investigations (Ulfman et al., 2018). Fifty percent of the whey is made up of β-lactoglobulins, which aid in the binding of calcium as well as zinc. Moreover, it shares a portion of its sequence with proteins such as retinol binding proteins (Layman et al., 2018). Contrarily, it is actively campaigned to add α-lactalbumin to meals and newborn formulas in order to promote diets high in

protein. Fatty acids as well as immunoglobulins (IgA, IgG1, IgG2 and IgM) can bind to serum albumin, which aids in the development of active immunity in humans (Chen et al., 2014). Certain protein components, such as lactoferrin, which enhances absorption of iron in the gastrointestinal tract to prevent undesirable enteric microbes as well as encourage the development of beneficial microorganisms, are iron-binding proteins (Sharma et al., 2013). It is also believed to be the main nonspecific disease resistance element in the mammary gland that affects the immune function. A peptide made from lactoferrin called lactoferricin is employed to fight intestinal infections. In order to prevent acid production in milk during refrigeration storage, lactoperoxidase, an enzyme having antimicrobial capabilities, is utilized (Nongonierma & FitzGerald, 2015). Amino acids that are necessary such as cysteine, leucine, isoleucin, valine etc. and bioactive peptides are all abundant in whey proteins (Li-Chan, 2015). Bioactive peptides are primarily created by using different enzymes via enzymatic hydrolysis. Proteases are the most commonly utilized enzymes, which can either be specific or non-specific to the target protein. When it comes to food production, hydrolyzing whey proteins with enzymes is the preferred method due to its short reaction time, the specificity of the enzyme's action, and the abundance of enzyme sources from animals, plants, and microorganisms. Trypsin, pepsin, chymotrypsin, and bromelain are the most commonly used enzymes, each having its own specific reaction conditions such as temperature, pH, and time (Agyei & Danquah, 2011). However, to achieve the best possible outcome, enzyme-substrate ratio, reaction conditions and the type of enzyme used must be optimized prior to hydrolysis. The selection of an enzyme is crucial as it determines where and how peptide bonds are broken. Enzymatic changes can result in peptides with consistent weights and enhanced functional and biological properties. Various proteases are manufactured and employed in labs to produce bioactive peptides. (Cheison & Kulozik, 2017). Researchers studied the effects of diet called MHN-02, which contains antioxidants as well as whey peptides, on rats to see if it had any anti-inflammatory properties. The rats fed this diet had a significantly better survival rate (90%) than the control group (55%) because the MHN-02 group exhibited increased superoxide dismutase activity, which converts harmful superoxide radicals into harmless substances like hydrogen peroxide and oxygen, and also had fewer pathological damages. (Takayanagi et al., 2011). The scientists also investigated the potential of whey protein derivatives to enhance the production of glutathione in neurons and alleviate neurological disorders. They discovered that whey protein isolates as well as hydrolysates, which contain peptides with antioxidant/anti-inflammatory properties, were capable of suppressing the production of IL-8 as well as ROS (reactive oxygen species) in human colorectal adenocarcinoma Caco-2 cells exhibited to H_2O_2. Use of whey protein isolate demonstrated a greater impact, suggesting that isolates are more proficient in decreasing inflammation as well as oxidative stress inside the intestinal cells. (Kong et al., 2012).

1.10 Health Benefits of Whey

Milk consumption is a tradition that has existed since the animal was domesticated in ancient times and has benefited from the natural product's rich nutritional content. Whey, a significant component of milk, has historically received less attention than milk. This is likely because whey is a metabolic end of the production of cheese and has been devalued for a long time (Solak & Akin, 2012) Intriguingly, ancient civilizations already praised the health benefits of whey in classical times; as early as the middle ages, whey was thought to be not only a medicine but also an intoxicant as well as a skin balm. In fact, it was a common ingredient in serums as well as tonics to heal various illnesses, ease the pain burns, and instill vitality (Mangano et al., 2019). Whey proteins' impact on hypertension is a result of how they affect the Renin-Angiotensin System and inflammation. Inhibitors of the angiotensin converting enzyme have anti-inflammatory qualities. In a study, whey protein consumption was observed to lower plasma concentration of pro inflammatory cytokines in comparison to casein consumption. Consequently, after consuming whey proteins as well as its amino acids, a decrease in pro inflammatory cytokines may potentially be linked to weight loss (Price et al., 2022). Certain carbohydrate components produced from whey have prebiotic action. Lactose aids LAB like Bifidobacteria as well as Lactobacilli. Stallic acids (a kind of oligosaccharide present in whey) are usually coupled to proteins which have been demonstrated to have prebiotic characteristics. In furthermore, three numerous different non-carbohydrate prebiotics derived from whey are glycomacropeptide, which is inferred from the selective enzymatic conversion of kappa-casein all through cheese production as well as promotes the development of bifidobacteria. Lactoferrin supports the growth of lactobacilli and bifidobacteria and calcium (calcium phosphate) (Gupta et al., 2017). It increases the development of intestinal lactobacilli preferentially and decreases the frequency of Foodborne disease in rats. Many variables, including age, genetic makeup, overweight, reduced physical activity, alcohol consumption, and the quality of saturated cholesterol, are linked to cardiovascular disease. Milk comprises of sphingolipids, free sterols, lipid, as well as oleic acid, among more than 12 other forms of fat. Consuming dairy goods and milk lowers blood pressure and minimizes the likelihood of hypertension, according to several research (Al-Sheraji et al., 2013). Age-related muscle loss and the detrimental effects it has on health are becoming more and more of a worry, both within regard to volume and monetary expenses. Strong muscles may be maintained as we age with proper nutrition and a sufficient intake of high-quality whey protein, especially when paired with resistance exercise and strength training. Because of the substantial amount of Branched—chain amino acids as well as essential amino acids in whey protein, it aids in the maintenance of muscle tissue. Seniors, physically active people, and anyone seeking to maintain or decrease weight may find this to be particularly beneficial. Older persons can prevent unwanted alterations to their physiques as well as numerous illnesses that are typically associated with ageing, like heart disease, dementia, diabetes, as well as other disorders, by preserving or boosting lean body mass

(Kadam et al., 2018). Due to the fact that the myelin sheath which surrounds as well as insulates neurons includes significant quantities of sphingomyelin and also its metabolites, nutritional Milk phospholipids, and especially sphingomyelin, are noteworthy for their function in neuronal transmission as well as intellectual growth. Moreover, dietary sphingomyelin supplementation reversed a reduction in brain weight as well as myelination in rats when de novo sphingolipid production was inhibited (Oshida et al., 2003). Moreover, sphingomyelin supplementation enhanced baby performance in behavioral, cognitive, including motor capacity assessments in human infants who were born preterm also with extremely low birth weights (Tanaka et al., 2013). Altered production of phospholipids in the brain is associated with age-related conditions such as Alzheimer's, schizophrenia and Parkinson's (Daz et al., 2018; Ojo et al., 2019), MPL may have neurological advantages as well. The intestinal barrier, the gut microbiota, as well as mucosal immunity are all important components of gut health that are combined by milk phospholipids impacts on the digestive tract (Rocha-Mendoza et al., 2021). Young artificially raised rats and mice given a high-fat diet have shown evidence of increased crypt thickness in the intestine when exposed to (Milard et al., 2019). In elevated diet containing fat diet for mice, dietary SM also lowers blood levels (Norris et al., 2017). Moreover, MPL supplementation has been shown in certain studies to enhance the number of *Bifidobacterium* in the gut, possibly via a prebiotic like mechanism (Milard et al., 2019). The pathogenesis of various degenerative diseases such as cystic fibrosis, diabetes, atherosclerosis, pneumonia, myocardial infarction, cancer, as well as aging is associated with the harmful effects of inflammatory or oxidative stress. To mitigate these stressors, whey protein, a precursor of the antioxidant glutathione, has the potential to counteract the negative consequences (Piccolomini et al., 2012). The use of hyperbaric treatment on whey protein can speed up the release of bioactive peptides, resulting in higher levels of glutathione within cells and a decrease in the production of interleukin IL-8 in laboratory settings. IL-8 is a cytokine that plays a significant role in the advancement of respiratory tract diseases. A research study on individuals with cystic fibrosis showed that taking 20 g of pressurized whey as a dietary supplement for 1 month led to a notable decrease in serum C-reactive protein levels, an important indicator of bodily inflammation (Kong et al., 2012). Multiple research studies have indicated that whey protein can provide advantages to individuals who are suffering from cancer. Additionally, it has been proven that breaking down the protein molecules, a process known as hydrolysis, can enhance its ability to combat cancer. Rats with colon cancer were given whey protein hydrolysate and showed fewer large and small tumors compared to rats given regular whey protein. (Attaallah et al., 2012. Scientists examined the potential of whey protein hydrolysate in safeguarding rat pheochromocytoma PC12 cells from oxidative harm. The study found that the hydrolysate, when used at a concentration of 100–400 μg/mL, increased cell viability by 20–30% when compared to cells exposed to H_2O_2. This suggests that the hydrolysate has the ability to function as an antioxidant. (Zhang et al., 2012). According to Pérez-Cano et al., (2007), whey protein concentrates can boost natural defenses in early life and protect against certain immune disorders. One such disorder is atopic dermatitis, a chronic skin

condition that causes inflammation, flakiness, and itching. Infants are particularly vulnerable to this disease, which is becoming more prevalent worldwide. A comprehensive analysis of systematic reviews has demonstrated that infants who consumed partially hydrolyzed whey-based formula had a considerably reduced occurrence of atopic dermatitis compared to those who consumed formula based on bovine milk. Psoriasis is a long-lasting autoimmune disease that causes thick skin, red patches as well as dry scales. The study investigated if a bioactive whey protein isolate could boost glutathione levels and lessen the intensity of systemic inflammation linked to psoriasis. Patients who consumed 20 g of whey protein isolate per day saw an improvement in their condition, according to a study by Prussick et al., (2013). In simpler terms, scientists investigated if a particular protein could help reduce the inflammation caused by psoriasis and found that patients who consumed the protein showed an improvement in their symptoms.

1.11 Effect of Processing on Functional and Nutritional Quality of Whey

Dairy whey, which is a valuable protein source, can be effectively employed in the manufacturing of diverse complex food products in order to improve their consistency. It can be used to create gels, stabilize emulsions or foams, or function as a water-retaining agent for commercial applications. This is achievable due to the presence of β-lactoglobulin as well as α-lactalbumin protein fractions, along with other minor but significant components like proteose peptone fraction, within whey proteins. These constituents play a vital role in the hydration, emulsification, foaming properties and gelation of such proteins. In an experiment performed by Segat et al., 2014, Whey protein isolate powder was treated with high levels of gaseous ozone for various durations ranging from 30 min to 480 min. The study aimed to examine how ozonation affects the structure and functional properties of proteins. Various tests were conducted to evaluate the chemical and structural effects of ozone. These tests included assessing surface hydrophobicity, analyzing through HPLC, FTIR, turbidity, and free sulphhydryl groups. Furthermore, the study assessed the impact of ozonation on protein solubility and foaming properties. The results showed that the treatment reduced the number of free sulfhydryl groups and increased the surface hydrophobicity, indicating that the protein structure underwent self-rearrangement following ozonation. The research discovered that ozonation resulted in a more adaptable structure without the development of a robust disulphide bond network or clumps, as shown by turbidity analysis and SDS-PAGE. Furthermore, ozonation brought about changes that enhanced foaming ability and foam stability, although it slightly decreased solubility. In another experiment done by Sodini et al., 2006 yoghurt was fortified with whey protein concentrate. The main objective was to examine how the physical characteristics of stirred yogurts with added whey protein concentrates fortified to 45 g protein per

kilogram are influenced by two whey processing conditions, namely pH and heat treatment. Cheddar whey was subjected to different pH levels (6.4 or 5.8) and heated at 72 °C for 15 s. It was then heated to either 82–88 °C for about 78 s. The resulting whey protein concentrates had a protein content of 38%, and the extent of whey protein denaturation varied from 10% to 53%. Finally, the whey concentrates were ultrafiltered as well as spray dried. The whey protein concentrates fortified yogurts showed notable variations in their physical attributes, including their ability to retain water, which ranged from 33% to 46%. Similarly, the stiffness of the yogurts, measured as the elastic modulus, varied between 63 Pa and 145 Pa, depending on the method used to process the whey. Whey protein concentrates with low denaturation levels led to the production of yogurts having enhanced elastic modulus as well as water holding capacity. The functional properties of whey protein concentrates for yogurt production can be optimized by reducing the extent of heat treatment during the processing of whey. Zhao et al., 2020 investigated into how subjecting whey protein obtained from goat milk to heat treatment influences both its microstructure and functional properties. This research investigated the impact of various thermal processing techniques frequently employed in the dairy industry, along with extended exposure to different temperatures, on the alteration, structure, and functional characteristics of whey proteins derived from the goat milk. The findings indicated that whey protein denaturation was observed at 85 °C for a duration of 30 min, and denaturation became more pronounced as the temperature rose. The treatment involving extended periods of low temperature had minimal impact on the protein's structure, whereas the use of extremely high temperatures had the most significant effect, leading to a gradual reduction in regular structures over time. The most severe damage to the protein's structure developed upon treatment at a temperature of 85 °C for exactly 30 min, which came consistent with the result of denaturation. Wang et al., 2020 studied the impact of ultra-high pressure homogenization (dynamic) on the composition as well as functional characteristics of protein present in whey. Process involves subjecting the protein to extreme pressure in a dynamic manner, leading to changes in its structure and properties. By exploring the effects of this technique, researchers aim to gain a deeper understanding of how it can be used to modify the characteristics of whey protein for various applications. This study discovered that subjecting whey protein to dynamic UHPH treatment causes a change in its secondary structure. This modification facilitates an exchange process between disulfide bond as well as sulfhydryl group, resulting in a notable enhancement of the protein's hydrophobic characteristics on its outer surface. As a result, functional characteristics of whey protein are altered. Following dynamic UHPH treatment, there was a notable reduction in the average particle size of whey protein and emulsion, along with a substantial increase in solubility, emulsion stability as well as foaming abilities.

1.12 Role and Application of Whey and Its Derivatives in Food Industry

The food industry is increasingly interested in whey proteins because of their distinctive functional and nutritional characteristics. Whey proteins find extensive utilization as functional components in numerous food items such as baked goods, dairy products, sports nutrition, and beverages. These proteins contain abundant essential amino acids that play a crucial role in muscle growth and recovery. Moreover, their remarkable biological value ensures efficient absorption and utilization by the body (Krzeminski et al., 2014). Additionally, whey proteins have excellent emulsifying, foaming, gelling, and water-holding properties, which make them valuable ingredients for improving the texture and sensory quality of foods. For example, in yogurt, inclusion of whey proteins can help to increase the protein content, improve the texture and mouthfeel, and enhance the creaminess and flavor. In bakery foods, whey proteins can be used to ameliorate the dough handling and machinability, enhance the volume and keeping quality of bread, and improve the texture and softness of cakes and pastries. Overall, the versatility and functionality of whey proteins make them a popular choice for food manufacturers looking to enhance the nutritional and sensory properties of their products (Kuhn & Cunha, 2012). The enzymatic breakdown of whey proteins can create bitter peptides, which makes them less appealing for use in food. The procedure entails the fragmentation of protein components like α-lactalbumin, β-lactoglobulin as well as serum albumin in order to produce whey protein hydrolysates that include these undesirable peptides. Various types of inhibitors are employed to mask the bitter taste of these peptides. These inhibitors include sucralose, adenosine 5′ monophosphate, adenosine 5′ monophosphate disodium, (MSG), sodium chloride, sodium gluconate, sucrose, fructose as well as sodium acetate (Leksrisompong et al., 2012). To improve the taste of whey protein, researchers have developed several techniques to identify and remove these bitter peptides. One approach used by Liu et al., 2014 involves separating the peptides from the hydrolysate using ultra-filtration and chromatography, and then identifying them with LC-TOF-MS/MS. Additionally, Gad et al., 2011 found that supplementing whey protein concentrate with spirulina, a type of freshwater algae, improved its antioxidant and metal-chelating properties in vitro and in vivo experiments with rats (Minj & Anand, 2020). The use of water-soluble polymers derived from whey protein has unique features when making films as well as coatings. These characteristics include attractiveness, natural biodegradability, suitable mechanical and optical properties, potential for value addition, and the ability to include functional substances. However, whey protein is prone to moisture penetration and mechanical limitations. These challenges can be addressed by cross-linking through physical, enzymatic or chemical means and the addition of plasticizers to achieve the desired properties. (Ramos et al., 2012). Different products of whey with their ingredients and composition is shown in Table 1.2. Whey protein based edible coatings as well as films have been successfully employed in various

Table 1.2 Different whey based food formulations

Food product	Whey component	Type	References
Crackers	WPC + SWP + DWPC	Confectionary based	Kumar et al. (2018)
Mortadellas	WPC-80 + WPC-26	Meat based	Królczyk et al. (2016)
Chocolate flavor topping	DWPC + lactose + WPI + WPC	Confectionary based	Kumar et al. (2018)
Sweet rolls	WPC+ SWP + DWPC	Confectionary based	Kumar et al. (2018)
Bread	Whey + Banana flour + wheat flour	Confectionary based	Arya and Poonia (2019)
Rabadi	Whey + pearl millet + buttermilk	Fermented product	Poonia and Kumari (2018)
Surimi	WPC-80	Meat based	Królczyk et al. (2016)
White bread	WPC + SWP + DWPC	Confectionary based	Kumar et al. (2018)
Luncheon meat	WPC-80	Meat based	Królczyk et al. (2016)
Cakes	WPC + SWP + DWPC	Confectionary based	Kumar et al. (2018)
Sausages	WPC-80	Meat based	Królczyk et al. (2016)
Kaymak mass	DWPC + lactose + WPI + WPC	Confectionary based	Królczyk et al. (2016)
Protein bars	WPC + WPI + lactose	Confectionary based	Kumar et al. (2018)
Isotonic drink	Water(85.4301%)	Beverage	Jain et al. (2013)
Low pH juice drink	Water(80.73%) + fructose(9.40%) + WPI (4.70%) + concentrated apple juice (4.70%) + natural beery flavor(0.10%) + red color(0.02%) + phosphoric acid(0.35%)	Beverage	Jain et al. (2013)

food products, including peanuts, frozen, walnuts, fruits, and salmon, breakfast cereals, to enhance fat, moisture, aroma as well as gas barriers. Moreover, the integration of active ingredients, such as antimicrobials, probiotics, prebiotics, antioxidants, and flavorings, into whey protein films as well as coatings which is a novel trend in the industry aimed at promoting health benefits to consumers (Kandasamy et al., 2021). Whey protein gel may be used to package a range of foods, additives, aromatic oils, and nutrients to boost their stability and avoid rancidity. Nevertheless, due to challenges with accessibility, iron fortification of food might be difficult. To address this issue, the cold-set gelling properties of whey protein isolate were investigated, and iron was captured by cold-set gelation when ascorbate was present. The optimal balance of iron and ascorbate was achieved using

the TNO Intestinal Model. Both the recovery as well as the bio-accessibility for iron were effectively increased by the gel formation of whey protein induced by iron as well as ascorbate, raising it from 10% to 80%. (Martin & De Jong, 2012). Encapsulation of essential oils extracted from botanical sources in whey protein isolate was also studied. The aforementioned oils are vulnerable to instability while having several therapeutic advantages, thus encapsulation can help with this. It was investigated if it was possible to microencapsulate cardamom oil as an essential oil in a whey protein isolate, and it was discovered that the microcapsules kept the oil's essential qualities adequately all over storage at the temperatures under consideration (Patel, 2015). These microcapsules also had a smooth texture, regular contours, and spherical shapes. Another study examined the encapsulation capability of protein from whey isolate-nanoparticle desolvated suspensions both with as well as without methoxyl pectin. The pectin-infused suspension was stable and defied homogenization. When comparing whey protein isolate nanoparticle solution without pectin, this combination showed greater surface tensions during storage at pH 3, indicating their suitability as surfactants. (Christaki et al., 2021). Applications in the food and pharmaceutical industries favor nanoemulsions greatly. The development of food proteins as emulsifiers has been demonstrated in earlier investigations. In this work, the nanoemulsions were stabilized using whey protein isolates under various ionic intensities as well as heating procedures, which preserved stability throughout storage. The results indicated that another coating of polysaccharides, which included gum arabic, carrageenan, xanthan, or alginate, may not be necessary when utilizing a reasonably high protein content as the emulsifier for creating nanoemulsions (Li et al., 2014). A study done by Tippetts et al., 2012, aimed to improve the nutritional value of Cheddar cheese by fortifying it with vitamin D. However, the vitamin tends to degrade during the long ripening period. To address this, the researchers incorporated vitamin D3 as an oil-in-water emulsion to fortify the cheese up to 280 IU/serving. They used a mixture of sodium caseinate, whey protein as well as an emulsifier, which resulted in about 74–78% vitamin retention. A diagrammatic overview of whey and its derivatives in food industry in shown in Fig. 1.1.

1.13 Safety Aspects and Future Concern

The functional food industry has seen significant growth due to consumer interest in goods with natural components and health advantages. Edible films and coatings offer a promising how to broaden the market and serve as an alternative to traditional packaging. By incorporating whey protein and active ingredients into these films and coatings, various food products can benefit from improved quality, extended shelf life, enhanced safety, and reduced environmental impact. Whey protein-based films and coatings have been found to have exceptional optical and barrier properties, making them efficient gas barriers that can transport antioxidants, antimicrobials,

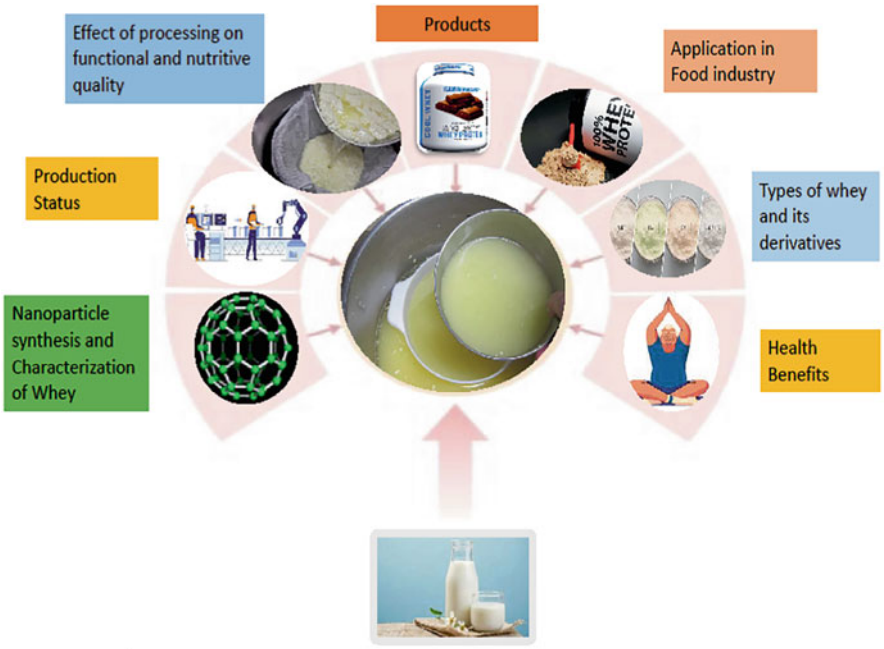

Fig. 1.1 An overview of various aspects of whey in food industry

and other nutrients (Kocira et al., 2021). However, their mechanical properties need to be improved through protein cross-linking or nanotechnology. The functional and bioactive substances in cheese, berries, as well as vegetables may be delivered by these films plus coatings, prolonging their shelf life and enhancing potential nutritious as well as sensory characteristics. This is the key to their economic success. Various food products have been effectively stored using multilayer laminates made of whey protein; the novel coating may be peeled off to make the films reusable. The utilization of packaging ideas that are based on whey protein could have a positive impact on the environment by allowing for the recycling of materials rather than burning them, which is typically done with synthetic laminates. This is conceivable because the raw ingredients for the packaging of whey protein are byproducts that come naturally from the food industry. It is crucial to remember that whenever it pertains to growth in industry, the processing of whey protein will always be cost-effective. Despite the potential of this new technology, additional scientific study is required to better comprehend how the film is made and to enhance the process's efficiency as a whole. Additionally, consumer studies and long-term toxicity assessments must be conducted to ensure that whey based packaging is safe for the environment and humans before it can gain a significant market share (Zhang & Sablani, 2021).

1.14 Conclusion

Nutritionists are increasingly seeing whey, previously thought to as a waste product from the manufacturing of milk, as a superior vitamin. For many populations, including newborns, persons at risk for heart disease, the elderly, diabetics, phenylketonuria sufferers, and athletes, whey protein-enriched formulas can be made. Efforts must be taken to overcome difficulties with taste and allergenic potential in order to maximize its use. There is promise for study into the use of whey protein in the treatment of cancer as it is now understood to be an immune-nutrient. We need to explore new ways of utilizing this valuable resource, including its potential to improve nutritional status as well as reduce metabolic syndromes. Nutritionists are increasingly recommending whey proteins and peptides as a great source of nutrition having several health benefits. Whey protein products that are recently being studied for their ability to formulate new drugs and functional food ingredients that can improve gut health and regulate nutrient absorption in the intestines due to their bio functional properties. Peptides derived from whey are being added as ingredients to functional food as well as fresh foods, alternatives as well as medicines to provide advantages and health benefits. Many among the bioactive peptides extracted from whey proteins have strong antioxidant activity along with other activities such as antihypertensive, antidiabetic as well as hypocholesterolemic properties. Once absorbed, these peptides act on determined target organs. These peptides can be consumed by various groups, including infants, seniors, diabetics, people at risk for heart disease, and athletes, when they are enriched in diets. Although there is a growing commercial interest in producing and using bioactive peptides, industrial-scale production is not yet established. Few market items have been introduced, asserting particular biological activity as well as therapeutic outcomes. Furthermore, whey proteins' useful qualities are crucial for applications in food systems. Nevertheless, due to considerable component interaction, the existing data is inaccurate in describing how the functional characteristics of whey proteins evolve in actual dietary settings.

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Chapter 2
Whey: Chemistry and Its Biotechnological Potential

Urmila Choudhary, Amrita Poonia, and Maricarmen Iñiguez-Moreno

Abstract Whey is a liquid that remains after casein has been coagulated by enzymes and/or acids and comes from the manufacturing of cheese or casein. Whey can be acidic or sweet depending on how casein coagulates. Whey typically has a 93% water content, a 6–7% solid content and lactose makes up the majority of the dry matter in whey (70%). There are moderate to low concentrations of whey proteins, minerals, milk fat, and minor substances including water soluble vitamins. The biologically most valuable components of whey have generally been identified as its nitrogen components, which also account for its high potential to be regarded as a functional food. Whey proteins are made up of thermostable fractions of proteose-peptones as well as intact globular fractions like α-lactalbumin, β-lactoglobulin, bovine serum albumin, and immunoglobulins. Other glycosylated proteins, including as lactoferrin, lactolin, transferrin, and others, are also present in lower amounts. All of the essential and non-essential amino acids are present in whey proteins, which are distinguished by their good amino-acid composition. Cys: Met ratio, which affects the bioavailability of sulfuric amino acids, is substantially higher in whey proteins than in other proteins of animal or plant origin. Anticancerogenic, antibacterial, and antioxidative characteristics, immunomodulatory, antidiabetic effects, satiety regulation and weight management; bone health protection; and dermoprotective activity are some of the most investigated positive effects. Whey proteins are distinguished by great functional qualities as solubility,

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foaming, emulsifying, and water binding ability in addition to their extremely high nutritional content.

Keywords Whey · Whey protein peptides · Bioavailability · Therapeutical value · Functional properties

2.1 Introduction

The discovery that an acid-coagulated milk gel separates into curds and whey is said to have led to the invention of cheese some 8000 years ago in the Fertile Crescent (Tunick, 2008). Whey was highly valued from the seventeenth through the early nineteenth centuries, particularly as a treatment for a number of common maladies (such as wounds and digestive disorders) (Tunick, 2008; Guo & Wang, 2019). Whey is a liquid that remains after casein has been coagulated by enzymes or acids and comes from the manufacturing of cheese or casein (Tratnik & Božanić, 2012). It is described as a yellowish-greenish liquid. The acidity or sweetness of whey will depend on the casein coagulation type. Whey typically has a water content of 93% and a solids content of 6–7%. The many physical, chemical, nutritional, and biological benefits of whey components are now supported by a growing body of scientific research, and the inherent value of whey components, notably the proteins, has been recognized. Together with variations in acidity, certain ingredient proportions, such as calcium or the current whey protein fractions, also fluctuate. Nonetheless, lactose makes up the majority of the dry matter in whey (70%) and is almost totally recovered from the wasted milk. At moderate to low concentrations, whey proteins, minerals, milk fat, and ancillary substances such water-soluble vitamins are present (Jelen, 2003; Tratnik & Božanić, 2012). Legislation and a rising amount of scientific and technological understanding now support their significance as food and related substances. Industrial methods have been developed and are being developed for processing whey and cheaply isolating usable whey components (Smithers, 2008Whey generally has a strong potential to be regarded as a functional food, with its nitrogen components being acknowledged as its biologically most beneficial ingredients. Among the intact globular fractions found in whey proteins are immunoglobulins, bovine serum albumin, α-lactalbumin, β-lacttoglobulin, and thermostable proteose-peptone fractions. There are also lower amounts of lactoferrin, transferrin, lactolin, and other glycosylated proteins. Whey proteins have a superior amino acid composition that includes both required and non-essential amino acids. When compared to other proteins of animal or plant origin, whey proteins have a significantly higher ratio of the sulfuric amino acids (Cys, Met), which affects their bioavailability. Hence, whey proteins have a biological value that is around 15% higher than that of egg proteins, which was used as a benchmark when examining the quantities of essential amino acids (Smithers, 2008; Tratnik & Božanić, 2012). Such whey proteins as a-lactalbumin, −lactoglobulin, lactoferrin, lactoperoxidase, and bovine serum albumin have been linked to antimicrobial and antiviral effects, immune system activation, anticarcinogenic activity, as

well as other metabolic properties (Gupta et al., 2012; Mehra et al., 2021; Rocha-Mendoza et al., 2021).

2.2 Chemistry of Whey Components

Whey is a yellowish watery portion and by-product of cheese and paneer industry. Poonia (2020) reported that the Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of whey are very high, e.g. acid whey has a BOD value of 35,000–45,000 mg/L and COD values of 55,000–70,000 mg/L. Whey is the liquid that remains after milk has been processed to remove the fat and casein through isoelectric or rennet-induced coagulation of the casein. The majority of the lactose, 20% of the milk proteins, traces of fat, and 95% of the original water make up whey. The type of cheese manufactured affects the whey's composition. The type and health of dairy cattle as well as the season and geographic location all have an impact on whey composition. Whey is divided into two primary categories: acid whey and sweet whey, which are determined by how the milk protein coagulates. Sweet whey, which is a by- or co-product of the production of hard, semi-hard, or soft cheese as well as rennet casein, has a pH range of 5.9–6.6.

Acid whey with a pH of 5 is produced by manufacturing organic or mineral-acid precipitated casein, as stated in Table 2.1. Medium acid whey is produced when channa and paneer are made. When compared to sweet whey, acid whey has larger levels of lactic acid and calcium phosphate, while sweet whey has higher levels of fat, lactose, and proteins (Fox et al., 2017). Whey is often highly nutrient-dense, easily digested, and assimilable. It is also regarded as a superior source of functional proteins and a high supply of vitamins B, minerals (such as calcium, potassium, sodium, iron, copper, and magnesium), as well as lactose (Macwan et al., 2016; Papademas & Kotsaki, 2019).

The process of making curd, either acid precipitation or rennet coagulation, determines the mineral makeup of sweet and acid whey.

Table 2.1 Approximate chemical composition of whey

Constituents (%)	Sweet whey	Acid whey
Water	93–94	94–95
Dry matter/Total solids	6.0–6.5	5–6
Lactose	4.5–5.0	3.8–4.3
Total protein	0.8–1.0	0.8–1.0
Whey protein	0.60–0.65	0.60–0.65
Citric acid	0.1	0.1
Ash (minerals)	0.5–0.7	0.5–0.7
Lactic acid	Traces	Up to 0.8
pH	6.2–6.4	4.6–5.0
SH value	About 4	20–25

Source: http://www.dairyforall.com/whey.php

Table 2.2 Whey proteins profile and their characteristics

Whey protein	% of total milk protein (%) (percentage of total WPs %)	Molecular weight range (Da)	Estimated average molecular weight (Da)	Isoionic pH
β-Lactoglobulin	9–10 (50.0)	18,205–18,363	18,300	5.14–5.49
α-Lactalbumin	2–4 (20.0)	14,147–14,175	14,000/14200	4.2–4.8
Serum albumin	≈1 (5.0)	66,267–69,000	66,300	4.71–5.13
Immunoglobulins	≈2 (10.0)	153,000–901,000	–	5.5–8.3
Lactoferrin	1.0	–	80,000	8.7
Lactoperoxidase	0.25–0.5	–	78,000	9.6
Proteose peptone	2–4	4100–40,800	–	3.3–3.7
Miscellaneous	<2.5	–	–	–

Source: Madureira et al. (2010), McSweeney and Fox (2013), Guo and Wang (2019)

Depending on the type of milk used and the effectiveness of the cheese-making process, the fat content of the separated whey may range from 0.5% to 1%. Although skim milk is utilized in the production of most acid whey from fresh cheeses, such as cottage cheese or regular quarg, their fat content is insignificant. Numerous factors, such as the pretreatment of the cheese milk and the whey (pasteurization, centrifugation, mechanical handling, cultures used, pre-concentration, recovery of casein fines, processing aids (yellow colour), membrane processes), as well as the proximate composition of different whey, can cause significant variations.

Depending on the kind of whey, lactose (4-O-β-D-galactopyranosyl-D-glucose), the main component of the whey solids, makes up 4.4–4.9% of the whey (nearly 75% of the dry matter). Due to the fermentation process, in which some lactose is transformed to lactic acid, acid whey typically has a lower lactose level. About 20% of the total proteins in milk are made up of whey proteins. They are a mixture of globular proteins that can be extracted from whey and are one of the most useful nutritional component of the whey. They have a very uniform distribution of non-polar, polar, and charged amino acids. According to Blažić et al. 2018, they are primarily made up of the thermosensitive proteins such as β-lactoglobulin (β-lg), α-lactalbumin (la), bovine serum albumin (BSA), immunoglobulin (lg), thermostable proteose-peptones, and lactoferrin (Lf), which make up about 50, 20–25, 10–15, 6, 1, and < 1% of the whey protein fractions, respectively (Table 2.2). Whey proteins have a compact globular form and very distinct amino acid profiles than caseins, which are present as a micellar suspension.

2.2.1 β-Lactoglobulin

The main whey protein is β-lactoglobulin (β-lg), which accounts for 50% of the total whey proteins (Oldfield, 1996). About 5.2 is where the isoelectric point of β-lg lies. With 162 amino acids, β-lg has a molecular weight of roughly 18.3 kDa. Four β

sheets form a barrel-like structure in which β-lg naturally exists, making these structures good places for hydrophobic interactions (Croguennec et al., 2004). Moreover, there are five cysteine residues in the β-lg monomer, four of which are involved in the formation of two disulfide linkages and one of which is a free thiol group. Between Cys106-Cys119 and Cys66-Cys160, there are two disulphide bonds, and Cys121 has a free thiol group. The natural β-lg structure encloses the two disulphide bonds as well as the free thiol group. There are nine antiparallel strands in native β-lg. The "calyx" is created by wrapping eight of the β-strands together to form a flattened and conical barrel. Trp19 closes one end of this calyx, while strand A bends 90° nearby residues 21–22 to interact with strand H in an antiparallel manner to complete the calyx. The calyx is cylindrical and has hydrophobic walls. The calyx is always empty unless a ligand is present. The ninth strand, strand I, is situated outside of the monomer and, along with strands A and H, forms the dimer interface. Each monomer has a hidden dimer interface that involves antiparallel contacts between residues 146 and 150 and those of the other subunit (Sakurai & Goto, 2002). Above the strand H and on the calyx's exterior, there is a 3-turn α-helix (McSweeney & Fox, 2013). The α-helix protects the free thiol group, but depending on the denaturation circumstances, it may become exposed to the outside, starting a series of events that include sulphydryldisulphide exchange reactions. The estimated contents of a solution of β-lg's helix, sheets, and random coil are 8%, 45%, and 47%, respectively (McSweeney & Fox, 2013). There are a number of genetic variations of β-lg that have been identified, with the A and B variants often occurring at about equal rates. The position of different amino acids at 64 and 118, where Asp and Val are present in the β-lg A variant and Gly and Ala in the β-lg B variant, respectively, is the fundamental distinction between the two forms (McSweeney & Fox, 2013). The flexibility of Variant A exceeds that of Variant B.

2.2.2 α-Lactalbumin

The second most abundant whey protein, α-lactalbumin (α-la), accounts for 20% of the total whey protein content. The protein α-la occurs as a roughly spherical, compact globular structure at neutral and alkaline pH conditions. Unlike β-lg, α-la has 123 amino acid residues and a molecular weight of 14 kDa. It is mostly found as a monomer. A calcium-binding loop connects the two domains of native α-la, which are a large α-helical domain and a tiny β-sheet domain. Eight Cys residues make up the molecule, and four of those residues form disulphide bonds: Cys6-Cys120, Cys28-Cys111, Cys60-Cys77, and Cys73-Cys90. Cys6-Cys120 and Cys28-Cys111's disulfide links are positioned in the helical lobe, while Cys60-Cys77's disulfide bond is located in the lobe that contains the β-sheet. The remaining disulphide bond is created between Cys90 of the helical lobe and Cys73 of the β-lobe. α-la lacks a reactive free thiol group because all Cys residues have taken part in creating disulphide linkages. Due to its lower inherent stability, the disulphide

bond between Cys6 and Cys120 is discovered to be more susceptible to cleavage than the other three (Kuwajima et al., 1990).

According to a spectral examination of α-la in solution, α-la includes 20% helix, 14% sheet, and 60% unordered structures (McSweeney & Fox, 2013). There are two types of α-la, apo and holo, with apo being the Ca-bound form. The most prevalent type of α-la in milk is the holo form. High thermal stability is provided by calcium binding, which stabilizes the conformation (McSweeney & Fox, 2013). Moreover, the separation of αμand βμsubdomains and disruption of the hydrophobic contacts can result from these structural alterations (McSweeney & Fox, 2013). The first stage of the unfolding of α-la, which results in the development of a molten globule state, is known as this process.

2.2.3 Bovine Serum Albumin

Bovine milk has different amounts of bovine serum albumin (BSA) depending on the stage of lactation, with late lactation having the highest concentration. BSA is a monomer that weighs up to 66 kDa and has 583 amino acid residues (McSweeney & Fox, 2013). Nine loops in this structure are supported by 17 intramolecular disulphide bonds. At position 34, there is a free thiol group as well. Three domain structures-I, II, and III-each maintained by an intramolecular network of disulphide bonds-give BSA its overall oblate shape (McSweeney & Fox, 2013). Two huge double loops and one little double loop make up each domain, each of which displays varying levels of hydrophobicity and surface charge. Ligand binding is a key characteristic of BSA. In pH-neutral conditions, BSA has between 5 and 12 binding sites that allow it to bind a variety of ligands, including fatty acids and cations. BSA's secondary structure is made up of 10% β-turns, 23% extended chains, and 66% helix, without β-sheets (McSweeney & Fox, 2013). Due to the substantial quantity of disulphide bonds, the helix formation is constrained. These disulphide bonds, however, are relatively short and offer some flexibility.

2.2.4 Lactoferrin

A single chain protein called lactoferrin (LF) has 689 amino acids and a molecular weight of about 80 kDa (McSweeney & Fox, 2013). There are no reactive free thiol groups in the 14 intramolecular disulphide bonds that make up the molecular structure of LF. Compared to the other whey proteins, LF has a high isoelectric point of pH 8.7. Two lobes of the LF polypeptide chain share similar amino acid sequences. The amino acids in the N- and C-lobes, respectively, were 1–333 and 345–692. N1, N2 and C1, C2 are the two subdomains that make up each lobe. An extended α-helix connects the two lobes. Each lobe contains two glycan and iron binding sites. The affinities for binding iron vary depending on the conformations of

the N- and C-terminal lobes. The iron-free, apo state's conformation is more flexible and open.

2.2.5 *Immunoglobulins*

Immunoglobulins (Igs) are a class of intricate, massive glycoproteins with antibody function. About 10% of the whey proteins are made up of these proteins. Bovine milk contains IgA, IgM, IgE, IgD, and IgG, which are five different forms of Igs (McSweeney & Fox, 2013). These Igs have a fundamental structure and range in molecular weight from 150 kDa to 900 kDa. IgG1 and IgG2 are the two divisions of type IgG. With over 80% of all Igs found in bovine milk, IgG1 is the predominant form. Igs are four-chain polypeptide monomers or polymers that can be found in the body. Of those four polypeptide chains, two are small and have a low molecular weight (22 kDa), whereas the other two are larger and have a high molecular weight (50–70 kDa) (Patel, 2015).

2.2.6 *Lactoperoxidase*

A type of milk enzyme called lactoperoxidase makes up 0.25–0.5% of all the whey proteins.

It is the enzyme that is most prevalent in whey after the curdling process. It has the ability to decrease hydrogen peroxide, which can inhibit a variety of bacterial species, and to catalyse specific processes. This enzyme can tolerate heat up to 75 °C, however after 30 min at those temperatures, it started to lose activity (Xiong et al., 2020). Moreover, whey protein contains a small number of highly active proteins such as growth factors, lactoperoxidase, milk fat globule membrane (MFGM) proteins, and vitamin binding proteins (Guo & Wang, 2019).

2.3 Utilization of Whey

A significant portion of whey is converted into whey powders due to the superior nutritional and functional qualities of whey solids, with the remainder being used to create sweet whey powder, demineralized whey, delactosed whey, whey protein concentrate (WPC) (35–80% whey protein), whey protein isolate (WPI) (at least 90% whey proteins), lactose and hydrolysed whey protein, or lactose (Alves et al., 2014; Jeewanthi et al., 2015; Celik & Onur, 2016). The sole difference between the hydrolyzed whey protein and the isolate one is the hydrolysis process of the whey protein molecules, which increases its digestion (Jeewanthi et al., 2015). Whey proteins can be separated and fractionated by precipitation, in conjunction with

acid treatment, high temperature treatment, centrifugation (the centri-whey process), and as part of a membrane process (usually ultra- or microfiltration followed by a diafiltration and spray drying). Poonia and Pandey (2023) reported that whey protein contains all nine essential amino acids and has high biological value as compared to other sources of dietary proteins. It can be used in the production of industrialized foods, different beverages and protein supplement.

Whey powder manufacture typically requires a number of steps:

- Clarification of whey
- Separation of cream and pasteurization
- Concentration of total solids (40–60% by using evaporation)
- Lactose crystallization
- Drying of whey (removal of water by spray drying, freeze drying)

The solid mass of the generated bulk powder, if lactose crystallisation is not done, is only acceptable for animal feed as a cheap source of superior proteins and carbohydrates. The final composition of whey protein concentrates or isolates is influenced by pre-treatments of milk or whey, such as heating or acidification, as well as the manufacturing techniques used, such as precipitation, ion exchange chromatography, gel filtration, and membrane fractionation.

Whey powder products are used in a wide variety of food-related industries due to their high nutritional value; the most common use is as an additive in the production of a variety of foods and beverages (e.g., meat products, infant formula, soups, beverages, sauces,, creamers, toppings, pressed nuts, cheese-based sauces, nut coatings, potato chips, savoury flavours & pastries, specialty bakery products like pizza, biscuits, and macaroni as well as souffles and cake) (Božanić et al., 2014). The technological advantages of adding whey protein to foods and beverages have also been examined in order to improve formulations' solubility, gelation, emulsification, foaming, stability, as well as sensory qualities like flavour, colour, and texture (Jeewanthi et al., 2015; Camargo et al., 2018; Soares et al., 2018). Table 2.3 shows the characteristics of whey protein as they alter as a result of hydrolysis as well as their technological functionality in food applications. Whey powders can also be utilized as a carrier for fat and oil and as an adsorbent. The whey is additionally processed by membrane separation when creating the higher-grade whey protein powders, typically via ultrafiltration or diafiltration (Blažić et al., 2018). Whey-derived components like WPC, WPI, and whey protein hydrolysate (WPH) are added to drinks with a high protein content, primarily sports drinks and beverages for the undernourished (Lappa et al., 2019).

2.4 Biotechnological Potential of Whey

There are several strategies for managing whey sustainably, most of which are focused on biotechnological and gastronomic applications for the creation of value-added goods like whey powder, functional foods and drinks, lactic acid,

Table 2.3 Modifications in whey protein characteristics due to hydrolysis and their techno-functional potential in food applications

Changes in protein characteristics due to hydrolysis				References
Altered molecular properties		Molecular characterization		
• Molecular charge • Molecular weight • Exposure of hydrophobic groups reactive amino acid side chain groups		• Degree of hydrolysis • Molecular weight distribution • Reverse phase chromatography • Surface hydrophobicity		Jeewanthi et al. (2015)
Techno-functional properties of whey protein hydrolysates				
Hydration properties • Solubility • Water binding • Cohesion, • Adhesion, • Elasticity	Aggregation and gelation properties • Gel formation • Film formation	Interfacial properties • Emulsification • Whipping formation • Foaming and aeration	Sensorial properties • Colour • Flavour • Texture	Ruann et al. (2017)

bioethanol, single cell protein, hydrogels, bioplastics, and biogas. While whey in large quantities can be converted to bioethanol, whey in smaller amounts is best used to make fermented or unfermented beverages. While bioactive proteins are more frequently used in both the food and pharmaceutical industries, whey and its components are included in dietary and health goods (Blažić et al., 2018). Because whey proteins are sensitive to heat treatments at temperatures above 60 °C and are vulnerable to microbial deterioration because of the high water content, non-thermal methods are used to produce whey beverages (Barukčić et al., 2015; Amaral et al., 2018; Režek et al., 2018).

2.4.1 Whey as a Sustainable Source of Lactose

Because it functions as dietary fibre and has prebiotic qualities, lactose offers a number of advantages from a health and nutritional standpoint. In this way, lactose helps the body absorb different minerals like calcium, phosphorus, and magnesium through the intestinal tract (Kwak et al. 2012). Moreover, it is used by intestinal bacteria as a food source and a substrate for the formation of lactic acid and short carbon cycle fatty acids (SCFA), which creates a moderately acidic reaction in the intestine and inhibits the growth and spread of dangerous bacteria (Francavilla et al., 2012). Also, because to its low glycaemic index (which is half that of glucose), it has less of an effect on blood sugar levels (Musci, 2016). By isolating lactose from deproteinized whey (for example, whey permeate obtained by ultrafiltration), lactose can be recovered from whey using a number of techniques, including evaporating whey to concentrate it, crystallising lactose from concentrated whey (Simone et al., 2019), and centrifuging or decanting the crystals to separate them (Božanić et al., 2014). As lactose is the primary factor contributing to the high BOD and COD levels in whey, its recovery might more than 80% lower the BOD value (Das et al., 2016).

In this approach, lactose recovery may be able to address both environmental and waste management issues. The recovered lactose may also be supplied to the food, pharmaceutical, dairy, and beverage (e.g., food-grade or pharmaceutical-grade) businesses, depending on its quality. It is typically employed as an excipient in the pharmaceutical business as well as in the food and confectionery industries, particularly in baking as a crust browning enhancer. Furthermore, by using microbes to break down lactose, new whey-based products can be created. To extract lactose from whey and turn it into products with industrial value, such as single cell proteins, probiotic starter cultures, organic acids and alcohols like lactic and citric acids, ethyl alcohol, fermented whey beverages that resemble kefir, biogas, bioplastic, and ethyl lactate, various biotechnological processes have been developed (Celik et al., 2016).

2.4.2 Whey as a Source of High Quality Proteins

It is well recognised that whey proteins have excellent nutritional and functional properties, including bioactive peptides, antioxidants, and immunopotentiators as well as necessary amino acids. In addition to it, whey proteins contain high level of essential amino acids and considered as a source of high quality proteins (Arya & Poonia, 2019). These proteins have a better biological value and are easier to digest than other proteins of animal origin because they have a lesser proportion of Glu and Pro and a higher Cys/Met ratio (Božanić et al., 2014). Many nutritional and biological advantages have been attributed to them, most of which are connected to the bioactive peptides that result from the proteolytic breakdown of whey proteins. Such bioactive peptides are essential for managing chronic diseases through nutrition (cardiovascular, digestive, immune and nervous systems). Whey proteins have a variety of positive health effects that can be categorised as antibacterial, antioxidant, antithrombotic, antihypertensive, or immunomodulatory (Brandelli et al., 2015). The academic community has become more aware of the distinctive biological and functional properties of whey proteins as a result of the development of methods for protein separation, purification, and drying (membrane separation and chromatography, electrodialysis, spray, and freeze drying), which has broadened their application (Khaire & Gogate, 2019). Whey proteins are distinguished by great functional qualities as solubility, foaming, emulsifying, and water binding ability in addition to their extremely high nutritional content. As a result, they are frequently utilised as natural stabilisers, emulsifiers, foaming agents, chemicals that bind to aromas, carriers for minerals, etc. (Ruann et al., 2017). Whey proteins can be easily formed into various bases and matrices (macro-, micro-, and nanostructures) that are appropriate for carrying a variety of bioactive chemicals, varied flavours, or compounds with high nutritional value, in addition to being used in food and beverages. Some literature also suggest the targeted use of whey proteins as emulsifiers, foaming and gelling agents, thickening agents, surface-active components, and texture modifiers (Fu & Nakamura, 2017; Andoyo et al., 2018; Khalifa et al., 2018).

2.5 Recent Trends in Whey Utilization

The focus of recent research in this area has been on the creation of novel whey protein-based value-added products, such as edible films, hydrogels, nanoparticles, and microencapsulated goods (Khaire & Gogate, 2019). The body of scientific evidence supporting the bioactivity of whey protein peptides produced by digestive enzymes, proteolytic bacteria, or plant proteases is expanding. The blood pressure-lowering impact, anticancerogenic, antibacterial, and antioxidative characteristics; immunomodulatory effects; satiety regulation and weight management; bone health protection; and dermoprotective activity are some of the most researched positive effects. Glycomacropeptide's (GMP) advantageous qualities are frequently linked to whey's medicinal benefits. GMP is a casein hydrolysate that is produced by enzymatic hydrolysis and is exclusively found in sweet whey. It is distinguished by the absence of phenylalanine and frequently appears in preparations intended for the phenylketonuric population. Moreover, GMP benefits the gut flora and is connected to the control of satiety and weight management (Brandelli et al., 2015; Patel, 2015; Smithers, 2008; Krol et al., 2017). Table 2.4 and Fig. 2.1 respectively illustrate the biological characteristics of whey components and their therapeutic use.

2.5.1 Edible Films and Coatings

The demand for so called'green' packaging has accelerated research on active bio-based packaging. Edible or biodegradable films are the most suitable green substitutes for traditional plastics and thus help in combating environmental pollution. The employment of latest technology for recycling and reuse of whey have inspired noteworthy scientific interest concerning the utilization of whey protein toward creating edible coatings and films. de Castro et al. (2017) reported that the edible biofilms have numerous advantages, as they may reinforce or replace existing natural layers, aid in preservation of moisture and prevention of loss of important components (e.g. flavours). Above all, these edible films can be consumed together with the product without prior removal.

Mishra et al. (2022) reported that whey and casein proteins can be effectively used in the development of edible coatings with improved physicochemical and textural properties. Plasticisation is required during the production of whey and casein protein-based coatings and films as they often lack flexibility. Sustainable oxygen barrier properties of whey protein films have made them suitable substitute material for replacement of traditionally used polyester and nylon films. Moreover, whey proteins can form clear films and coatings with improved mechanical and barrier properties compared to polysaccharide-based films and may provide surface sterility (Basiak et al., 2017). Apart from providing improved barrier properties, such films and coating also biodegrade in a shorter time period. In order to develop a new eco-efficient food packaging product exhibiting enhanced resistance to moisture

Table 2.4 Biological properties of whey constituents

Whey constituents	Biological properties	References
Whole whey proteins	Prevention of cancer (breast, intestinal cancer and chemically induced cancer)	Brandelli et al. (2015), Patel (2015), Krol et al. (2017), Shahraki et al. (2018), Teixeira et al. (2019), Xiong et al. (2020), Murali et al. (2021) and Falsafi et al. (2022)
	Antimicrobial activities	
	Targeted delivery and controlled release of bioactive components	
	Increment of satiety response (increment in plasma amino acids, cholecystokinin and glucagon like peptide)	
β-Lactoglobulin	Transporter of retinol, palmitate, fatty acids, vitamin D and cholesterol	Gupta et al. (2012), Loch et al. (2012), Sousa et al. (2012), Brandelli et al. (2015) and Tai et al. (2016)
	Enhancement of pregrastic esterase activity	
	Transfer of passive immunity	
	Regulation of mammary gland phosphorus metabolism	
α-Lactalbumin	Prevention of cancer	Patel (2015), Krol et al. (2017), Shahraki et al. (2018), Teixeira et al. (2019) and Murali et al. (2021)
	Lactose metabolism and synthesis	
	Treatment of chronic stress-induced disease	
Bovine serum albumin	Fatty acid binding	Blažić et al. (2018), Shahraki et al. (2018), Teixeira et al. (2019), Xiong et al. (2020) and Murali et al.(2021)
	Anti-mutagenic function	
	Prevention of cancer	
	Immunomodulation (disease protention through passive immunity)	
Immunomodulation	Antibacterial activity (HIV)	Barukčić et al. (2015), Amaral et al. (2018), Režek et al. (2018) and Xiong et al. (2020)
	Antifungal activity	
	Opioid activity	
Glycomacropeptides	Interaction with toxins, viruses, and bacteria (mediated by the carbohydrate fraction)	Patel (2015), Krol et al. (2017), Gupta et al. (2012) and Xiong et al. (2020)
	Control of acid formation in dental plaque	
	Immunomodulatory activity	

transfer and enhanced flexibility, whey proteins are required to be blended with appropriate plasticizers, such as glycerol or sorbitol.

2.5.2 Whey as Functional Food

Whey is a fantastic source of folic acid and vitamin B. The most variable aspect of the whey dry matter is its mineral makeup, which can range from 0.5% to 0.83%, depending on the process used to make the cheese. The minerals sodium, potassium, and calcium are the most prevalent in whey, and they are all present as salts in the

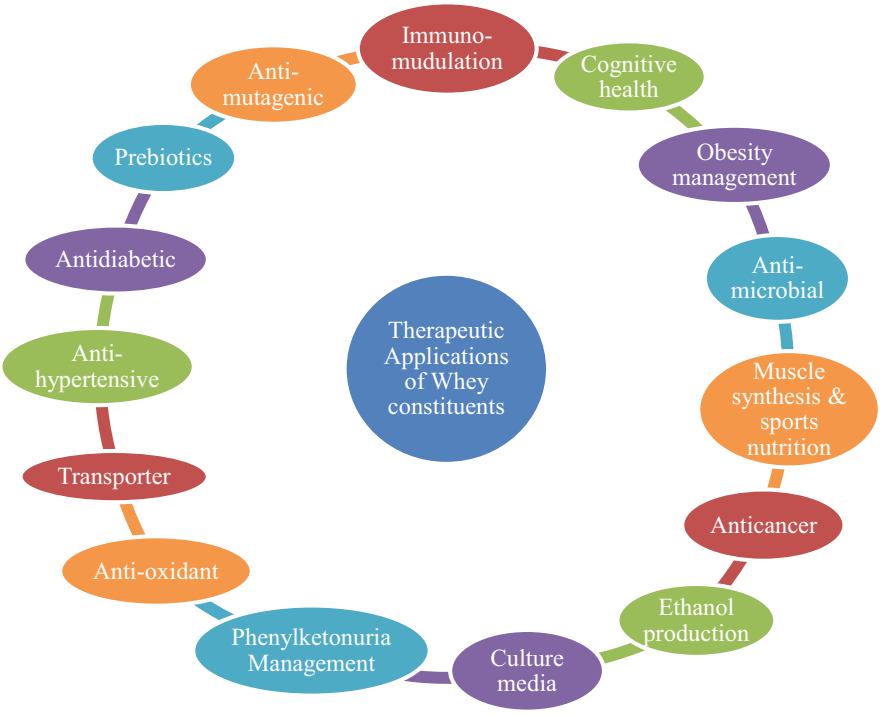

Fig. 2.1 Therapeutic applications of whey and whey constituents

forms of chlorides, phosphates, citrates, and sulphates (Barukčić, 2013). As a result, whey is a great source of lactose, a sugar with a low glycaemic index, functional proteins, peptides, and vitamins and minerals. Hence, it may be viewed as a functional food for a variety of consumer categories, focusing on athletes in particular but also targeting young children and the elderly.

2.5.3 Whey as Source of Bioplastics

Because to the ease with which the lactose in whey permeate may be transformed into polyhydroxyalkanoates (PHAs) and polylactic acid (PLA), the use of cheese whey as a substrate for the manufacturing of bioplastics has recently gained attention (Ryan & Walsh, 2016). Whey protein films stand out as a sustainable biodegradable alternative to conventional plastic films thanks to their better mechanical and barrier qualities (Choudhary et al., 2021; Zandona et al., 2021). Whey proteins can also create polymeric 3D networks, such as hydrogels, in addition to biofilms (Lappa et al., 2019; Zandona et al., 2021). The use of these proteins in the diet and supplements has the potential to treat many diseases caused by metabolic

imbalances, so it seems essential to take advantage of their potential. Research on bioactive compounds and nutrients propelled whey protein to the forefront of the functional food sector.

2.6 Conclusion

Whey is a fantastic food that contains a variety of bioactive components. Whey protein makes about 20% of the total protein in milk, and its principal constituents, which are present in varying concentrations, include β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, and others. It is abundant in bioactive peptides, antioxidants, immunoglobulins, and branching and essential amino acids. It offers protection from a variety of metabolic illnesses, including phenylketonuria, cancer, diabetes, obesity, and cardiovascular problems. The protein has been proven to speed up healing from injuries caused by resistance training, promote gastrointestinal physiology, and shield skin from harmful radiations. Whey protein has demonstrated its appropriateness as a fat replacement and emulsifier in addition to energising the body. Moreover, its potential for edible and antimicrobial packaging makes it very desired in both the food and pharmaceutical industries. The dairy sector adopts sustainable methods and reduces its environmental impact as a result of using and exploiting whey. To develop and grow the future of functional dairy beverages in the global dairy business, more research into inventive uses of whey will be necessary.

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Chapter 3
Utilization of Whey: Sustainable Trends and Future Developments

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Abstract Whey is produced in huge quantities by the dairy industry as a byproduct, and as non-food leads to serious environmental issues due to its high organic matter content. There has been a lot of research done over the last several decades how to use whey in a more sustainable and cost-effective way. The creation of value-adding goods including whey powders, functional meals, edible films and coatings, lactic acid, alcoholic beverages, sports drinks, and other biochemical, bioplastics, and biofuels is the core objective of sustainable whey management. In recent years, researchers have looked at different ways to use whey in a more affordable and ecologically friendly way, with the main goal of turning undesirable end products into useful materials. It is a source of several bioactive ingredients with various physiological and functional characteristics. It also provides an opportunity to food industries to develop functional foods with potential health benefits. Whey's active components are advantageous because they offer antibacterial and antiviral activities, boost antioxidant activity, support bone and immune system health, improve athletic performance, and prevent cancer and cardiovascular disease. This chapter describes how to use whey and its components sustainably while using integrated processes and refining techniques to create high-value whey-based products. This is done in accordance with many international initiatives for improved planetary health, such as the EU Green Deal and the Sustainable Development Goals (UN, General Assembly. Transforming our world: The 2030 agenda for sustainable development. 2015).

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Keywords Whey protein · Dairy waste · Byproduct · Whey utilization · Sustainable development goals

3.1 Introduction

Milk is the primary source of essential nutrition and most adaptable food available to humans, which may be ingested either raw or in the form of processed dairy products. In general, milk is used to make a wide variety of food products, including protein-rich and cream-based products, beverages, powder, cheese, yoghurt, butter, and fermented milk. Asia is the world's largest milk-producing region expected to produce nearly 419 million tons of milk in 2022, an increase of 2.1% from the previous year (2021). India, Pakistan, China, Uzbekistan, Kazakhstan, and Japan, among other countries, are expected to contribute significantly to this increase. The projection for milk production in India is 221 million tons, growing at a slower rate than in previous years due to the emergence of Lumpy Skin Disease, which had a severe impact on small-scale producers (Chourasia et al., 2022; FAO, 2021). Whey, a milk constituent, is a byproduct of cheese and casein production in the dairy industry. Many food items now identify whey as a value-added component (Solak & Akin, 2012). There are significant amounts of by-products created, primarily whey, due to the dairy industry's ongoing expansion. Cheese whey is a severe biological pollutant, if not adequately handled, that might be hazardous to the environment (Zandona et al., 2021). About 90% of the milk byproduct lost during the manufacturing of dairy products like curd and cheese is made up of whey. Worldwide production of fresh whey reached 117 million tonnes in 2019, with the European Union and the United States accounting for 69% of the total production (FAO, 2020). Due to the high concentration of nutrients in whey, a byproduct is created that, if released into the environment, would pose a serious threat to the ecosystem due to its high biological oxygen demand (BOD) (35–60 g/L) and chemical oxygen demand (COD) (50–102 g/L) values (Zotta et al., 2020). Milk's whey retains around 55% of its nutrients but still an appreciable loss in food energy occurs due to inappropriate whey waste disposal. The majority of the abundant nutrients include 0.1–0.8% lactic acid, 1% mineral salts, 0.8% soluble proteins, and 4.5% lactose. Whey protein contains all nine essential amino acids and has high biological value as compared to other sources of dietary proteins. It can be used in the production of industrialized foods, different beverages and protein supplement (Poonia & Pandey, 2023). Due to its high nutritional value, whey is utilized for production of a variety of products with added value, including bioactive peptides, prebiotics, bacteriocin, enzymes, dry whey powder, paste, and lactose (Chourasia et al., 2022; FAO, 2022). Whey holds its industrial value due to high-quality protein, therefore used for the development of protein supplements. Despite the substantial research into several potential benefits of using whey in industry, a significant part of the world's whey production is still goes waste as an effluent (Verma et al., 2023). In order to meet the majority of the Sustainable Development Goals (UN, 2015) and lessen pollution of the air, land, and water, this research intends to offer an overview

of the sustainable use of whey, lactose, and whey proteins for the development of high value-added goods. Along with new breakthroughs and improvements in refining technology for sustainable whey management, several environmental efforts aimed at lowering ecological effect are examined in this context.

3.2 Nutritional Composition of Whey

The watery, yellow-greenish portion of milk that remains after the curd has been separated during the cheese-making process is known as whey. It makes up close to 85–90% of the milk's volume and contains around 55% of the nutrients. The following is a typical breakdown of whey dry residue: According to the whey acidity, milk contains 70% lactose, 14% proteins, 9% minerals, 4% lipids, and 3% lactic acid (Zandona et al., 2021, Baskaran et al., 2009). Due to their strong functional qualities, which have found several uses as components in food compositions, whey proteins have received special attention. Table 3.1 depicted the nutritional compositions of whey proteins.

3.3 Utilization of Whey Protein

Twenty percent of the total protein in milk comes from whey proteins. They are made up of globular proteins that may be extracted from whey and are among the nutritionally most advantageous parts of whey. The distribution of non-polar, polar, and charged amino acids is quite consistent in them. (Zandona et al., 2021). Whey proteins serve a crucial function in the immunological defense of infants (Han et al., 2023). The -lactoglobulin (Lg), -lactalbumin (La), bovine serum albumin (BSA), immunoglobulin (Ig), thermostable proteose-peptones, and lactoferrin (Lf) make up most of the whey protein fractions, each of which makes up around 50, 20–25, 10–15, 6, 1, and less than 1% of the total protein, respectively. Whey proteins have an extremely compact and globular structure and significantly different amino acid profiles from caseins, which are present in a micellar suspension. When compared to

Table 3.1 Nutritional composition of whey protein fraction

Protein	Approx. content g/L whey
Alfa-lactalbumin	0.6–1.7
Beta-lactoglobulin	2.0–4.0
Serum albumin	0.2–0.4
Immunoglobulins	0.5–1.0
Protease-peptones	0.2–0.4
Other (caseins, glycoproteins)	0.1

Source: (Francis & Wiley, 2000; Jeewanthi et al., 2015; Chandrapala, 2018; Chavan et al., 2020)

animal-derived proteins, they have a greater ratio of cysteine and methionine and a smaller fraction of the amino acids, which gives them a superior biological value and makes them simpler to digest (Božanić et al., 2014). Due to its high protein content, safety, and biodegradability, whey has several agricultural uses, including the encapsulation of bacteria to protect plants from pests and illnesses (Riseh et al., 2022). Regardless of calorie restriction strategies, adults and the elderly should consume high-protein diets, mostly composed of animal protein sources. Whey protein is a high-biological value supplement that contains necessary amino acids that help with hunger management, glycemic parameters, and the preservation of muscle mass (Giglio et al., 2022). In addition to its nutritive components, whey may also include pollutants from the environment or possibly harmful compounds that come from cleaning procedures i.e., cleaning-in-place residues and disinfection by-products (Tsermoula et al., 2021). The production of powdered whey and good separation of the components of whey are the results of the progress of processing techniques, notably membrane filtration. Protein isolation uses selective precipitation, which when combined with centrifugation and electrodialysis might provide pure protein fractions (Ramos et al., 2015). Ion exchange purifies proteins and demineralizes; microfiltration and ultrafiltration remove fat and protein from whey; electrodialysis causes demineralization; nano-filtration and reverse osmosis concentrate. Diafiltration is used to remove minerals and low-molecular-weight molecules. After concentration, spray-drying is used to create premium powders that are frequently used in gourmet food products (Panghal et al., 2018; Nishanthi et al., 2017). The creation of underutilized products like whey permeate and de-lactose whey permeate, which are mostly made of lactose, minerals, and nitrogen that is not obtained from proteins, has been facilitated by the growing production of whey-derived compounds. The potential of these products as additives in food and medicinal goods is limited by the lack of knowledge regarding the non-protein nitrogen and other unidentified molecules in these products; hence, a complete study of their composition is essential (Riseh et al., 2023; Yiğit et al., 2023).

3.4 Health Benefits of Whey

It has been established that whey is a source of a number of bio-active substances with unique physiological and functional characteristics. The food sector is given the chance to provide nutritious meals or foods that are useful for human health. Among other benefits, whey-derived bioactive compounds include antibacterial and antiviral properties, enhance immune system and bone health, raise antioxidant activity, boost athletic performance, and help prevent cancer and cardiovascular disease. (Solak & Akin, 2012; Zaky et al., 2022 & Gupta & Prakash, 2017).

3.4.1 Anti-microbial, Anti-viral and Anti-carcinogenic Activities

Several ingredients in whey have the potential to provide protection against pathogens, bacteria, and viruses. Igs—its peptide derivative, lactoferricin, lactoperoxidase, and sphingolipids are some of these components (Wakabayashi et al., 2003; Floris et al., 2003). Furthermore, whey protein may undergo proteolysis during gastrointestinal transit to produce antimicrobial peptides. The inhibitory action of LF, -LA, and -LG against type 1 human immunodeficiency virus has been evaluated (HIV-1) (Yalcin, 2006; Chatterton et al., 2006). Due to nausea and appetite loss, cancer patients receiving radiation or chemotherapy often struggle to satisfy their daily nutritional needs. Because it is relatively mild on the body and simple to digest, whey protein is a great protein alternative for cancer patients (Solak & Akin, 2012; Hakkak et al., 2001). Whey protein offers greater protection against the growth of intestinal cancers. When compared to other protein sources, dairy proteins, especially whey, give protection against intestinally generated cancers. Intestinal, breast, and colon cancers have been demonstrated to be decreased by whey-containing diets (Akın, 2006).

3.4.2 Cardiovascular Health

There is some evidence that whey proteins are beneficial to cardiovascular health. Milk peptides have been shown to be beneficial in the treatment of hypertension. The levels of triglycerides in the blood may be lowered by drinking fermented milk that contains whey protein concentrate. Additionally, those who are overweight or obese who consume whey protein see improvements in their blood pressure and vascular function (Pal & Ellis, 2011; McNally, 2008).

3.4.3 Immune Modulating Activity

It has been shown that whey products and their components have a role in the immune system of the host. Whey contains several bioactive substances, some of which have been demonstrated to improve immunity and provide protection against viruses, infections, certain cancers, and infections. Particularly, the synthesis of glutathione is increased by three of the peptides found in whey, which are known to strengthen the immune system (Chourasia et al., 2022; Solak & Akin 2012).

3.4.4 *Physical Performance, Bone Health and Weight Control*

According to the literature study, those who lead physically active lives may take advantage of whey and its components. In infant formula, sports nutrition snacks and drinks, and other food products, whey and whey constituents act as value-added ingredients (Sharma & Chauhan, 2018). The quantities of branched chain amino acids (BCAAs) found in whey protein are thought to be the greatest of any natural dietary source, making it an excellent supply of these essential amino acids. They are digested directly into muscle tissue, unlike other essential amino acids. BCAAs are vital for athletes because they are the first amino acids consumed during exercise and resistance training. This makes them the first ones to be depleted after intense workouts (Ha & Zemel, 2003). When calories are limited; whey protein is superior to other forms of protein for promoting fat reduction. It influences the hormones that govern appetite as well as hunger (Solak & Akin, 2012). A high-protein diet reduces both the number of calories consumed and the amount of body fat stored, and whey protein is more effective than red meat in preventing weight gain and improving insulin sensitivity (Baer, 2006). Whey protein helps control blood glucose levels and manage weight, both of which are typical issues for people with type-II diabetes. Whey protein can help with both problems by regulating blood sugar. The meal's contribution to maintaining lipid oxidation and ensuring rapid access to amino acids for utilization during exercise increases the effectiveness of exercise training in reducing adipose tissue (Akal, 2017). Since whey protein includes similar components as found in human breast milk, it is an essential component in a varied range of baby formulae, including those that are intended for children who were born prematurely. In addition, pregnant women, who are required to consume a greater quantity of protein, may benefit greatly from consuming whey protein, which is an outstanding option among protein sources. The stomach mucosa is defended against the damaging effects of ethanol by whey protein concentrate. Sulfhydryl compounds, which are stimulators of glutathione formation, are responsible for the protective qualities of the molecule. Because it is easily digested, whey protein contributes to an increase in plasma amino acids almost immediately (Yiğit et al., 2023).

3.5 Sustainable Utilization of Whey

The dairy sector will experience a variety of difficulties as whey production expands globally in the upcoming years. The dairy industry has to look for innovative, sustainable, and environmental friendly methods of whey usage in order to decrease the negative environmental effects of whey disposal and elevated operating expenses of whey processing (Zotta et al., 2020; Asunis et al., 2020; Addai et al., 2020; Pires et al., 2021). The utilization of by-products may help in the boost up economy with

protection of environment and new product development (Papademas & Kotsaki, 2019).

There are several environmentally friendly whey management techniques, most of which are targeted towards biotechnological and gastronomic applications for the development of goods with added value. Large volumes of whey may be processed into bioethanol, although it is more cost-effective to manufacture fermented or unfermented whey-based drinks with lesser quantities. Thus, sustainable management of whey might assist to the achievement of some of the UN Agenda 2030 of Sustainable Development Goals, more particularly SDG 6: Clean water and sanitation; SDG 9: Industry, innovation, and infrastructure; and SDG 12: Responsible consumption and production. Making whey into useful raw material and then processing it further to create high-value goods may help to limit the number of dangerous compounds released into the environment and so lessen the environmental pollution and climate changes. Additionally, it may dramatically increase safe recycling and reuse globally and cut the volume of untreated wastewater by half. Additionally, resource efficiency would be increased, clean, environmentally friendly industrial practises would be adopted, and there would be a 50% reduction in the quantity of food lost globally in production and supply chains per person (Zandona et al., 2021).

Whey protein is increasingly recognised as a component that adds value to several food items. Whey and whey components are viewed as value-added ingredients in infant formula, sports nutrition snacks and drinks, and other food products. (De Castro et al., 2017; Yadav et al., 2015; Kong et al., 2022). Table 3.2 presents the previously developed food products using whey protein as a sustainable source. Whey proteins are rich in bioactive peptides and essential amino acids. (Yiğit et al., 2023). Due to their physiochemical properties, whey proteins may enhance the texture and sensory qualities of food (Kilara & Vaghela, 2018). Creating unique functional foods using whey proteins might treat various non-communicable illnesses and provide advantages for sensory and textural qualities as well (Keri Marshall, 2004; Batista et al., 2018). As an emulsifier, fat-replacer, gelling, and encapsulating agent, whey proteins are also known to improve the sensory and textural properties of food (Yiğit et al., 2023). They also exhibit functional features that play a significant role in food processing. Additionally, whey protein is used to create edible coatings and films based on whey protein that are then enhanced by the addition of additives and active ingredients, their technological characteristics, and some potential uses in food or could be used as carriers for other bioactive compounds, as depicted in Fig. 3.1. (Yin et al., 2020; Daniloski et al., 2021; Soleimanifar et al., 2021; Kandasamy et al., 2021; Etxabide et al., 2023; Vasiliauskaite et al., 2023; Zhong et al., 2023).

Table 3.2 Whey protein-based products and their key advantages

Whey protein product	Product type	Process	Key findings	References
Milk-like beverage (way-mil)	Create by combining liquid or powdered whey with buttermilk, skim or whole milk, certain vegetable oils, hydrocolloids, and emulsifiers	Emulsification	It has a similar look to milk and a different flavour. It contains around 2–4% milk fat, 1–1.5% proteins, 4–5% lactose, minerals, and water-soluble vitamins	Jeličić et al. (2008)
Flavoured milk	Whey protein concentrate (WPC) amino acids and whey protein hydrolysates (WPH) are added to strawberry and chocolate-flavored milk	Milk produced with strawberry and chocolate flavours and 1–2% WPC/WPHs added before pasteurisation	Up to 42% more antioxidant activity was produced WPH has the potential to be used as a natural bio functional element to enhance the antioxidant capabilities of food products	Mann et al. (2014)
Chocolate	Whey based Milk chocolate	Whey was added to the original milk chocolate to change the intricate microstructure of the chocolate	By lowering the melting peak temperature, thermal and rheological behaviour changes the crystallinity of cocoa fat. a quantifiable drop in hardness that shows the plasticizing action of the whey particles and the transition from brittle to more ductile fracture processes	Lapčíková et al. (2022)
Probiotic milk	Combining pasteurized acid whey with different type of milks	Developed using probiotic cultures *Lactobacillus acidophilus* LA-5 or *Bifidobacterium animalis* ssp. *lactis* BB-12	Throughout the storage period, the bacterial count was more than 8 log cfu/mL, especially higher than the minimal therapeutic dosage	Skryplonek et al. (2019)
Fruit-flavoured beverages	Cheese whey beverage with strawberry concentrate	Fortification	The beverage helps to lower the	Da Silva Miglioranza et al. (2003)

(continued)

Table 3.2 (continued)

Whey protein product	Product type	Process	Key findings	References
	and iron bisglycinate added for flavour		prevalence of anaemia in kids and teenagers	
Sports drinks	Utilizing cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, acid whey (3.32% lactose) was fermented	Fermentation	It helps with mineral replacement following intense exercise	Abella et al. (2016)
Alcoholic whey beverages	Deproteinizing whey, concentration of whey, lactose fermentation (often using *Kluyveromyce sfragilis* and *Saccharomyces lactis* yeast strains), flavouring, sweetening, and bottling	Deproteinizing and fermentation	70% of the dry matter in whey is made up of lactose, which is converted into lactic acid to give the product its refreshingly sour flavour. The remaining 20% ferments to alcohol	Macwan et al. (2016)
Regular whey powder	Powder-based (particle sizes of 150–200 μm)	Spray or roller drying	The performance of those who are physically active is improved by whey-derived bioactive components with antibacterial and antiviral capabilities. These components also increase immunological defence, bone health, and antioxidant activity	Solak and Akin (2012)
Deproteinized whey	Powder-based	Produced by heating whey to temperatures between 70 °C and 80 °C, followed by acidification and the removal of the flocculants by centrifugation, decantation, or filteration	The entire solids of the original whey are included in roughly 90% of deproteinized whey and whey UF permeate	Macwan et al. (2016)
Edible film or coating		WPI self-assemble into nanofibrils	Reduce browning, maintain phenolic	Feng et al. (2018)

(continued)

Table 3.2 (continued)

Whey protein product	Product type	Process	Key findings	References
	WPNF-based edible coatings for apple	(WPNF). WPNF-based edible coatings plasticized with glycerol and trehalose	compounds, maintain weight, be low cost, and be highly biocompatible	
Whey protein concentrate (WPC) based high-protein fat-free dairy desserts	Skimmed-milk powder, various whey protein concentrations, sucrose, and κ—carrageenan	Membrane filteration and ion exchange technology used to make WPC	High-protein, fat-free dairy desserts are made with whey protein, which could replace fat and have positive effects on health	Kusio et al. (2020)
Ricotta-type whey cheese or Whey quark	Cheese	Heat-induced coagulation of whey proteins	Greater affordability, longer shelf life, and enhanced sensory attributes	Jelen (2011)
Norwegian-style whey cheese Mysost	Cheese	Controlled crystallization and then drying	Longer shelf life	Jelen (2011)
Fermented soy whey (FSW) drink	*Cordyceps militaris* SN-18 used for fementation	Fermentation	In addition to removing the oligosaccharides from soy whey, this fermentation boosted the amount of essential amino acids, total phenolic and flavonoid, and isoflavone aglycones	Dai et al. (2021)
Animal feed	Liquid whey for cattle	–	Milk yield was increased slightly when liquid whey was fed to cows. Whey consumption significantly reduced consumption of hay or grain	Schingoethe (1976)
Yogurt	WPC+ starter cultures *(Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*)	Inoculation and incubation	Sensory changes caused by WPC as well as physicochemical changes (acidity, nutritional value, water activity, water-holding capacity, texture, and colour,	Brodziak et al. (2020)

(continued)

Table 3.2 (continued)

Whey protein product	Product type	Process	Key findings	References
			including whitening and yellowing indices)	
Non-fat unflavored cup set-style yogurt	Acidic fresh liquid whey protein concentrate + sweetened skim milk	Homogenization	It had flavour characteristics that were comparable to those of fresh liquid sweet whey protein addition, but it had less gel strength, which led to variances in trained panel texture characteristics and lower customer approval ratings for fat-free yoghurt manufactured with added acid whey protein component	Wherry et al. (2019)
Cookies	Blackcurrant concentrate and WPI were mixed via freeze-drying (FWB) and spray-drying (SWB). The cookie batter included both SWB and FWB	Spray-drying and freeze-drying	A higher protein and lower carbohydrate diet alternative was made possible by combining the protein components with cookies	Wu et al. (2021)

3.6 Conclusion

Environmental concerns have compelled governments to pass laws governing the disposal of whey, which has prompted the dairy sector to look for alternative strategies and prospects for the management of dairy wastes. In the attempt to reduce dairy waste and be in accordance with global initiatives like the Sustainable Development Goals of UN Agenda 2030, the high potential for contamination of whey makes its reuse and recycling a serious scientific concern (UN, 2015). The development of several sustainable whey management solutions as a result of extensive scientific research has changed whey from a byproduct of cheese production to a raw material with added value. In the context of functional foods that promote health, whey proteins have attracted increasing commercial attention. In order to create functional foods and nutraceuticals that operate as active medicinal agents, whey components, especially proteins and peptides, will become more and more popular.

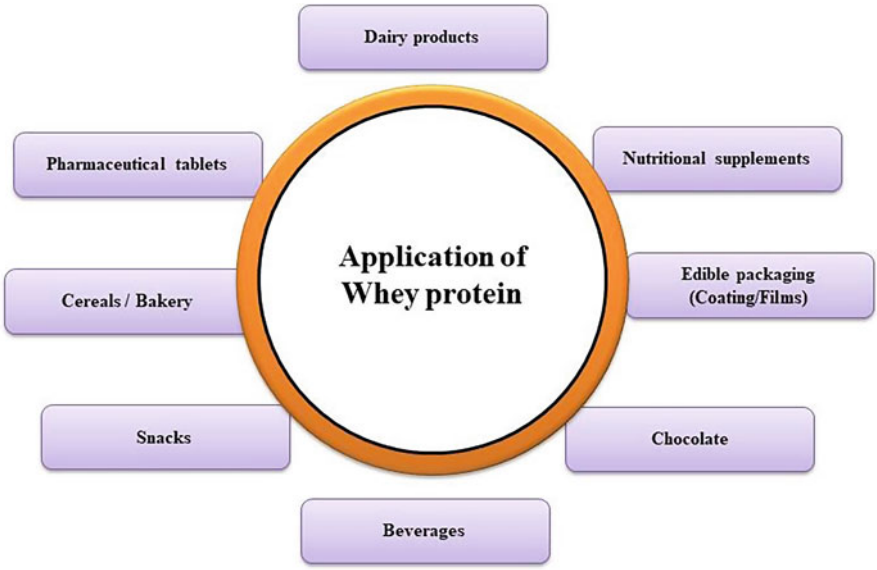

Fig. 3.1 Some potential applications of whey protein in food (information adopted from: Kandasamy et al., 2021, Llamas-Unzueta et al., 2021, John & Ghosh, 2021)

In addition to ongoing research confirming the biological activity of whey components, they capitalized on the strong consumer trends toward health and wellbeing. The extensive use of whey proteins as functional food components, nutraceuticals, and nutritional supplements will certainly lead to significant breakthroughs in the food and healthcare industries.

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Chapter 4
Green Technologies for Treatment and Utilization of Whey Towards Sustainable Exploitation

Maricarmen Iñiguez-Moreno and Amrita Poonia

Abstract Whey is a byproduct produced in high amounts in the non-dairy and dairy industries. It can turn into a significant contaminant in a short time if disposed of untreated in the environment. Whey is an important source of nutrients, including carbohydrates, proteins, isoflavones, and micronutrients. Whey valorization using various green technologies can improve the management of this agricultural waste and lead to the economic and efficient production of value-added products. Whey valorization is carried out through physical, enzymatic, and microbial processes that facilitate its use to release compounds such as isoflavone aglycones, polysaccharides, prebiotics, organic acids, bioactive peptides, and bacteriocins. Bioprocesses enable the large-scale manufacture of value enzymes such as β-galactosidase, protease, and amylase. Whey can be utilized to improve the functional and nutritional value of meals, as well as to create unique functional products with health-promoting properties. Whey waste may thus be effectively utilized not only by returning it to the food chain. It also acts as a paradigm for the evolution of sustainable technologies that offer a dependable option for addressing one of the world's most serious issues. To illustrate the importance of green utilization of whey, industrial applications are presented at the end of this chapter.

Keywords Emerging technologies · Microbial activity · Industrial applications · Green technologies · Whey valorization

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4.1 Introduction

As the world's population grows, so does the need for food, particularly protein goods. The world's population is expected to grow by 2 billion during the next 30 years, from 7.7 billion now to 9.7 billion in 2050, and might reach over 11 billion by 2100 (United Nations, 2019). Dairy products account for a large portion of the food produced and consumed globally daily (Chourasia et al., 2022). World milk output in 2022 is estimated to be over 930 million tons, up 0.6% from 2021, owing mostly to volume increases in Asia and a modest gain in Central America and the Caribbean, offset by a considerable fall in Europe. Cheese exports reached 3.5 million tons (FAO, 2022).

Whey is a food industry by-product that accounts for 90% of the liquid by-product of milk disposed of during the process (Kaur et al., 2020), with the global output of fresh whey reaching 183 million tons of this waste in 2020 (Valdez Castillo et al., 2020). The two most frequent forms of whey are acid and sweet whey. Acid whey is the result of the creation of cottage cheese or Greek yogurt, whereas sweet whey is a consequence of the production of hard and semi-hard cheeses (Rocha-Mendoza et al., 2021; Zandona et al., 2021). The content of carbohydrates, fats, and proteins constitutes the main organic fraction of whey (Macwan et al., 2016). Improper management of this waste contributes to the eutrophication of water bodies and the acidification of soils due to its high lactose content (44–46%). These changes have a severe impact on aquatic life, food production, and human health, and they constitute a significant danger to the world economy. The composition of whey, including lactose concentration, depends on the conditions used in cheese production (Kaur et al., 2020). On the other hand, soy whey is a nutrient-rich byproduct produced during the production of tofu, soy cheese, and soy protein isolates (Chua et al., 2018). Soy whey contains proteins, carbs (sucrose, raffinose, and stachyose), and polyphenolic substances (isoflavones) that can be enzymatically transformed into bioactive compounds with health benefits (Jadaun et al., 2019). Moreover, soy protein has bioactive peptides that can be activated after intake by hydrolysis by digestive enzymes (Chourasia et al., 2022).

The vast volumes of untreated whey that are thrown, as well as the pollution created by these milk leftovers, have prompted governments all over the globe to encourage enterprises to focus on the clean production of products and services (Osorio-González et al., 2022). Furthermore, due to the high nutritional content of whey, which also includes vitamins and isoflavones, and its health advantages, roughly 55% of the nutritional value of milk is recycled for the development of value-added products in the food and chemical sectors (Panghal et al., 2018). As a result, whey disposal represents a large loss of potential nutrients and energy. Whey has unique nutritional value and hence must be processed judiciously into the edible as well as non-edible value added products. The disposal of these by-products has now become a challenge and danger to the environment (Poonia, 2020). To harness the nutritional value of whey while minimizing the environmental impact disposal, it is critical to driving whey management toward cost-effective and sustainable

recovery and development of new valued products (Macwan et al., 2016). In this context, several environmentally friendly technologies, including ultrafiltration, fermentation, and enzymatic processes, have been tested for whey recovery (Osorio-González et al., 2022).

In recent decades, potential uses for whey have been explored that are more cost-effective and environmentally friendly, especially to convert unwanted end products into valuable raw materials. The production of value-added products such as whey powder, whey proteins, functional foods and beverages, edible films and coatings, lactic acid and other biochemicals, bioplastics, biofuels, and other comparably important bioproducts is the focus of sustainable whey management (Macwan et al., 2016; Zandona et al., 2021).

4.2 Emerging Technologies for Whey Obtention

Researchers are focused on the use of residues to obtain biopolymers for the elaboration of food packaging aimed at the replacement of synthetic (Ranganathan et al., 2020). Whey protein has many applications in the food, cosmetic, and pharmaceutical industries due to its chemical and mechanical properties, some of them include the elaboration of functional composite nanofibers (Drosou et al., 2018), films (Feng et al., 2018), and coatings (Tavakolipour et al., 2020). In addition to this, the demand for creating new food and drink products or reformulating existing ones with improved nutritional value has increased in recent years. The growing interest in adding natural products with bioactive ingredients to foods, to provide a positive impact on one or more physiological processes when consumed is becoming popular in the food market. In this sense, whey proteins and whey protein hydrolysates have wide applications in the protection and stability of bioactive compounds from natural sources (Feng et al., 2018; Tavakolipour et al., 2020). Cheese waste and soy products are important sources for the production of biopolymers by direct extraction or fermentation. Numerous alternative processing techniques for whey protein extraction have been extensively tested. In general, there are three ways to extract or obtain whey byproducts, including direct extraction from biomass, microbial fermentation, and the enzymatic process (Cermeño et al., 2020). Some of the most common methods are reviewed in the next sections.

4.2.1 Physical Methods

Physical separation procedures are appropriate for removing suspended solids from effluents or separating solid particles from mixtures. Being separation and concentration the most important operations units in the food processing environment (Pouliot et al., 2014). Physical technologies, particularly membrane technology, are used in the dairy industry to concentrate and separate milk and milk

by-product components, yielding products of added economic value. Indeed, whey processing is the most common application of membrane technology in the dairy sector, accounting for more than 75% of all membrane applications (Pouliot et al., 2014; Cassano & Conidi, 2017).

4.2.1.1 Micro-, Ultra- and Nanofiltration

Currently, technologies such as microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and diafiltration are worldwide renowned membrane technologies that, in terms of uniformity, economy, and standard operation, have potential uses in the food business. Whey separation favors the recovery of protein and lactose-rich concentrates that have high economic value in the market (Yadav et al., 2022). Microfiltration enables the separation of particles with molecular weights greater than 200,000 Da, whereas ultrafiltration, which uses membranes with a pore size of 1–100 nm, allows the selection of macromolecules weighing 1×10^3–2×10^5 Da. Nanofiltration, on the other hand, separates low molecular weight substances (200 Da and 1000 Da) from bigger molecules. Tiny ionized molecules, such as dissolved mineral salts, are eliminated at an inversely proportional rate to their valence. Water, lactose, soluble minerals, non-protein nitrogen, and water-soluble vitamins are all present in the resultant permeate, also known as an ultrafiltrate or nanofiltrate. In contrast, retentate comprises proteins, lipids, and colloidal salts (Mistry & Maubois, 2017).

Nano- and ultrafiltration are widely known membrane technologies that may be employed in the food sector in a consistent, cost-effective, and consistent manner. Both are of particular interest to the food industry due to their lower pressure requirements compared to the reverse osmosis process (Mistry & Maubois, 2017; Yadav et al., 2022). The dairy sector has increased its usage of nanofiltration technology because it promotes the development of consistent food products. It is commonly employed in whey processing (Alfano et al., 2022; Yadav et al., 2022), lactose recovery (Esfandian et al., 2019), whey demineralization or desalination (Myronchuk et al., 2018, 2019), and lactic acid separation (Alexandri et al., 2018; Talebi et al., 2020). A nanofiltration membrane has been used effectively to concentrate and demineralize liquid whey to produce alternative whey products such as whey powder. For example, an integrated downstream process for cheese whey fractionation employing micro-, ultra-, and nanofiltration. The 10 kDa permeate was focused using nanofiltration membranes in stages. The fundamental issue in the application of membranes in the food sector, however, is the development of cost-effective technology, as well as highly efficient and long-lasting membranes (Alfano et al., 2022; Yadav et al., 2022). Because of their high mineral and organic acid content, these sites are of special relevance in whey processing. Pre-desalination of whey appears to be the best method for dealing with the problem of greater mineral content and higher organic acid content. The dairy sector employs electrical and pressured technologies for preliminary whey desalination, such as electrodialysis, ion exchange, ultrafiltration, nanofiltration, dia-nanofiltration, or the combination of

two processes (Talebi et al., 2020; Alfano et al., 2022). Ozone pretreatment of waste from a cheese process increased the desalination rate by electrodialysis and nanofiltration, up to 80% was removed in 5 h and 40 min, respectively (Myronchuk et al., 2018).

4.2.1.2 Ultrasound-Assisted Extraction (UAE)

UAE, also known as ultrasonication if the extraction is carried out in an aqueous media, uses powerful shock waves to produce microbubbles in the liquid medium. These structures violently expand and collapse; this process is known as cavitation, and it creates high-energy shock waves that damage molecules. Processing time, temperature, and intensity are the most important factors affecting the process of hydrolysis recovery by this technique (Costello et al., 2021; Huu et al., 2021). However, the use of ultrasound (US) on a large scale is complicated because it requires a high-energy input (Soto-Sierra et al., 2018). Also, certain reactive hydroxyl radicals, such as H^+ and OH^-, may be produced during the process and react with hydrolysates. As a result, specific molecules (for example, nitrogen) need to be introduced to the medium to minimize oxidative free radical damage (Show et al., 2015; Zupanc et al., 2019). Cavitation (20 kHz) causes whey protein to unfurl. This approach also changes the secondary structure of proteins by reducing the number of—helices and increasing the number of—sheets and—turn structures (Ozuna et al., 2015; Figueroa Pires et al., 2021). In addition, the US helps to homogenize the particle size and increase the solubility of the treated whey protein. Moreover, the thermal stability of the treated samples increases sixfold due to the homogenization of the secondary structure of the hydrolysates (Khatkar et al., 2018).

4.2.1.3 Microwave Assisted Extraction (MAE)

MAE is based on irradiation with microwaves (2450 MHz), which directly activates most molecules that have dipole rotation or ionic conductivity. The interactions between the material and the electromagnetic field cause a rapid temperature rise. The disruption of H-bonds and migration of solute ions increase solvent permeation into the biomass, facilitating specific compound extraction (Cermeño et al., 2020). In recent years, MAE has been widely employed as a strong tool for quick and efficient chemical synthesis (Sameut et al., 2020) and natural product extraction (Espada-Bellido et al., 2019; Vázquez-González et al., 2020). This technology has replaced traditional heating with three-dimensional heating of the reaction mass and may complete the process in minutes rather than hours or even days as in traditional methods (Gala et al., 2020). The parameters used in hydrolysis, pH, temperature, time, and solvent, affect the peptide structure (Mellinas et al., 2020). The effects of US (400 W), MAE (75 °C/15 min), and US coupled with microwave heating on the structural, physicochemical, and functional characterization of transglutaminase-induced WPI were investigated. MAE had the greatest impact on the structural

and functional features of TGase-induced WPI. The peptides' zeta potential, emulsion stability, and foam stability were all enhanced by 7.8%, 59.27%, and 28.95%, respectively. When compared to ultrasound or the combination of ultrasound and microwave hydrolysates, MAE enhanced the reaction of WPI with TGase and improved its functional characteristics (Zhang et al., 2022).

4.2.2 Enzymatic Treatments

Whey protein hydrolysates have been used to improve the properties of foods and beverages by modifying their solubility, viscosity, emulsification, and foaming capabilities (Brandelli et al., 2015). Whey byproducts have received a special interest in sports medicine. In terms of skeletal muscle protein anabolism, protein hydrolysates comprising mostly di- and tripeptides have been shown to outperform intact (whole) proteins and free amino acids (Manninen, 2009). Enzymatic bioprocesses may be used to treat whey and create nutraceuticals including bioactive peptides, prebiotics, exopolysaccharides, organic acids, bacteriocins, and isoflavone aglycones, as well as industrially essential enzymes like galactosidase, protease, and amylase. (Chourasia et al., 2022).

Enzymatic hydrolysis is the most frequent method for whey protein hydrolysis. The functionality and active characteristics of these hydrolysates are affected by various parameters, including enzyme type, pH, temperature, time, and enzyme/substrate ratio (Ballatore et al., 2020; Sakkas et al., 2022). For the hydrolysis of whey proteins, enzymes from microbial, plant, and animal sources have been utilized (Sáez et al., 2019; Chourasia et al., 2020; Kaushal et al., 2021). Enzymatic hydrolysis is often carried out under relatively moderate circumstances (temperature 20–70 °C, pH 6.0–8.0) (Sáez et al., 2019). Some of the enzymes that can convert whey into bioactive molecules with beneficial qualities include dextransucrase, levansucrase, glucosidase, and galactosidase (Chourasia et al., 2020; Kaushal et al., 2021). Microbial proteases are the focus of protein hydrolysate production at the industrial level. Biocatalysts for the production of protein hydrolysates at the industrial level are very interested in microbial proteases. Commercial proteases of microbial origin have been used to produce hydrolysates from whey proteins with great success. A commercial enzyme frequently used for protein breakdown is trypsin. Due to its superiority over other proteolytic enzymes, which includes its higher activity, increased cleavage specificity, and excellent stability under various processing conditions (Andriamihaja et al., 2013; Brandelli et al., 2015).

Trypsin was used to hydrolyze WPC 35 (processing conditions 4.31 h, 41.1 °C, and 0.017 enzyme/substrate ratio). Peptides with molecular weights less than 3 kDa showed strong antioxidant activity and were more efficient at HO-radical scavenging than peptides with molecular weights more than 3 kDa. Because of its strong reactivity with biological systems, this radical is extremely important. Moreover, both peptides >3 kDa and ≤ 3 kDa demonstrated significant cytoprotection against oxidative stress caused by menadione (48% decrease cell viability) in IEC-18 cells

(Ballatore et al., 2020). Camel whey protein hydrolysates were created using the enzymes pepsin, trypsin, and chymotrypsin. The chymotrypsin-derived peptides have greater DPPH and ABTS radical scavenging activities. Proline, tryptophan, histidine, and tyrosine are among the hydrophobic amino acids that impart antioxidant activity to whey-derived bioactive peptides (Feng et al., 2018; Ballatore et al., 2020). Compared with unhydrolyzed protein, trypsin hydrolysates showed higher metal chelate activity. Pepsin-based products, on the other hand, showed greater lowering power in FRAP activity. In terms of antibacterial action, hydrolysates inhibited bacterial growth substantially more than unhydrolyzed whey protein (Kamal et al., 2022). Furthermore, because whey proteins are widely regarded as safe (GRAS), they may be used to make dietary supplements and as bioactive components in functional meals. Bioactive peptides with antioxidant, antihypertensive, immunomodulatory, and antibacterial activities were produced when whey proteins were hydrolyzed by commercial proteases (Zhao & Ashaolu, 2020; Bustamante et al., 2021).

4.2.3 Microbial Activity

Generally, whey is either discarded into the environment or utilized as animal feed. Whey, on the other hand, is a viable source for generating a variety of value-added products due to its high concentration of lactose, functional proteins, peptides, and other nutrients. Lactose is a disaccharide made up of galactose and glucose that are joined by a -1,4-glycosidic bond (Zandona et al., 2021). Whey has been used in a variety of ways, including microbial conversion. Lactose acts as a carbon source for microbial growth, making this feasible. As a result, whey has the potential to be employed as a substrate for the production of a wide range of value-added products via various microbial routes (Kaur et al., 2020; Chourasia et al., 2022).

4.2.3.1 Microbial Fermentation

Fermentation techniques to convert whey into value-added products have emerged as a viable path for biorefinery development and nutraceutical food manufacturing. Whey can be bio-transformed using biotechnological methods such as microbial fermentation, mainly by lactic acid bacteria (LAB) and yeasts (Chua et al., 2018; Chourasia et al., 2021). Whey can be valorized with proteolytic LAB strains to produce bioactive compounds and functional foods (Kaur et al., 2020). In addition to this, microorganisms such as yeasts, *Bacillus* spp., fungi, and algae can be utilized to ferment whey to create high-value chemicals for the food sector (Chua et al., 2018; Valdez Castillo et al., 2020; Chourasia et al., 2022). LAB and yeasts are commonly employed due to their strong proteolytic activity and capacity to multiply in acidic settings (Daliri et al., 2018; Chourasia et al., 2021).

LAB is one of the most essential bacteria for the generation of bioactive peptides due to its GRAS classification and effective proteolytic system (Chourasia et al., 2021; Guo et al., 2023). LAB Cell membrane proteases hydrolyze whey proteins to generate extracellular oligopeptides that, when consumed, can have biological benefits and add value to fermented foods. These oligopeptides are taken in by active transporters in LAB cells and degraded into smaller peptides and free amino acids by intracellular endopeptidases (Daliri et al., 2018; Chourasia et al., 2021). As a result, LAB strains may ferment whey proteins to produce highly concentrated). As a result, LAB strains ferment whey proteins to produce highly bioactive peptides that aid in the absorption of essential amino acids (Guo et al., 2023). As a result, *Pediococcus acidilactici* SDL1414 peptides generated from whey might be employed to make functional meals for hypertension or refined and used as dietary supplements to treat hypertension. This is because these peptides inhibited 84.7 ± 0.67% of angiotensin-converting enzyme activity (Daliri et al., 2018).

Whey is a typical substrate for single-cell protein (SCP) synthesis due to the presence of lactose (Suman et al., 2017). Because of its quick growth on this substrate, yeast is the most commonly utilized microbe in the biotransformation of whey into single-cell products (Kaur et al., 2020) GRAS *Kluveromyces*, *Candida*, and *Saccharomyces* yeasts have created biomass from whey and whey permeate. Yeast-derived SCP includes B-complex vitamins, dietary fiber from—glucans, decreased levels of low-density lipoprotein cholesterol, and beneficial changes in blood glucose and insulin levels, in addition to a protein composition of 30–50% (Ritala et al., 2017; Suman et al., 2017). *Kluyveromyces marxianus* biomass has been shown to have an 83.33% crude protein content after 24 h of whey fermentation (Nayeem et al., 2017). This suggests that whey is a low-cost feedstock for the manufacture of SCP utilizing yeast (Nayeem et al., 2017; Ritala et al., 2017). Moreover, several GRAS yeasts, such as *K. marxianus* and *Saccharomyces cerevisiae*, have demonstrated superior milk protein proteolysis to LAB, resulting in bioactive peptides and essential amino acids (Zhang et al., 2017). Whey fermentation by proteolytic LAB and yeast cultures may result in the production of probiotic functional foods loaded with bioactive peptides or be an efficient and dependable source of proteins for human consumption.

4.2.3.2 Dark and Photo-Fermentation

Photo-fermentation is an anaerobic fermentation process carried out by photoheterotrophic bacteria capable of creating biohydrogen and carbon dioxide from organic acids in the presence of light. Dark fermentation, on the other hand, is carried out under anaerobic circumstances by strictly anaerobic or facultatively anaerobic bacteria. Hydrogen (H_2) produced biologically from renewable sources (biomass, wastewater, organic waste, etc.) is referred to as biohydrogen (Pankaj & Singh, 2016; Łukajtis et al., 2018). The dark fermentative *Enterobacter aerogenes* MTCC 2822 and the photo fermentative *Rhodopseudomonas* BHU 01 produced biohydrogen by combining dark and photo-fermentation using cheese whey as

substrate, yielding 3.40 mol/mol and 5.88 mol/mol of lactose each step, respectively. This suggests that two-step fermentative H_2 generation has a better conversion efficiency, which may contribute to a reduction in organic load in wastewater (Rai et al., 2012). The creation of biohydrogen from agricultural waste has been identified as a promising step toward bioremediation, green energy production, and a circular economy approach (Łukajtis et al., 2018).

4.3 Industrial Applications of Whey Obtained by Green Technologies

The simultaneous integration of numerous operational units into one process, lowering the environmental effect of whey, is a financially and environmentally viable alternative to the use of whey for the manufacturing of more valuable items. Whey supplemented with bioactive compounds can be utilized to increase the nutritional content and functionality of meals, as well as to develop novel functional foods with health benefits (Fig. 4.1) (Figueroa Pires et al., 2021; Chourasia et al., 2022). Whey wastes were used to develop various products (Table 4.1), including *Lactobacillus plantarum* MTCC 5690 and *Kluyveromyces lactis* NCDC 257, which were proposed to ferment fresh paneer whey and obtain a naturally carbonated probiotic beverage (Kadyan et al., 2021). Similarly, *Lactobacillus* spp., *S. cerevisiae*, and *Pichia membranifaciens* have been used for the biotransformation of soy whey into kefir with antioxidant properties and inhibitory activity of angiotensin-I converting

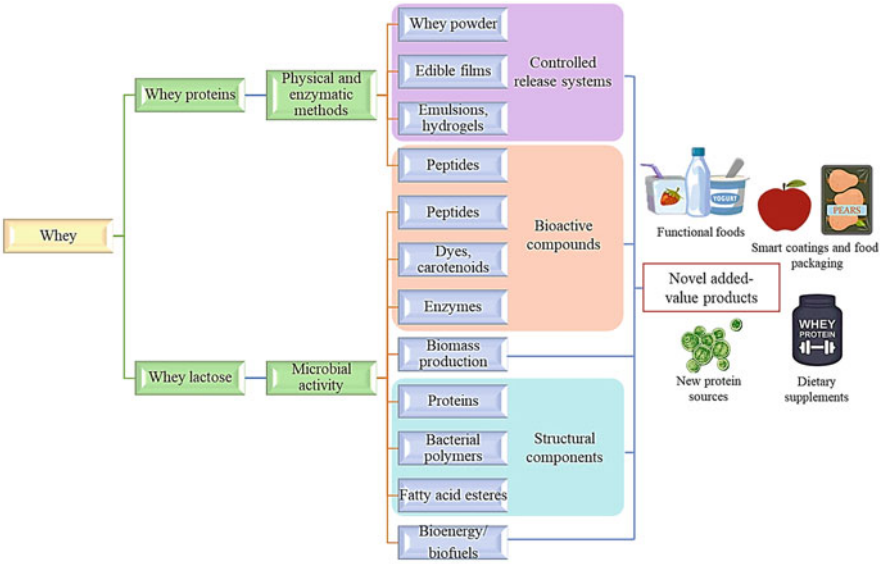

Fig. 4.1 Transformation of whey waste in added-value products by emerging technologies

Table 4.1 Added-value products obtained from whey waste

Source	Extraction technique/ microorganism	Product obtained	References
Goat cheese whey	Filtration, centrifugation, ultrafiltration and nanofiltration	Lactose	Macedo et al. (2021)
Cheese whey	Freeze concentration	Whey protein and lactose	Uald Lamkaddam et al. (2023)
Sweet whey	Fermentation, ultrafiltration-diafiltration, ultrasound pretreatment and transglutaminase crosslinking	Whey protein concentrate	Gantumur et al. (2023)
Cheese whey	*Lacticaseibacillus casei* BL23	Lactic acid	Catone et al. (2021)
Cheese whey and microalgal biomass	*Lactobacillus plantarum*	Lactic acid	Nagarajan et al. (2020)
Tapioca starch, sugar from beet molasses and bread, alfalfa press green juice, and acid whey	*Bacillus coagulans* A534	L-lactic acid	Olszewska-Widdrat et al. (2020)
Whey permeate	*Lactobacillus delbrueckii* and *engineered Lactococcus lactis*	D-lactic acid	Sahoo and Jayaraman (2019)
Cheese whey	Membrane filtration and fermentation with *Acetobacter aceti*	Acetic acid and whey protein	Nayak and Chakrabortty (2023)
Istrian albumin cheese whey	*Kluyveromyces marxianus subsp. marxianus*	Whey distillate	Bendelja Ljoljić et al. (2023)
Ricotta cheese exhausted whey	Membrane filtration and fermentation with *Haloferax mediterranei* DSM1411	Poly (3-hydroxybutyrate-co-3-hydroxyvalerate)	Raho et al. (2020)
Cheese whey	A mixed photosynthetic consortium of bacteria and algae	Polyhydroxyalkanoates with a hydroxyvalerate	Fradinho et al. (2019)
Cheese whey permeate	*Kluyveromyces lactis* CBS2359	Bioethanol	Sampaio et al. (2020)
Cheese whey	*Saccharomyces cerevisiae*	Bioethanol	Beniwal et al. (2021)
Mozzarella cheese whey and sugarcane molasses	*Candida tropicalis* and *Blastoschizomyces capitatus*	Bioethanol	Balia et al., (2018)
Cheese whey	β-Galactosidase then *Saccharomyces*	Bioethanol and galactonic acid	Zhou et al. (2019)

(continued)

Table 4.1 (continued)

Source	Extraction technique/ microorganism	Product obtained	References
	cerevisiae and *Gluconobacter oxydans*		
Cheese whey permeate	*Escherichia coli* BL21	Recombinant proteins	de Divitiis et al. (2023)

enzyme (Azi et al., 2021). Another increasing alternative to the use of whey is the production of kombucha, which has antioxidant properties and antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* (Vohra et al., 2019).

Nowadays, whey is mainly used in the industrial production of lactose, dry whey, and whey paste (Israni et al., 2020; Figueroa Pires et al., 2021). The global whey protein market was valued at US $5.33 billion in 2021 and is expected to grow at a Compound Annual Growth Rate of 10.48% from 2022 to 2030. The industrial value of whey is related to the high content and quality of protein used in the production of protein supplements (Grand View Research, 2020). Spray drying is the most commonly used method for obtaining a powder from liquid whey on an industrial scale, accounting for 70% of annual processing, and is the production of whey powders. Whey clarification, cream separation, pasteurization, evaporation of total solids (40–60%), lactose crystallization, and whey drying are the typical steps in whey powder production. If lactose crystallization is not carried out, the solid mass of the resulting powder is only suitable for animal feed as an inexpensive source of high-quality proteins and carbohydrates. Spray-drying, on the other hand, needs significant financial investment to acquire the necessary equipment and uses a significant amount of energy during the process, whereas whey powders have a relatively modest selling price that increases depending on the protein concentration (Figueroa Pires et al., 2021; Chourasia et al., 2022).

Depending on the protein concentration, the powder is referred to as whey protein concentrate (25–80%) or whey protein isolate (greater than 90% protein by dry weight) (Morr & Ha, 1993). Spray drying was also shown to be a viable way of recovering solid whey proteins from fresh Paneer whey. The optimum conditions established were a 1350 rpm aspirator rate, 1.5 mL/min feed flow rate, and 3 bar air pressure, yielding 0.50 g. The researchers enhanced the yield (0.98 g) by using an ultrasonic nozzle at 40 Hz, owing to excellent cake layer removal and fouling reduction based on the physical effects of cavitation (Prabhuzantye et al., 2019). The generation of bioactive peptides is one of the benefits of using whey protein. Its breakdown with trypsin yields antibacterial peptides against *Listeria monocytogenes* and *Staphylococcus aureus* (MIC 10–20 mg/mL) (Demers-Mathieu et al., 2013).

Due to their high nutritional value, whey powder products are used throughout the food industry; however, the most common use is as an additive in the creation of a wide variety of foods and beverages, including infant formula, meat products, baked goods, dairy products, cereals, beverages, soups, sauces, toppings, chocolates,

creamers, nut coatings, pressed nuts, cheese-based sauces, potato chips, savory flavors and pastries, special bakery products like pizza, and biscuits (Grand View Research, 2020; Zandona et al., 2021). When whey powder is added to recipes, it can improve the sensory characteristics and physical features of the dish (foaming or acid stability) (Díaz-Ramírez et al., 2016; Zhang et al., 2022). The use of these products is growing as a result of the Covid epidemic and increased awareness of the need of living a healthy lifestyle (Grand View Research, 2020). In addition to boosting the value of the by-product, the development of such functional meals will help to lower whey treatment costs and effluent volume (Chourasia et al., 2022). Technological advancements and the development of new cleaner technologies led to the exploration of alternative methods to turn whey into essential value-added goods, allowing the reinsertion of this waste into the value chain.

The production of exopolysaccharides (EPS) by microbial fermentation is a growing alternative for the use of dairy residues. Microbial fermentation of whey lactose can lead to the synthesis of EPS such as xanthan gum and alginate, which are useful in a variety of industries including food, agriculture, pharmaceuticals, and medicine (Efremenko et al., 2022). Lactose-rich whey is used as a low-cost substrate for LAB cultures to produce EPS. LAB in recent years, many cultures, including Lactobacillus spp., Lactiplantibacillus spp., Streptococcus spp., and Lactococcus spp., have used whey to produce EPS (Efremenko et al., 2022; Meruvu & Harsa, 2022). The interest in replacing synthetic plastics and chemical compounds leads scientists and industry to constantly search for natural polymers to develop environmentally friendly packaging and coatings. Since whey is an important source of protein, many studies have evaluated its use for this proposal (Feng et al., 2018; Avramescu et al., 2020; Tavakolipour et al., 2020). Edible or biodegradable films are a practical way to extend the shelf life of foods and improve their quality while reducing environmental impact. These films, in addition to acting as selective barriers for moisture and gas migration, may also serve as carriers for a variety of functional components. Antioxidants, antibacterial agents, flavors, spices, and colorants are examples of such substances that increase the functioning of packaging materials by adding innovative or additional capabilities (Schmid & Müller, 2019). Nanoemulsions obtained by the US containing thyme essential oil, whey protein, and polysorbate were used as coatings for zucchini preservation. Coatings reduced water loss, higher firmness, better sensorial profile, and better peel in comparison to controls. This result was attributable to the coating acting as a dynamic membrane allowing plant breathing and reducing reactive oxygen species occurrence in the peel increasing the shelf-life until 200% at 10 ± 1 °C and 75 ± 1% relative humidity storage conditions (Bleoanca et al., 2022). Also, it has been used for the development of bioplastics, usually in combination with other natural compounds. Eco-friendly bioplastic sheets were fabricated by the compression molding method using whey protein isolate and potato four. The obtained bioplastic had increased twice the tensile strength and three times elongation at break, and better damping properties compared with the control bioplastic (potato flour). Furthermore, the blended bioplastic had a lower glass transition temperature than the control, indicating a reduction in energy usage throughout the bioplastic formation process

(Omrani-Fard et al., 2020). Another recent and particular application of whey wastes is aimed to reduce the cost production of recombinant proteins, being possible of using them as an effective and low-cost alternative inducer for the synthesis of those important products (de Divitiis et al., 2023). Whey protein can be reinserted into the value chain by the use of one or more unit operations resulting in added-value products mitigating environmental impact (Table 4.1).

4.4 Conclusions

Environmental issues and the decline of sustainable protein sources have forced governments, scientists, and the industry sector to address the appropriate disposal of whey and work on alternatives. As a result, encouraging approaches and opportunities for appropriate waste management have been explored. The reuse and recycling of whey have become a major issue in waste reduction due to its high pollutant content. Several initiatives have led to the development of various sustainable strategies for managing whey, changing the perception of whey from waste to a valuable resource. Whey ingredients have high nutritional, functional, and therapeutic value, making them an excellent basis for improving the quality of commodities or developing new products such as beverages, fermented, probiotic, alcoholic, and feed products. Whey production offers a variety of benefits in terms of quality, energy savings, and reduction of water consumption and environmental impact. This chapter focuses on sustainable whey extraction technologies and their applications to reintroduce this by-product into the value chain, particularly in the developing emerging fields like biochemicals, biofuels, and bioplastics.

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Chapter 5
Whey: A Potential Source of Bacterial Cellulose and Xanthan Gum

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Abstract Expeditious growth of dairy industry comes along with large quantities of by-products including whey, to be processed. Whey is the greenish translucent liquid fraction that remains following the manufacture of cheese, *chhana*, *paneer* and casein. It is recognized as the major by-product of the dairy industry with the production figure of 165 million tons per year. Whey is classified into two categories based on the method of milk coagulation i.e., sweet and acid whey. Sweet whey is a result of enzymatic milk coagulation by rennet (pH 5–6) while, acid whey is the result of milk coagulation by mineral acid (max. pH 5.1). Earlier whey was considered as a waste by the dairy industry and its disposal was a significant problem owing to the environmental regulations. However, being rich in nutritional components, discarding of whey brings significant loss of potential nutrients along with high biological oxygen demand of 30,000–50,000 mg/L and chemical oxygen demand of 60,000–80,000 mg/L. Furthermore, the lactose content of whey is higher as compared to protein content, thus, paving the way for utilization of whey as a source of lactose for a variety of applications. One such application lies in the synthesis of bacterial polymers including cellulose and xanthan gum. Recently, the main focus has also been shifted to the development of biodegradable environmental friendly polymers as an alternate to synthetic fossil derived plastics with diverse applications in food, medical science and technology areas. However, high cost of carbon source constitutes the main limitation for the bulk production of microbial-synthesized polymers. Furthermore, the limitation diverted the attention of researchers towards food grade industrial by-products and wastes. Whey, being the significant by-product of dairy industry with potential limitations including difficulty in its disposal. It has been demonstrated that almost 1 L of whey can be utilized to produce

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50 tons of lactose, which can further act as main carbon source. Lactose obtained from whey can not only serve as substrate in biotechnological production of polymers but also save the environment from harmful effects of plastic in long run. Additionally, cheese whey has also been explored as a carbon source for the xanthan gum production. To date, several studies aimed at evaluating xanthan gum production on whey substrates using gram negative bacteria *Xanthomonas* (mainly *X. campestris*pv. *campestris*) have been reported. Bacterial cellulose, a homopolysacchride biopolymer, has also been produced using wheyas an alternative growth medium; however limited studies have been reported. Processing interventions and suitable approaches including use of recombined bacterial strains, pretreatment of whey (hydrolysis of lactose) have also been used to enhance the yield of polymer with whey as a substrate. Further, extensive research is required to provide the clear perspective for industrial scale production of biopolymers by the valorization of whey.

Keywords Whey utilization · Biopolymers · Xanthan gum · Cellulose production · Cheese whey

5.1 Introduction

Dairy sector in India holds an incomparable space in country with highest milk production in the world. India is agriculture based country where dairy industry prepares the base of source of income and employment in rural areas. India secures first position in milk production contributing 23% of world milk production followed by the United States of America, China, Pakistan and Brazil. India shows a compound annual growth rate of 6.2% with 209.96 mn tones milk production (NDDB, 2021) in 2020–2021 in comparison to 146.31 mn tones in 2014–2015 due to advancement of technology, proper nutrition and proper management practices. The per capita availability of milk has also increased from 176 g/day in 1990s to 427 g/day in 2021–2022 and it is estimated to increase to 592 g/day by 2023–2024. Out of total milk production, 45% of the milk production is contributed by Indigenous/Non-Descript Buffaloes followed by 28% by crossbred cows, 3% by exotic cows and 3% by goat respectively (DAHD, 2021). As per 20th livestock census, total livestock population in India includes 192.49 million cattle, 109.45 million buffalo, 72.46 million sheep, 148.88 million goats, 9.06 million pigs and 851.81 poultry respectively (DAHD, 2021). Dairy sector in India has contributed about Rs. 14,899.8 Billion in 2022 and is expected to earn about Rs. 31,185.7 Billion by 2028 with 13.2% growth rate (CAGR) during 2023–2028. Traditional dairy products make large portion of total production to be consumed by Indian consumers, The consumptions pattern of dairy products in India is chiefly skewed towards traditional products; however, western dairy products are also gaining popularity in the metro cities now with technology advancement and consumer awareness towards nutrients. The percentage consumption pattern in India shows that 45.7% of total milk output is consumed as liquid milk, whereas 39%, 6.9%, 6.5%, 3.7%, 1.9%, 0.6% and

remaining 0.5% of total milk production is converted into ghee and butter; dahi; khoa and sweets; milk powder including infant milk food; paneer, chhana and cheese; ice cream and kulfi; and others dairy products respectively. Different types of dairy byproducts are also obtained during the production of primary dairy products which normally contain higher nutrient content with great potential to cause environmental and health hazard. Therefore, it becomes very much necessary to utilize these byproducts in to prevent hazards and to fetch better returns. These dairy products are utilized for edible, pharmaceutical and industrial purposes by primary and sometimes secondary processing. They can improve the economics of dairy products to many folds and can also be a potent source of nutrients to fight against hunger and malnutrition.

5.2 Whey as Potent Dairy Product

Whey is such an important dairy products, obtained during preparation of cheese, casein and paneer. It is actually the yellow-green watery serum that separates from the curd during conventional cheese or paneer making. It contains approximately 93% water and 7% solid content, out of which lactose, minerals and whey proteins makes around 70–72%, 12–15% and 8–10% respectively. The chemical oxygen demand (COD) of cheese whey ranges between 50 g/L and 80 g/L, while biochemical oxygen demand (BOD) varies from 40 g/L to 60 g/L (Chatzipaschali & Stamatis, 2012). According to the nature and acidity of whey, it is classified into sweet and acid whey. Sweet whey is usually obtained during cheese or industrial rennet coagulated casein production with a pH range between 6.0 and 6.5, whereas acid whey with pH $<$ 5.0 is produced during casein coagulation by fermentation or addition of organic or mineral acids. The normal composition of both types of whey is given in Table 5.1.

Whey is a potent effluent with higher major organic nutrient content which requires an excellent focused management for development of biological treatments without valorization; biological treatments with valorization; physicochemical treatments and direct land application. Past studies have been mostly oriented for sustainable whey management with recent processing techniques for preparation of whey powders, whey proteins, biodegradable films, edible coatings, lactic acid,

Table 5.1 Composition of different types of whey

Components	Sweet whey (g/L)	Acid whey (g/L)
Total solids	63.0–70.0	63.0–70.0
Lactose	46.0–52.0	44.0–46.0
Protein	6.0–10.0	6.0–8.0
Calcium	0.4–0.6	1.2–1.6
Phosphate	1.0–3.0	2.0–4.5
Lactate	2	6.4
Chloride	1.1	1.1

biopolymers and other value added bio products. The interlinkage of processing techniques with recent biotechnological tools is a key strategy to maximize the use of dairy byproducts like whey. The production of bioplastics/biopolymers with efficient whey management may increase the potential revenue of the entire bioprocessing chain in dairy industry.

5.2.1 Biopolymers

Biopolymers are biocompatible and biodegradable organic substances present in natural sources and have many industrial uses like biodegradable packaging films in food industry as well as medically implanted organs, tissue transplant, drug transportation material and dressing materials in phama companies. Polymers as common biomolecules comprise nucleic acids, proteins, carbohydrates and lipids as well as a variety of functional chemical side chains. Biotechnological tolls like tissue engineering and medication delivery have been customized the development of bio polymers through genetic modification of beneficial microorganism Polylactic acid and polyhydroxy alkanoates (Baranwal et al., 2022). Bio polymers may be of three types according to their origin and method of synthesis (Fig. 5.1).

Synthetic and semi-synthetic polymers prepared for food and pharmaceutical industries application have various environmental concerns in terms of pollutants, solid waste and gas emission etc. Therefore, now a days biopolymers prepared from natural (plant, microbial and animal) sources are getting popularization with technology advancement and consumer awareness towards environment. Bacteria are prime cell factories that can efficiently convert carbon and nitrogen sources into a large diversity of intracellular and extracellular biopolymers such as polyamides (amino acids linked by peptide bonds), polysaccharides (sugars or sugar acids linked by glycosidic bonds), polyphosphates (inorganic phosphates connected by anhydride bonds), and polyesters (hydroxyl fatty acids connected by ester bonds) (Moradali & Rehm, 2020). These polymers are regulated with environmental stimulus during their synthesis and they also actively participate as adhesives and energy

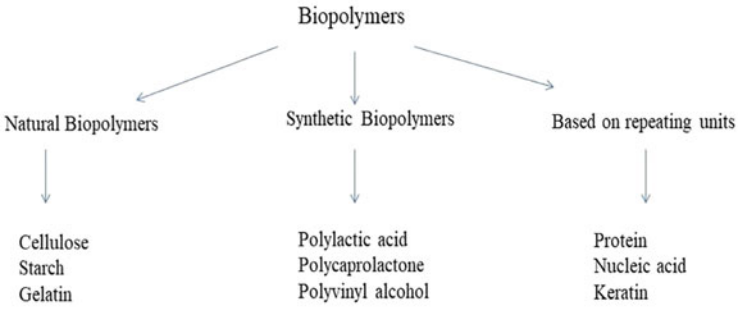

Fig. 5.1 Classification of biopolymers based upon their origin

protectors in various biological functions. Their physico-chemical properties are important for bacterial behaviors, such as translocation, attachment onto biotic or abiotic surfaces, invasion, protection and persistence. Bacterial biopolymers possess several intriguing properties with useful applications in biomedicine and food packaging; therefore, biopolymer factories featuring bacteria engineered to optimize production are increasingly utilized in the pharmaceutical and food industry.

The production of various polymers like cellulose and xanthan gum from bacterial source is difficult due to high production cost (about 30%). Hence, various approaches have been made in recent times to decrease production cost of these biopolymers with use of agricultural wastes and by-products like crude glycerol remaining from biodiesel production, grape bagasse, molasses, corn steep liquor, rotten fruits, and milk whey (Semjonovs et al., 2017). However, use of alternative low-cost carbon sources for biopolymers production changes the properties like crystalinity, O_2 and H_2O transmission, and the degree of polymerization (Salari et al., 2019). It provides an immense opportunity for food technologists and chemical scientists to produce safe and excellent quality biopolymers like cellulose, xanthum gum etc. from milk byproducts i.e. whey without any adverse effect on quality characteristics.

5.2.2 Whey as a Carbon Source

Whey is the dairy product that is produced in huge amount during preparation of paneer, chhana and casein at industrial level. In India, channa and paneer production are major source of the byproduct whey (Macwan et al., 2016). Whey contains higher amount of nutrients with (> 35,000 ppm BOD and (>60,000 ppm COD, thus may cause serious hazards if not efficiently utilized. It's disposal not only causes loss of valuable nutrients even may post a negative economic growth in dairy industry due to increase cost of effluent treatment and waste disposal (Kumari & Rani, 2019). Therefore, utilization of whey has been felt a necessity in view of the current requirements for alleviating environmental pollution as well as recovering available nutrients. By using whey in food and other uses it will boost global competitiveness, promote sustainable economic growth and generate employment (Poonia, 2020). However, nutritively rich whey have the scope of transformation from waste to best functional ingredients, if appropriately nutrient is extracted or converted into useful ingredient. Major whey macronutrients lactose and proteins have been explored by pharmaceutical as well as by food industries. Whey Proteins which accounts for 20% of bovine milk protein was considered a byproduct earlier. However, researchers established the role of whey protein in human health especially, satiety, energy balance, metabolism and inflammation (Boscaini et al., 2023). Whey proteins are rich in essential amino acids, branched-chain (L-isoleucine, L-leucine, and Lvaline) amino acids, and sulphur-containing amino acids (cysteine, methionine). Extraction of whey protein from whey by membrane processing or chromatographic separation methods helped to convert it into bioactive ingredient specifically peptides. In light

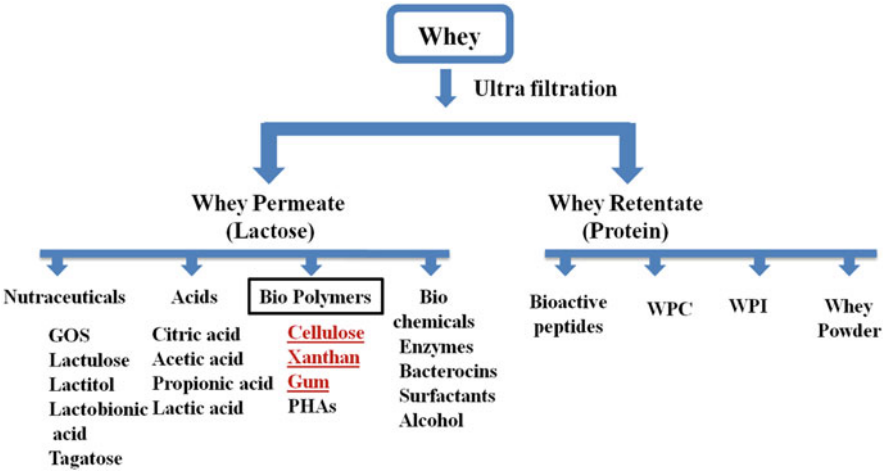

Fig. 5.2 Biotechnological and physico-chemical intervention in whey nutrients utilization

of whey protein, desirability of lactose is less as functional ingredient due to low ferment ability, low sweetening power, and low solubility. Another big challenge for lactose is growing lactose intolerance across the globe (approximately 75%) (Rao et al., 2021). Whey lactose content can be utilized as a substrate for a variety of bio chemical applications. *One such application is polymer synthesis* (Lappa et al., 2019) by ultrafi*ltration of whey, as depicted in* Fig. 5.2 *for synthesis of cellulose and xanthan gum.*

Thus, the application of whey for biopolymer production is beneficial for lowering not only environment pollution, but also production cost of biopolymer. The major limitation in biosynthesis of biopolymers is availability of economic nutrient media and whey is a best suitable alternative as it is comparatively less expensive source (Gahlawat & Srivastava, 2017).

In last decade whey and its derivatives has been explored for synthesis of biopolymers with major focus on xanthan (Mollea et al., 2013; Özcan and Öner, 2015) or bacterial cellulose (BC) (Lappa et al., 2019). The work has been mentioned in reviews focused on whey valorization, however specific publication particularly for compounds such as xanthan or bacterial cellulose are not available. *Different methods of whey valorization for synthesis of biopolymers are shown in* Fig. 5.3.

5.2.3 Xanthan Gum Production from Whey

Xanthan gum is a natural polysaccharide produced by the bacterium *Xanthomonas campestris* and contains linear (1–4) linked b-D-glucose backbone (as in cellulose) with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue linked (1–4) to a terminal mannose unit and (1–2) to a second mannose

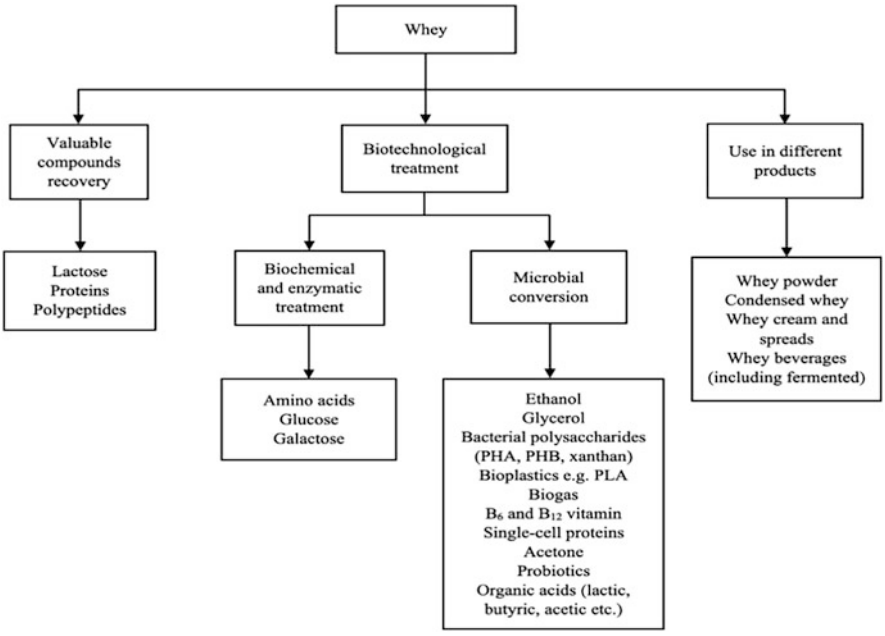

Fig. 5.3 Different ways of whey valorization for production of biopolymers

that connects to the backbone (Melton et al., 1976). Bacteria is preserved for long term storage and inoculum is obtained by growing on suitable solid or liquid media in big lbioreactors at 28–30 °C with pH 7.0, >0.3 (v/v) aeration rate and > 1 kWm^{-3} specific power input for agitation. Whole fermentation process takes about 100 h where 50% of glucose is converted into product. Medium composition, pH, temperature and dissolved oxygen concentration in culture and type of bioreactor as well as mode of operation (batch/continuous) are main factors influencing microbial growth and xanthan production. Broth is produced at the end of fermentation containing a mix of bacterial cells, xanthan and different chemical compounds. Bacterial cells are removed by filtration or centrifugation from the broth, followed by precipitation using water-miscible non-solvents (Isopropanol, ethanol, acetone), addition of certain salts and pH adjustments. The moisture is then mechanically removed from product and then dried to remove extra water. The dried product with constant moisture content is pulverized and finally packaged in suitable containers with lower water permeability. *The whole process of xanthum gum production from whey is shown in* Fig. 5.4.

The xanthan production process is energy-intensive and highly expansive in conventional stirred-tank fermenters due to difficulty in agitation and aeration because of more viscosity in xanthan broth. Main steps of microbial cells recovery process include deactivation and lysis, biopolymer precipitation, moisture removal and pulverization. Centrifugation and heat treatment are not effective to isolate pure gum without cells due to high viscosity and xanthan degradation. Therefore

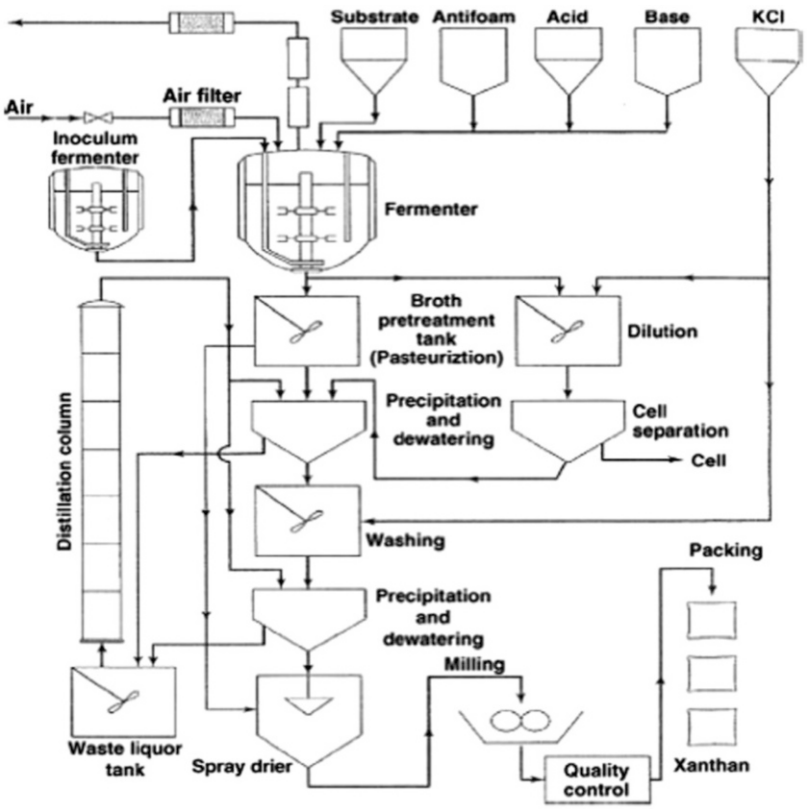

Fig. 5.4 Xanthan production in conventional stirred tank fermenter (Rosalam & England 2006)

enzymatic lysis of microbial calls in broth is preferred to maintain the gum structure even at higher viscosity (Suresh & Prasad, 2005). United States Food and Drug Administration have approved xanthan gum safe for food and pharmaceutical industries on the basis of various toxicology tests. Being polysachharide in nature, gums are hydrophilic and exhibit binding properties however, they become insoluble in organic solvents. In relation to this, exopolysaccharides have been identified to coat the surface of cells and to perform a variety of biological processes, including resistance to drying, adhesion, immunological response, and act as agents for transferring the information (Bazaka et al., 2011). Xanthun gum is one of such exopolysaccharides that has been commercialized successfully and was approved for use in food in 1968 (Petri, 2015). Owing to its ability to dissociate polymer and release of elctrolystes in aqueous solution, it exhibits higher solubility in both cold and hot water. Further, it has been recognized as GRAS by US FDA (Soccol et al., 2013).

Aerobic fermentation process mainly involving the bacterial strains including Xanthomonas campestris, Xanthomonas pelargonii, Xanthomonas phaseoli and

Xanthomonas malvacearum is involved in the synthesis of xanthan gum (Candido da Silva et al., 2017). However, among these strains, most commonly and commercially used strain belongs to X. campestris. This strain is considered as a plant pathogen affecting many plants such as cabbage, alfalafa and beans (García-Ochoa et al., 2000). The synthesis of xanthan gum by X. campestris involves various sequential biochemical steps such as consumption of simple sugars followed by their conversion to derivatives of nucleotides, absorption of monosaccharide subunits followed by pentasaccharide subunits linked to an isopentyl pyrophosphate carrier assembled and polymerized together, and lastly, their secretion. It is believed that D-glucose-1-phosphate units and D-glucose from 2 moles of uridine diphosphate glucose are added sequentially to form the backbone of xanthan (Rosalam & England, 2006; Alkhateeb et al., 2016; Freitas et al., 2011; Schmid et al., 2015). This is followed by utilization of guanosine diphosphate mannose and uridine diphosphate glucuronic acid to generate monosaccharide units namely, D-mannose and D-glucuronic acid, respectively. Further, blocking of all these steps is possible if the step lacks the presence of specific enzymes and substrates for the biochemical reaction to occur. Further, three sub-units namely, glucose, mannose and glucuronic acid in the ratio of 2:2:1 during their absorption, forms the larger and repeated structure of pentasaccharide units. In addition to certain enzymes and substrates, genes also form the significant components for the synthesis of certain nucleotide precursors (Becker, 2015; Lopes et al., 2015). Mainly, three cycles are recognized to be significant in synthesis of xanthan gum from bacteria such as Entner-Doudroff pathway, tricaboxylic acid pathway and pentose phosphate pathway (Bhat et al., 2022).

5.2.3.1 Processing Parameters and Yield of Xanthun Gum Production

It is thought that xanthan is created as a defence mechanism against stress from the environment. It has a significant role in the immunology of microbial cells in host plant tissue in addition to its function in cell lifespan. Xanthomonas campestris is initially cultured in a fermenter that is well-aerated and well-agitated in preparation for the production process (Becker, 2015; García-Ochoa et al., 2000). A sufficient nitrogen source, nutritional salts, and a source of carbohydrates like glucose or sucrose are all included in the medium. The operational and environmental circumstances affect the xanthan yield and the structure of the gum produced. In addition to these, the production is also influenced by other variables such as the concentration of the main medium constituents i.e. carbon and nitrogen and processing parameters like inoculum size, agitation, aeration rate, temperature, pH and the type of impeller utilized. By the time the fermentation process is through, the production medium has been contaminated with microbial byproducts, xanthan gum, and numerous other chemical agents (Zahidah et al., 2020). First, the germs are eliminated, either through heating or another process like centrifugation or filtration. In order to precipitate, organic solvents like ethanol, isopropanol, and acetone must be used, along with

certain salts and pH modifications. The product is mechanically dewatered, dried, and packaged after precipitation.

Many inexpensive nutrients and substrates have been utilised to produce xanthan gum on an industrial scale. Sucrose, sugarcane molasses, and whey are examples of carbohydrate sources that have been successfully incorporated into the production medium (Silva et al., 2009). Whey offers sufficient nitrogen as well as a few other development elements. For the efficient conversion of carbon sources to the necessary polysaccharide synthesis, a high carbon to nitrogen ratio is needed. For effective gum production, batch cultivation with complex media is preferable. Maintaining the stock culture with the microbial source adequately is necessary for the consistent synthesis of xanthan. Accumulation of polysaccharides begins throughout the growth phase and continues after growth. When organic acids are produced during fermentation, the pH decreases. The ideal pH for fermentation medium must be kept 7.0 by using buffer or adding base as lower pH <5.0 significantly inhibits formation of gum (Kerdsup et al., 2011).

5.2.3.2 Factors Affecting Xanthan Gum Production

There are several factors which influence the production of xanthan gum directly or indirectly such as microorganism used for fermentation, substrate, source of carbon and nitrogen, temperature, pH, stirring speed and aeration rate, time and process of fermentation and certain agents that can enhance the production of xanthan gum. The bacterium *X. Campestris*, gram negative bacteria that develops a yellowish tint upon colonisation, produces xanthan gum (Ryan et al., 2011). Microbial strain should be particular as it utilizes different substrate and finally influences the inclusion of side chain linkages, functional group and sugar sequence to control gum formation. As per Gunasekar et al. (2014), amino acid sequence, appropriate carbon and nitrogen sources, minerals like potassium, calcium, phosphorous, magnesium iron etc. are the important nutrient content. To lower the cost of producing xanthan, a variety of agro-industrial wastes have been employed as a substrate, including citrus remnants, carob extract, vegetable leftovers, olive oil waste waters, sugar-beet pulp waste, maize steep liquor, apple juice waste, whey permeates, etc., (Bardone et al., 2016). For large-scale xanthan manufacturing, the most common commercial carbon sources are glucose and sucrose. The ideal carbon concentration for making xanthan is between 2% and 4%; lower or higher carbon source concentrations impede growth. For the most part, sucrose is preferred while making xanthan gum. Although it is possible to use other commercial substrates such xylose, galactose, and lactose, the yield will be minimal because bacteria cannot consume these substances. On media with lactose as the carbon source, X. campestris produces little xanthan because it lacks the enzyme—galactosidase, which ferments lactose. The synthesis of xanthan is thought to be enhanced by a high C/N ratio. The maximum yield, 14.744 g/L, is produced by glucose, followed by sucrose (Leela & Sharma, 2000).

Compared to inorganic ones source of nitrogen, organic ones cost less. Peptone, yeast extract, corn steep liquor, and soybean meal are examples of organic sources. Ammonium or nitrate salts are examples of inorganic sources. The best sources of peptone and yeast extract for xanthan synthesis are organic sources. Ammonium salts are regarded as a better substrate for biomass accumulation among inorganic sources, although nitrate is recommended for the highest gum yields (Chavan & Baig, 2016). It was discovered that a temperature between 28 °C and 30 °C was ideal for producing the most yield. According to a report, the ideal temperature for microbial growth and gum production varies slightly, with *X. campestris* growing best at 25–27 °C and xanthan gum producing best at 25–30 °C. The structural composition is impacted by temperature variations during cultivation, which may ultimately adversely affect the gum viscosity. The temperature range from xanthan gum production may vary between 25 °C and 35 °C, as higher temperature may negatively affect the production of biomass and xanthan gum. The pH varies from 6 to 7.5 where *X. campestris* grows best and from 7 to 8 where xanthan is produced best. With a pH of 7, Xanthomonas cultivation is possible. The pH range of 6.0–8.0 using strong alkali like sodium hydroxide, potassium hydroxide or ammonium hydroxide is the best for effective manufacture of xanthan gum. Maximum gum formation with appropriate viscosity is best improved at pH around 8.0 and temperature 30 °C, whereas pH ranging between 6.0–7.0 °C and 25–27 °C temperature range were considered to encourage bacterial growth. Regulated pH was observed to promote the microorganism's growth without significantly affecting the gum output (Murad et al., 2019).

The extracellular xanthan deposition causes the oxygen mass transfer rate to decrease as xanthan synthesis rises. Moreover, the viscosity of culture media is rising, which affects its uniformity and nutrient distribution by reducing the aeration rate of the media. The formation of xanthan gum is directly correlated with the dissolved oxygen rate, according to several studies. Because of Xanthomonas's aerobic nature, medium must include a lot of oxygen in order for bacteria to function effectively and produce more gum. As a result, it becomes important to keep an eye on the airflow rate and stirrer speed because they eventually have an impact on gum production. For the manufacturing of xanthan, batch or continuous fermentation can be used. Batch-scale fermentation results in high substrate to gum conversion efficiency (75–80%), however the process takes longer than 2 days. However, for the manufacturing of xanthan, batch or continuous fermentation can be used. Batch-scale fermentation results in high substrate to gum conversion efficiency (75–80%), however the process takes longer than 2 days (de Mello Luvielmo et al., 2016). It has been discovered that citrate increases xanthan synthesis regardless of the necessary pH range. Pyruvate content in xanthan has a significant impact on the functionality of polymers. Some scientists came to the conclusion that increasing nitrogen concentration increases the degree of pyruvilation, especially if an organic nitrogen source was used. The yield and viscosity of xanthan gum produced are increased, the cultivation period is shortened, and more sugar is utilised when maize steep liquor (1 g/L) is added. Citric acid is added as chelating agents to stop salt from precipitating during heat sterilisation, which raises the productivity of xanthan. As acetic

acid is a weak carboxylic acid and xanthan is soluble in it, the addition of acetic acid can increase the solubility of xanthan in the solution (Palaniraj & Jayaraman, 2011).

5.2.3.3 Application of Xanthan Gum

Xanthomonas campestris-produced xanthan gum is used for various industrial activities which are linked with its unique properties like it exhibits higher viscosity at lower concentration, it is soluble in both hot and cold water, it is resistant to enzymatic degradation, stable in wide pH range, temperature and salt solutions as well as it can interact with other polymers too. Food, pharmaceutical manufacturing, personal care, the oil and textile industries, wastewater treatment, and water-based paints are just a few examples of the many industrial applications (Nwodo et al., 2012). Xanthan is utilized to control rheological properties in solutions as stabiliser, thickener, emulsifier in emulsions and suspensions solutions because of its exceptional viscous properties. It is also used gel formation, suspending and flocculation agent for development of toothpaste and in both culinary and non-food applications due to it's pasty consistency. Xanthan is also added in pesticides, fungicides herbicides and other agricultural products for uniform suspension of solid components in formulations (DeAngelis, 2012). Xanthan gum has been employed as an elicitor in barley cultivars coupled with fungicides to prevent the Bipolaris sorokiniana-caused barley spot blotch. Because to its quick dispersion and hydration, non-pollutivity, and good colour production, xanthan has found use in jet injection and printing. Because it is environmentally friendly, xanthan gum has been used in the creation of new generation thermo-set coatings. Moreover, the oil industry employs xanthan gum for pipeline cleaning, oil fracturing, and drilling.

5.3 Cellulose Production from Whey

Bacterial cellulose is produced by several microbial genera, *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina* (Seo et al., 2014) and a cell-free system (Ullah et al., 2017). Bacterial cellulose has higher water holding capacity (WHC), excellent mechanical, crystalline and tensile properties, better thermal and slow water release rate. It can combine with different organic or inorganic metals/compounds, nanoparticles and biocompatible polymers to form composite materials to improve its existing properties as well as to impart additional features (Ul-Islam et al., 2014). Various carbon molecules are polymerized by microbial cells to form single β-1,4-glucan chains during cellulose production, which are protruded from the small pores present on terminal complexes on cell membrane surface. These perfectly newly formed β-1,4-glucan chains are grown in culture medium to form protofibrils which are crystalized into micro- and macro-fibrils, bundles, and finally transformed into ribbons-like structures. This ribbon like sturcture is composed of about thousands of individual

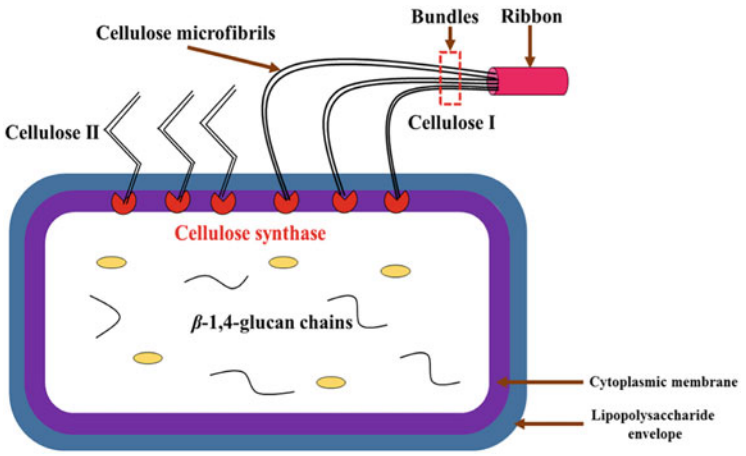

Fig. 5.5 Formation of cellulose chains formation in microbial cells (Ul-Islam et al., 2015)

β-1,4-glucan chains. Ultimately cellulose is formed as thick gel like membrane at the air–medium interface in a static cultivation (Czaja et al., 2006). *In short, cellulose can be synthesized by bacterial microbial cells in following four-step process, which is also shown in* Fig. 5.5.

(a) Glucose nucleotides formation resulting into monosaccharide activation
(b) Sequential addition of glucose repeating units through polymerization
(c) Addition of acyl groups (if present) to individual glucose units
(d) Excretion of cellulose fibers through wall/membrane complex into extracellular environment

5.3.1 Xanthum Gum from Bacterial Origin

Various bacterial strains has been explored for the production of cellulose (Table 5.2), however, strains of acetic acid bacteria (AAB) *Komagataeibacter xylinus* are most commonly used. The major limitation in production of BC is the cost of raw material as C and N source and less expensive alternative media are being explored for ease of large scale production (Rehm, 2010; Kreyenschulte et al., 2012; Amaro et al., 2019; Gorgieva & Trček 2019). One of the alternatives source is whey, however limited focus has been given to it as C and N media in this respect (Marangoni et al., 2002). The major limitation of whey utilization for BC production is its major carbon source i.e. lactose which can only be utilized either after hydrolysis (chemical or enzymatic) into glucose hydrolysis (Torres et al., 2010; Kucera et al., 2018) or by using recombinant strains AAB with β-galactosidase

Table 5.2 Bacterial cellulose production using whey as a substrate

Substrate	Bacterial strain	Yield (g/L)	References
Whey	*G. sucrofermentans* B-11267	5.45	Revin et al. (2018)
Cheese whey hydrolyaste	*Komagataeibacter rhaeticus* P 1463	6.55	Semjonovs et al. (2017)
Cheese whey hydrolyaste	*Gluconacetobacter xylinus* PTCC 1734	3.5	Salari et al. (2019)
Cheese whey permeate (CWP) with β-galactosidase (0.5 U/mL, 30 °C)	*Komagataeibacter xylinus*	6.77	Rollini et al. (2020)
Lactose hydrolysed cheese whey	*A. xylinum* strain 15,973	5.5	Lappa et al. (2021)
Rotten fruit extracts + non-hydrolysed milk whey	Mutant strain *A. xylinum* ITz3 with *lac*Z gene	50	Jozala et al. (2015)
Corinthian currant grapes extract (sugars 20 g/L), cheese whey (50.4%) and yeast extract (1.7%)	*Komagataeibacter sucrofermentans* DSM 15973	8.4	Bekatorou et al. (2019)
Whey medium (no pre-treatment)	*K. rhaeticus* P-1463 Hydrolised	1.95	Kolesovs and Semjonovs (2020)
Whey (β-galactosidase pre-treated)		2.41	

activity to obtain glucose from CW lactose (Battad-Bernardo et al., 2004; Pescuma et al., 2015; Lappa et al., 2019).

5.3.2 *Factors Affecting Bacterial Cellulose Production*

The critical factor affecting the BC production process is to select the suitable microorganism. The strains like *Pseudomonas, Salmonella* and *Sarcina ventriculi* from genera *Azotobacter, Gluconacetobacter* (formerly known as *Acetobacter*, as well as *Komagataeibacter*) used for BC synthesis. Among these strains of Gluconacetobacter such as Gluconacetobacter xylinus, Gluconacetobacter hansenii, and Gluconacetobacter pasteurianus has been described by by Adrian J. Brown in 1886 for better BC production (Kubiak et al., 2014; Torres et al. 2010). The Gluconacetobacter sp. is the only acetic acid bacteria capable of producing BC at large scale among all the gram negative stains (Lin et al. 2013). This elitism of acetic acid bacteria is associated with its ability to produce cellulose as a predominant metabolite during biochemical synthesis reaction leads to high cellulose yield (Castro et al., 2011). Gluconacetobacter sp. has been isolated from various acidic food by product like citrus juice fungus (Kim et al., 2017), vinegar (Du et al., 2018; Škraban et al., 2018), fruits (Numata et al., 2019), Kombucha tea (Revin et al., 2018). Literature reveals the higher yield of BC when *Gluconacetobacter* sp. Strains

is used. Revin et al. (2018) observed the yield of 5.45 g/L BC when whey was fermented as nutrient medium. Literature indicates that AABs can produce BC, however use in whey is not feasible as they are not good in utilizing lactose. Semjonovs et al. (2017) found the Pretreatment of whey with β galactosidase enzyme or microbial strain as the best approach to increase the yield of bacterial cellulose. They reported that higher amount of cellulose 2.90 g/L (0.024 g/L/h) and 6.55 g/L (0.019 g/L/h) was obtained using cheese whey hydrolyaste as carbon source for strain *Komagataeibacter rhaeticus* P 1463, when 50 mL medium in 300-mL flasks or 300 mL medium in 3000-mL jars were used as identical static processing conditions at 28 °C for 5 or 14 days, respectively. Salari et al. (2019) observed 3.5 g/L BC (0.0104 g/L/h) within 14 days of fermentation in hydrolysed cheese whey (reducing sugars 30 g/L) using the producer strain *Gluconacetobacter xylinus* PTCC 1734. Cheese whey permeate (CWP) supplementation with minimal amount of β-galactosidase (0.5 U/mL, 30 °C) boosted the BC yield (6.77 g/L) from the acetic acid bacteria *Komagataeibacter xylinus* (Rollini et al., 2020). Lappa et al., 2021 used lactose hydrolysed cheese whey as raw material for BC production using A. xylinum strain 15,973 leading to the production of up to 5.5 g/L of BC, which is a remarkable concentration, taking into account previous cited research activities. The composition of nutrient media should be compatible with strain and fermentation conditions, and this leads to the increased search of new culture medium which enhances the strain productivity within fermentation system (Campano et al., 2016). Further genetic modification in BC producing bacterial strains may ease the process along with yield increase. Battad-Bernardo et al. 2004 modified the *Acetobacter xylinus* strain by inserting the *lac*Z gene to develop a mutant strain *A. xylinum* ITz3. The mutant strain when grown in lactose based medium, showed a 28-fold increase for production of bacterial cellulose with 160 times increase in β-galactosidase activity. Combination of carbon sources was another approach cited in literature to enhance the microbial cellulose yield in media. Rotten fruit extracts use along with non-hydrolysed milk whey shown promising results of BC production (about 50 mg/mL, 0.52 mg/mL/h) (Zikmanis et al., 2020). Bekatorou et al. (2019) explored comprised extract of corinthian currant grapes (sugars 20 g/L), Cheese whey (50.4%) and yeast extract (1.7%) as low cost carbon sources for bacterial cellulose production using *Komagataeibacter sucrofermentans* DSM 15973 strain with higher (8.4 g/L) cellulose yield.

5.4 Conclusion

Indian dairy sector has made remarkable achievement during last few decades and emerged from small traditional basket to the 'Oyster' of the global dairy industry. With the production of various dairy products, a large amount of dairy products like whey is produced in a huge amount. Depending upon the primary product and pH, the whey may be classified as sweet or acid whey. Whey contains large amount of lactose and may be used for production of various biolpolymers like cellulose and

xanthan gum using various bacterial strains. Thus processing of nutritionally dense whey in various ways may be fruitful for dairy industry to enhance the economics with production of biodegradable environmental friendly polymers. These biopolymers are gaining popularity over synthetic or semi synthetic polymers with diverse application in pharmaceutical and food industry. However increased cost of carbon source limits the production of microbial fermentation based polymers at large scales. Whey, as a significant diary byproduct can produce good amount of lactose and is an excellent source of carbon at lower cost. Few studies have been conducted on production of xanthum gum on whey substrates using gram negative bacteria *Xanthomonas*. Bacterial cellulose is one of the most common homopolysacchride biopolymer is now a days produced from whey as an alternative growth medium with specific biotechnological tools. Different methods of whey pretreatments, selection of bacterial strains and whey valorization should be priority research area for whey based biopolymers production at large scale for various industrial uses at minimal cost with desirable quality characteristics.

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Chapter 6
Bioplastic Production Using Whey (Polyhydroxyalkanoates and Polyhydroxybutyrates)

Ananya Rana, Vikram Kumar, Tejpal Dhewa, and Neetu Kumra Taneja

Abstract It has been discovered that bio-based plastics, which are derived from organic and renewable resources, are effective substitutes for polymers derived from petroleum. Unfortunately, few of them, especially those made from whey, have been evaluated economically. The world needs alternatives to plastic since its production and buildup have adverse effects on the environment. Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrates (PHBs) act as viable alternatives to conventional plastics given their biodegradability, biocompatibility, and potential for biological synthesis. PHAs are produced by bacteria, and one of the primary costs related to the production of plastic associated is the carbon supply that the bacteria use to ferment food. As a result, a number of industrial waste streams, such as whey, an effluent from the dairy sector, have been explored as potential carbon sources for bacterial growth and production of PHA and PHB. When whey is utilized to produce PHA, the process may become less expensive and better for the environment. Whey pre-treatments and the selection of a PHA or PHB-producing strain are just two issues that continue to impede the use of whey as a carbon source for PHA and PHB synthesis. In this chapter, the current state of knowledge on the use of whey for the production of PHA and PHB was reviewed, and creative solutions to the challenges faced throughout this manufacturing process were proposed.

Keywords Bioplastic · Renewable · PHA · PHB · Biodegradability · Biocompatibility

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6.1 Introduction

The majority of the manufacture of plastics, that is indispensable to all market capitalism, is accomplished through the use of fossil feed stocks with severe environmental consequences. However, the global output of plastics is soaring (204 million tonnes in the year 2002, up to 335 million tonnes in the year) and is expected to regular to rise. Because the plastic is not easily biodegradable and accumulate in a variety of situations, their production has led to environmental problems (European Commission, 2019). Nevertheless, despite impressive efforts to enhance recycling rates (recycling grew by 79% in Europe between 2006 and 2016); in 2016, 31.1% of European plastics were recycled. The rest of the plastics are dumped inside landfills (27.3%) or can be used for energy rehabilitation (41.6% European Bioplastic) (European Commission, 2019). Therefore, it is imperative that these "conventional" plastics be replaced with bioplastic, which are plastics that are organically generated and/or biodegradable. Despite the fact that it is believed that around 85% of total plastic wares might replaced by bioplastic, only about 1% of the total plastics manufactured worldwide are bioplastic (European Bioplastic) (Rosenboom et al., 2022). The output of bioplastic is anticipated to increase, reaching 2.44 million tonnes in 2022, with only 1.086 million tonnes being biodegradable (European Bioplastic). Unfortunately, this expected growth falls short of the ever-growing worldwide demand for plastics. Polyhydroxyalkanoates (PHAs) are biosynthesized polyesters came to be regarded as highly advantageous alternatives to conventional polymers manufactured from petroleum. It has been found that these molecules serve as carbon storage and reducing equivalents, and that they do so by aggregating in granules within bacterial cells. PHAs are biocompatible and biodegradable and have properties similar to those of conventional polymers (Geyer et al., 2017). As a result, PHAs are now manufactured commercially and used in many different kinds of products, such as packaging and medical equipment. Despite their superior qualities and environmental benefits, the high production costs of PHAs prevent their widespread application. The use of costly fermentation carbon sources, which account for about 40% of total PHA production costs, is a major factor in these high prices (Chaharsooghi et al., 2011). In order to reduce the cost of both PHA synthesis and garbage disposal, it has been suggested that waste products be used as carbon sources for PHA production by microorganisms. PHAs have been successfully produced using a wide variety of waste materials (Castilho et al., 2009). Wastewater from homes, food scraps, molasses, olive oil mill effluents, palm oil mill effluents, tomato cannery water, lignocellulosic biomass, coffee waste, starch, waste from the biodiesel industry, used cooking oil, pea shells, wastewater from paper mills, bio-oil from the fast pyrolysis of chicken beds, and cheese whey are some of the materials that can be used. The main byproduct of the dairy industry is whey, which is derived by precipitating and removing casein from milk during cheese-making (Nielsen et al., 2017). Over 120 million tonnes of whey are released from industries per year globally, but only 50% of it is used for human and animal feed. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of whey

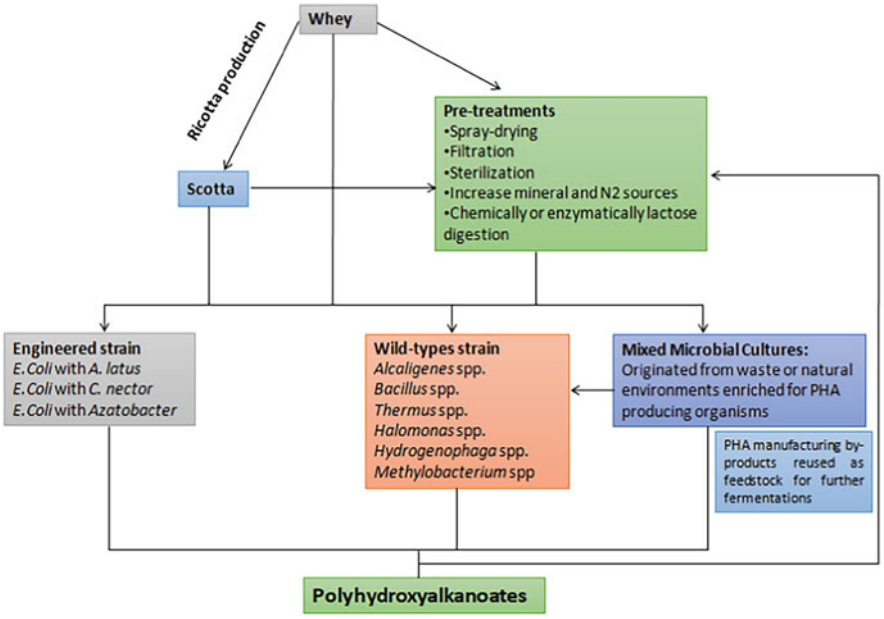

Fig. 6.1 Diagram showing probable whey-based polyhydroxyalkanoates routes. PHA fermentations use whole whey. Nevertheless, pre-treatments usually increase PHA production. Ricotta cheese whey, or scotta, is produced from whey. Scotta can produce PHA directly or after pre-treatments

are very high, e.g. acid whey has a BOD value of 35,000–45,000 mg per litre and COD values of 55,000–70,000 mg per litre (Poonia, 2020). The disposal of the residual whey creates environmental problems resulting from the relatively large organic load it carries, as whey is constituted primarily of lactose (39–60 g/L), lipids (0.99–10.58 g/L), proteins (27–60 g/L), and mineral salts (4.6–8 g/L). Lactose accounts for the majority of cheese whey's biochemical oxygen requirement; consequently, it is crucial to develop a biotechnological application for lactose. Whey lactose would significantly lower PHA production costs without affecting food production or the environment. This chapter summarizes current understanding regarding the production of PHA and PHB using cheese whey (Fig. 6.1). In addition, the challenges and potential of employing the industrial production of PHAs uses whey.

6.2 Microorganisms Used to Produce PHAs and PHBs from Whey

Whey is a nutrient-dense medium ideally suited for microbial growth. Yet, it has been demonstrated that locating bacteria capable of producing PHAs efficiently while growing on whey is incredibly tough. Sadly, it has been demonstrated that several well-explained PHA-producing microbial species are incapable of synthesizing PHAs directly from whey (Amaro et al., 2019). *Cupriavidus necator*, formerly known as *Ralstonia eutropha*, *Wautersia eutropha*, and *Alcaligenes eutrophus*, is capable of collecting PHAs around 80% of total dry weight while growing in the presence of glucose, but is incapable of efficiently growing and producing PHAs in presence of lactose, the primary carbon source of whey. Another instance is the halophilic archaeal bacteria *Haloferax mediterranei,* which has the capacity for PHAs accumulation to the tune of nearly 60% of its dry weight (Sharma et al., 2016). Despite the fact that *H. mediterranei* can consume lactose, but it does not used as an efficient source for growth and PHA production. *Alcaligenes latus* stands out as an exception because it is a commercial PHA synthesizer and can extract PHAs from up to 70% of its dry weight from a wide range of sugar sources. *Alcaligenes latus* may produce whey lactose to PHA (0.11 $g\,L^{-1}\,h^{-1}$), according to a recent study (Amaro et al., 2019). *Alcaligenes latus* can manufacture PHAs from whey, but more research is needed to maximize its potential. In certain cases, pre-fermenting whey lactose into glucose and galactose helped highly PHA-producing bacteria make PHA from it (Surendran et al., 2020). Utilizing this hydrolyzed whey lactose, it was demonstrated that high PHA producers, such as *C. necator* and *H. mediterranei*, may manufacture PHAs. Yet, from an industrial standpoint, it is preferable to minimize the additional cost of PHAs that result from transforming whey lactose to glucose and galactose before to fermentation. Through the use of genetic engineering techniques, these conversion steps have already been omitted. For example, lactose-consuming *Escherichia coli* cells were engineered to express genes involved in PHA biosynthesis pathway from bacteria with a high PHA production rate (Bosco et al., 2021).

Instead, lactose degrading genes have been introduced into high-producing bacteria. While, Genetic engineering can improve whey PHA production, but it requires more carefully regulated production facilities (Mitra et al., 2020). Genetically modified microorganisms (GMMs) are widely used in scientific research facilities and the biotechnology industry, raising worries about unintentional release into the environment, as well as the transfer of genes to other types of microbes/non-microbes hosts. In light of this, biosafety legislation of particular nations has deemed it important to limit their use in order to prevent any potential problems they may offer. The order mandates the notification, risk assessment, and permission of work involving GMOs, as well as the approval of laboratories and facilities where such work will occur (Mitra et al., 2020). There are four classes of laboratories for working with GMMs, each with its own set of regulations regarding containment, association of the laboratories and area nearby laboratories, work execution plans,

technical facilities, internal supervision, and control (Council Directive 98/81/EC of 26 October 1998 amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms) (Amaro et al., 2019). Thus, biotechnology laboratories and biosafety precautions are necessary for businesses to protect their employees, the public, and the environment against hazardous bacteria and substances, as well as qualified personnel, which raises plant management expenses and PHA manufacturing prices. Whey-based PHA production using mixed microbial cultures (MMCs) has recently gained attention (Amaro et al., 2019). MMCs do not need sterile conditions and can adapt to altering industrial effluent, although they produce less PHA. MMCs directly generate PHAs from cheese whey lactose or after lactose digestion by another MMC. MMCs are typically collected from waste sources and increased for PHA synthesis via feast and famine cycles to develop strains that can produce PHA-based carbon storage (Valentino et al., 2015). The utilization of an unknown microbial population to manufacture PHAs, in medical applications, PHAs' production efficiency, polymer characteristics, and biocompatibility pose issues, despite their remarkable versatility and low cost. To the best of our knowledge, a study isolating single organisms for PHA synthesis from whey has not yet been conducted. Another approach involves using MMCs to break down the lactose into organic acids, which are then used as a substrate for a high-PHA strain. But still, PHAs from whey have not been produced using this method (McAdam et al., 2020).

6.3 Pre-treatment of Whey

For direct PHA synthesis, whey poses various challenges as a substrate. First, it has been reported that whey has a low C-N ratio, which significantly impedes PHA formation. In addition, lactose sugar is not a good carbon source for PHA-producing strains, as was previously mentioned. However, as whey is a complicated, non-sterile, and frequently changeable by-product, its immediate application in research laboratories and manufacturing processes leads to (Amaro et al., 2019). Consequently, most research on PHA production from whey has used whey derivatives that have been pretreated (Fig. 6.1 and Table 6.1). To adjust lactose concentration, whey powder is diluted with water before fermentation. Spray-dried powdered whey may be stored longer and doesn't vary seasonally. Spray-drying a PHA production pipeline will increase costs (Chang et al., 2021). Based on the reasoning for making use of whey powder in screening and enhancing PHA production, fermentations with whey may be comparable to that using whey powder. Ultrafiltration removes most of whey's proteins and other particles, leaving a lactose-rich permeate (Amaro et al., 2019). To remove protein, whey is ultra filtered, acidified to a pH close to 4, heat treated, centrifuged, and filtered to produce a supernatant. Whey permeates or supernatants simplify production but cost more. *Methylobacterium* sp. ZP24 used whole whey and whey supernatant to grow biomass and PHA on a complex mineral medium. (Pantazaki et al., 2009). When

Table 6.1 A summary of the research aiming at manufacturing PHAs from whey, broken down by the type of whey pretreatment

Types of whey	Type of culture	Microorganisms type	Type of PHA
Permeate of fermented whey powder	MMC	Not define	P-3(HB-co-HV)
Fermented whey supernatant	MMC	Not define	PHB/P-3 (HB-co-HV)
Supernatant of whey + additives	Pure cultures	*Bacillus megaterium* *Thermus thermophilus HBB* *Methylobacterium* sp. *ZP24*	PHB Diverse PHB
Fermented whey powder	MMC	Not define	P-3(HB-co-HV)
Hydrolyzed whey + additives	Pure cultures	*Halomonas halophila*	PHB
Hydrolyzed whey powder	Pure cultures	*Haloferax mediterranei*	P (3HB-co-3HV)
Fermented whey powder	MMC	Not define	P (3HB-co-3HV)
Supernatant of whey powder + additives	Pure cultures	*Alcaligenes latus*	PHB
	Formulated cultures	Engineered *E. coli* with *Alcaligenes latus* PHA biosynthesis genes	PHB
		Engineered *E. coli* with *Azatobacter* spp. PHA biosynthesis genes	PHB

extrapolating from using whey concentrates or supernatants to whole whey, care should be used.

Minerals and nitrogen sources are other sorts of additives that are commonly employed in PHA synthesis research including whey (Table 6.1). Instead of permeates or supernatants, whole whey might eliminate these additives. In other studies, it is unclear if the microorganisms can It would be excellent for industrial production if large quantities of PHAs could be accumulated utilising whey without additions. The addition of salts ((NH4)2SO4, 5 g L^{-1}; Na2HPO4, 2.5 g L^{-1}; KH2PO4, 2.5 g L^{-1}; MgSO4, 0.2 g L^{-1}; and MnSO4, 0.01 g L^{-1}) to whey supernatant nearly doubled *Bacillus megaterium's* biomass and PHA production by tenfold (Koller et al., 2008). Thus, it would be worthwhile to over look the use of other affordable waste materials as whey additions if whey were to be supplemented in order to increase PHA synthesis; this subject will be addressed later in the chapter.

The manufacture of PHA should make greater use of whole whey as a research tool. However, using entire whey presents significant difficulties in the experimentation process, one of which is sterility concerns. When employing MMCs, these concerns are less important; nonetheless, sanitation is absolutely necessary when working with single cultures. It is impossible to sterilize complete whey by heating it to high degrees because the total whey protein will precipitate at those temperatures

(Bosco & Chiampo, 2010). Moreover, filtering of whey is problematic because of the large quantity of suspended particulates that it contains. It is possible to apply a procedure called low-temperature pasteurization, but doing so typically requires multiple cycles of heat and cold treatments, which are operations that are both time-consuming and expensive. In addition, the method of pasteurization does not guarantee complete sterilization, which may result in sterility issues during the fermentation phase of the process (Webb & Whittier, 1948).

Hence, UV radiation or high-temperature-short-time pasteurization may be utilised prior to fermentation with pure strains in entire whey. These procedures could kill any whey bacteria (Shabbir et al., 2021). In addition to that, the utilization of antibiotics as a potential solution has also been investigated. *Vancomycin* was proven to minimize *Bacillus cereus* contamination in *Hydrogenophaga pseudoflava* PHA synthesis using whey without considerable compromising the production efficiency of PHA. This was accomplished by inhibiting the growth of the *Bacillus cereus*. The utilization of untreated entire whey results in a high lactose concentration, which is another issue that occurs (Koller et al., 2008). It was demonstrated that the content of lactose in the whey was extremely important for the synthesis of biomass and PHA by *B. megaterium* CCM 2037. When grown in 20 g/L diluted whey supernatant, biomass and PHAs increased to 2.51 g/L and 0.79 g/L, respectively (Amaro et al., 2019). This strain achieved a biomass of about 1.5 g/L when cultured in 40 g/L lactose-containing whey supernatant and synthesized <0.5 g/L PHAs. Whey lactose must be converted into galactose and glucose before fermentation, in addition to the aforementioned pre-treatments (Bosco & Chiampo, 2010). This is necessary for the growth of certain bacteria (Table 6.1). A few of the authors have circumvented the need for lactose hydrolysis by employing a medium that imitates hydrolyzed whey and contains galactose and glucose as carbon sources.

6.4 Alternative Strategies to Enhance PHAs Production from Whey

This chapter focuses on two essential elements that must be considered in order to achieve commercially viable PHA generation from cheese whey: microbe selection and whey pre-treatment needs. Despite the fact that not all parameters may be discussed in this article, it is necessary to note that a number of additional crucial factors must be considered for the efficient generation of PHAs from whey.

6.4.1 Recycling of Whey to PHAs Production

As stated in the preceding sections, the manufacture of PHAs from whey requires the addition of a number of chemicals. Thus, it would be quite intriguing if these

compounds could be derived from other waste sources. *H. mediterranei* was utilized to assess the viability of reusing organic material as substrate in future PHA biosynthesis pipelines by fermenting enzymatically digested whey lactose to produce PHA (Khatami et al., 2021). *H. mediterranei* is a severe halophile, and as a result, it's utilization has the benefits of decreasing disinfectant charges and facilitating differential osmotic pressure-based PHA extraction methods. Despite this, *H. mediterranei* fermentations for PHA generation generate huge quantities of very salty waste streams (Mitra et al., 2020). Thus, writers have evaluated the viability of incorporating these waste streams into succeeding fermentations. In spite of the modest yields achieved, the recycling of wastes enabled the manufacture of PHA, presenting intriguing opportunities for future research on the re-use of organic waste in PHA biosynthesis processes. Applying these flows to additional biotech manufacturing techniques are additional intriguing possibility that, to our knowledge, has not yet been detailed (Koller et al., 2008). Consequently, to add value through eco-friendly procedures, research on recycling waste streams during PHA synthesis from whey is crucial.

It has been demonstrated that additions, such as nitrogen sources, are necessary for optimizing the generation of PHA from whey. As mentioned, these additions from food waste might be valuable. Whey has been used to make PHA from waste frying oils, however this additive source for PHA production is largely investigated (Caldeira et al., 2020). It has been demonstrated that protease-hydrolyzed whey functions as a complicated nitrogen source to increase PHA synthesis from wastage of fried by roughly 40% utilizing *C. necator*. Thus, the blending of waste sources offers virtually limitless opportunities to improve the effectiveness of PHA synthesis without the addition of expensive chemicals. Additionally, to testing different waste streams for PHA production increases, cheapest additives could also be tested (Obruca et al., 2014). For instance, adding 1% ethanol as an external stress chemical to cheese whey increased *B. megaterium* PHA synthesis by nearly 40%. For maximizing PHA synthesis from cheese whey, the usage of ethanol or other stressors, like PHA production boosters, must unquestionably be considered.

6.4.2 Improvement in PHAs Purification Process

Another potential issue associated using entire whey for microbial PHA production extracts and purifies PHAs. PHA extraction from bacterial cells started with two approaches. The first method utilised PHA's solubility in chloroform and insolubility in methanol. Solvent-based PHA extraction yielded above 90% (Amaro et al., 2019). Unfortunately, the usage of solvents makes these methods both environmentally and economically undesirable. The second method involves cleaning with enzymes and detergents to remove biological entities while preserving PHAs. These solutions are more eco-friendly, but typically result in less PHA production and high prices when enzymes are employed (El-malek & Steinbüchel, 2022). Many new solvents and extraction methods, including creating PHA-secreting microbial strains, are being

evaluated as potential alternatives to the most commonly used extraction methods. Except for a modified *E. coli* study that produced partial PHA, this strategy has not been explored. For the generation of PHAs from whey (Tan et al., 2014), Yet, cheaper PHA extraction methods have not been thoroughly examined. One and only ecologically acceptable method for PHA extraction from whey uses a single microbe to break down *H. mediterranei* cells using their high osmotic pressure (Pagliano et al., 2021). However, it has to be determined if while using whole whey, PHA extraction yields and purity are equivalent, which is rich in proteins and lipids and adds an additional degree of complexity to PHA extraction operations (Amaro et al., 2019).

6.5 Conclusion

To address the environmental issues caused by the manufacture and accumulation of traditional plastics, it will be necessary to produce bioplastic in a sustainable and cost-effective manner. PHAs have qualities that are desirable in the market and can be biologically synthesized from inexpensive waste substrates like whey, suggesting that they could play a key part in this bioplastic solution. While effective generation of PHAs from whey is a desirable goal, there are still several obstacles that must be solved. We have compiled a summary of the literature on whey pre-treatments and generating microbe selection for PHA generation from whey. Based on what we know presently, it seems likely that PHA can be extracted from whey using a variety of methods. The lack of research on the use of whey for the industrial production of PHAs, taking into accounts the associated costs and environmental impacts, is another key finding of this review. Thus, this research may inspire and direct efforts to develop commercially feasible methods for extracting PHAs from whey.

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Chapter 7
Potential of Whey for Production of Value-Added Products Using Microbial Fermentations

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Abstract Whey being the green watery liquid produced through casein coagulation using rennet. Whey constitutes of milk solids (45–40%), milk sugar, i.e., lactose (70%), minerals (70–90%), proteins (20%) and vitamins (B and C) are available in milk. Whey is the major waste from dairy industry accounting for higher organic value of about 1,00,000 mg O_2/L Chemical Oxygen Demand. So, food industries are keen to valorise this nutritional potential of whey. Whey with abundant nutrient and bioactive compounds can be used for formulation of nutraceutical and functional foods using various physical, chemical and biological approaches. In past few years, advanced biotechnological techniques have suggested new alternatives for processing of whey and its conversion into valuable products. Microbial fermentation refers as a green approach for bioconversion and valorisation for valuable outcome. Selective microbes can be used for converting this whey into organic acids, aroma compounds, bacteriocins, biopolymers, prebiotics, single cell proteins, enzymes and bio-alcohol etc. The selection of suitable microbial culture, process and ingredient optimisation is quite important in controlling the yield, quality and purity of finished product. So, there is huge scope of research in reinforce different microbes for whey valorisation for developing new sustainable products with some unique characteristics. This bioconversion of whey to value added products is most efficient in terms of product development and reduction in pollution level. This book chapter emphasises on microbial fermentation of whey as starting material for manufacturing of ingredients, beverage and eatables.

Keywords Single cell proteins · Bioplastics · Biofuels · Bioactive peptides · Bacteriocines · Enzyme production

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7.1 Introduction

The demand of healthy, nutritious and functional foods is tremendously increasing at global level. Health concern and societal awareness towards balanced diet has motivated the scientists to explore news sources of functional and novel foods. Our diet must include all major components of food for being regarded as balanced diet, out of which proteins play the major role in human metabolism and energy generation. They are composed of amino acids, some of which may either be synthesized by in-built human metabolism (non-essential amino acids) or may need to be out sourced through diet (essential amino acids). Thus, diet is considered important for human sustainability and life expectancy. Milk, soybeans, legumes, eggs, meat, etc. are some of the protein sources commonly used and out of which milk is the complete food accounting to the presence of all the essential amino acids. It is composed of proteins, that are, casein protein and whey protein. Generally, whey is a thin watery liquid produced after casein coagulation through rennet. Hence, can be regarded as the by-product of milk industry, with huge amount of production. The worldwide manufacturing of whey is around 170–200 million tons annually (Kumar et al., 2018) and about 40% whey is lost as waste causing significant nutritional losses and global issues (Panghal et al., 2018). The content of organic acid in whey is very high which makes it impossible to dispose in water bodies. The biological oxygen demand (BOD) and chemical oxygen demand (COD) of whey is 20–40 g/L and 50–80 g/L respectively (Buchanan et al., 2023). Lyso-alanine (toxic substance) is formed during chemical hydrolysis of whey (Sinha et al., 2007). Allergenicity can be treated using probiotic microorganism hydrolysis. Some studies reported that the cheese whey when released into the water body causes eutrophication leading to increase in acidity of sea water (Fernández-Gutiérrez et al., 2017).

Whey is available in two forms, that are, sweet and acid whey (Table 7.1). Whey is available in variety of colours depending upon the source of milk that is being used, the colour ranges from yellow/green to bluish tinge (Smithers, 2008). Whey is

Table 7.1 Whey composition and its production

Type of whey	Proteins (g/L)	Lactose (g/L)	Minerals (g/L)	pH	Production	References
Sweet whey (SW)	6–10	46–52	2.5–4.7	5.6–7	Cheese whey, produced during cheese manufacturing process through rennet coagulation	Mulcahy, (2017)
Acid whey (AW)	6–8	44–46	4.3–7.2	4.3 < 5.6	This is produced through fermentation process of lactose to lactic acid	Mulcahy, (2017) and Guo and Wang (2019)

produced almost 20–60% from cow milk (Krissansen, 2007). Basically, it contains water, lactose, soluble proteins, minerals, and trace amounts of lipids (Spălățelu, 2012; Panesar & Kennedy, 2012). Lactose is the important constituent of whey with the positive effect on calcium mineral absorption (Lifrah et al., 2000). Whey protein contains all nine essential amino acids and has high biological value as compared to other sources of dietary proteins. It can be used in the production of industrialized foods, different beverages and protein supplement (Poonia & Pandey, 2023). So, keeping in mind the nutritional importance and environmental issues, it is suggested to work on the possibilities to extract and isolate valuable components using physical, chemical and biological methods. Whey being quantitatively produced protein has a great potential in food industry. There is an immense scope of whey utilization in the manufacture of bakery products (Arya & Poonia, 2019). Whey valorisation is the future of proteinaceous supplements. There is vast majority of value-added whey products in food (beverages, bakery products, jams) and pharmaceutical industry.

Whey being considered as highly proteinaceous source with major potential in food technology. The proteins of whey are globular in their structure and heterogenous in nature. The amino acids are embedded in the protein structure with some alpha-helical motifs. These includes β-lactoglobulin (BLG), bovine serum albumin (BSA), α-lactalbumin (ALA), immunoglobulins, lactoferrin, lactoperoxidase enzymes, glycomacropeptides, and lysozyme with high biological value (Table 7.2) (Marshall, 2004; Madureira et al., 2007). Whey is considered to be functionally active as it contains valuable bioactive compounds, it is completely digested protein with the protein digestibility index of 99 and protein efficiency ratio of 3.2.

Despite the good digestibility of whey protein, it also has other unique characteristics including the presence of huge quantity of branched amino acids, occurrence of beta-lactalbumin, alpha- lactoalbumin, glycomacropeptides, with the functionality at wide range of pH. Whey proteins are known for their numerous health promising effects, that includes, the prevention of diabetes, reduction of asthma risks, prevention of bacterial respiratory infection, DNA damage prevention, positive effect on fatty liver (Chen et al., 2013; Loss et al., 2011; Osama et al., 2013; Hamad et al., 2011; Toden et al., 2007). Not only this, whey proteins are also known to enhance the free-radical scavenging activity of body with the positive impact on muscular strength. It is also known to prevent cancers, obesity and help in weight reduction. Inflammatory factors e.g. IL-1β IL-6, TNF-α may be reduced by using whey in obese people, diabetic patients (Alimoradi et al., 2016). The presence of valine, leucine, and isoleucine play potential role in metabolism of energy, insulin maintenance in blood and neural functionality. The synthesis of skeletal muscle protein is related to the amount of leucine and cysteine is responsible of glutathione manufacturing (Micke et al., 2002). It has progressive nature in reduction of oxidative stress and also helps to prevent redox imbalance-caused diseases (Trachootham et al., 2008).

Table 7.2 Composition and functional benefits of proteins in whey

Proteins	Content (%)	Molecular weight (kDa)	Isoelectric point	Nutritional & functional benefits	References
BLG	45–58	18–18.36	5.2–5.5	Contains essential amino acids. The uptake of retinol is increased. The gelling, emulsifying and foaming characters are enhanced. It is known to be an allergen with fatty acid binding property and possible antioxidant	Marshall, (2004), Ballard and Morrow (2013), Claeys et al. (2014), Parodi (2007), Laursen et al. (1990), Saarela (2007) and Korhonen and Pihlanto-Leppälä (2004)
α-lactalbumin (ALA)	13–25	14–14.15	4.2–4.8	Major human milk protein. Contains essential amino acids which binds calcium and exhibit non-gelling character, lactose production, immunomodulator; anticarcinogen	Ballard and Morrow (2013), Laursen et al. (1990), Saarela (2007), Korhonen and Pihlanto-Leppälä (2004) and Buchanan et al. (2023)
Glycomacropeptides	10–20	7–8.6	<3.8	Contains branched amino acids with antiviral properties	Claeys et al. (2014), Parodi (2007), Laursen et al. (1990), Saarela (2007), Korhonen and Pihlanto-

(continued)

Table 7.2 (continued)

Proteins	Content (%)	Molecular weight (kDa)	Isoelectric point	Nutritional & functional benefits	References
					Leppälä (2004) and Buchanan et al. (2023)
Bovine serum albumin	6–12	67–70	4.6–5.2	Contains essential amino acid with antioxidant properties, growth inhibition effect on human breast cancer cells, opioid agonist	Ballard and Morrow (2013), Claeys et al. (2014), Parodi (2007), Laursen et al. (1990), Saarela (2007), Korhonen and Pihlanto-Leppälä (2004) and Buchanan et al. (2023)
Immunoglobulins,	9–14	140–1100	5.2–8.3	May provide a 'passive immunity', anti-microbial, pathogen binding inhibition, activation of phagocytosis, anti-inflammatory	Marshall (2004) and Parodi (2007), Laursen et al. (1990), Saarela (2007), Korhonen and Pihlanto-Leppälä (2004) and Buchanan et al. (2023)
Lactoferrin,	1–2	77–78	7.9	Bactericidal protein and may inhibit tumour formation, antioxidant, anticarcinogen, antibacterial, antiviral,	Marshall (2004), Ballard and Morrow (2013), Claeys et al.

(continued)

Table 7.2 (continued)

Proteins	Content (%)	Molecular weight (kDa)	Isoelectric point	Nutritional & functional benefits	References
				antifungal, supports the growth of favourable bacteria	(2014), Parodi (2007), Korhonen and Pihlanto-Leppälä (2004) and Buchanan et al. (2023)
Lactoperoxidase	0.5	78–89	9.6	Possesses antibacterial activity	Ballard and Morrow (2013), Claeys et al.(2014) and Parodi (2007)
Lysozyme	0.0003	15.09	12	Antimicrobial agent, synergy actions with immu-noglobulins and lactoferrin	Marshall (2004), Ballard and Morrow (2013), Claeys et al. (2014), Parodi (2007), Laursen et al. (1990) and Saarela (2007)

7.2 Microbial Fermentation

Microbial fermentation is a process through which the breakdown of organic molecules take place via microorganism involvement into simpler ones. Fermentation led to change in physicochemical and sensory property of food. The nutritional profile is enhanced and biochemical changes also take place in food substrate. It is regarded as the natural transformation of food through which changes in flavours, colours, appearance occurs. The aesthetic appeal also increased with the enrichment in vitamins, essential amino acids, anti-nutrients, protein profile. It reduces the cooking time with increase in shelf stability of food product. There, are broad spectrum of microorganisms available for fermentation process, but from all the available varieties LAB (Lactic acid bacteria) and yeast are more preferred on

industrial scale. LAB are considered to be human friendly microorganisms and GRAS identified. They will contribute to the nutritional value via increasing digestibility of protein & lactose. The bioavailability of minerals will get a boost through fermentation process. It also possesses health benefits of improving immune system and reduce cholesterol (Sharma & Gautam, 2007). Therefore, microbial fermentation improves the texture, nutritional and sensory profile of food substrate.

7.2.1 Single Cell Proteins

The increasing population demands more convenient proteinaceous food sources to meet individual needs. This ever-lasting population food demands must be fulfilled by innovative and creative protein sources at economic values. In this direction, Single Cell Proteins (SCP) is the future of protein supplements in the growing world. It is one of the potent protein sources at convenient rate in comparison to soyabeans, milk, and legumes. It is the best alternative protein source in staple diet. SCP is the source of protein, produced with the help of microorganisms, or in simple words, SCP is the dead and dry cell biomass of microorganisms (Anupama & Ravindra, 2000). The most common type of microorganisms which are used in SCP production are yeast, bacteria, fungi and algae. These microorganisms grow or multiply on substrate which acts as the food source for their development (Khan et al., 2010). Whey being the protein complex derived from milk industry as by-product in higher amounts, are most suitable substrate for SCP production. Whey is highly beneficial to human health as it contains many biological properties, which includes, antioxidant property, anti-hypertensive property, anti-cancerous property, and anti-viral property (Marshall, 2004). Whey is bio-transformed into SCP, a value-added product using microorganisms.

The term SCP was coined in 1968 to replace the originally existing terms, that are, "microbial protein" or "petropotein". As living in a society where aesthetic values are more prominent, it is very problematic to accept a microbial based protein source. But with advancement in technology and eagerness to enhance health, this microorganism-based protein source is the best alternative to match-up the increasing globalization food needs. A variety of microorganisms are used in SCP production. The biomass production in SCP can be achieved either by solid-state or submerged fermentation. With completion of fermentation method, the produced cell biomass is subjected to downstream processing which includes cell washing, cell disruption and purification of the extract by applying various advanced membranous technology (Fig. 7.1) (Faust, 1987). In a study, *B. subtilis* (Bacteria) was known for its efficiency in protein usage from whey as compared to both yeast and fungi for the development of SCP. The inoculated whey samples were kept at 37 °C in rotatory shaker incubator for 48 h and the supernatant obtained was analysed for protein estimation by Lowry's method. The main reason for using LAB is the high rate of its cell division (Gomashe et al. 2014). The bacterial SCP is high in protein content with higher number of essential amino acids. Yadav et al., 2016, concluded

Fig. 7.1 Whey based single cell proteins production

that *Kluyveromyces marxianus* alone and *K. marxianus* and *Saccharomyces cerevisiae* in conjugation were used as inoculum in whey, to produce food-grade SCP using batch fermentation method. Fermentation process was carried out at 35 °C for 30 h using *Kluyveromyces marxianus* and at 30 °C for 30 h using mixed cultures, The other microorganisms that are used for SCP production with whey as substrate includes *Aeromonas hydrophylla, Penecillium cyclopium, Candida intermedia, Bifidobacterium* sp. The use of SCP is restricted due to its higher nucleic acid amount, as the high concentration of nucleic acid in human body lead to the development of gout disease. Therefore, to use SCP as human potent protein source, the amount of nucleic acid must be reduced down to below 2% by applying various methods including chemical treatment or enzymatic methods using sodium chloride, sodium hydroxide, ammonium hydroxide and ribonuclease, deoxyribonucleases respectively. Apart from nucleic acid content, toxicology is major aspect of SCP approval as many bacteria and fungi contain toxins which are secondary metabolites. Therefore, toxicity test must be done before marketing of SCP especially in case of human protein supplementation (Anupama & Ravindra, 2000). Hence, SCP is the nutritious protein supplement with wide range of utility in bakery sections, ready-to-eat section, and many more with high future demands.

7.2.2 Bioactive Peptides

Proteins are the polymeric chains of different amino acids joined together by peptide. Despite of the physiochemical role of proteins, these also perform major biological roles in human body (Samtiya et al., 2021). The sequences of amino acids are responsible to describe or identify its biological role. These amino acid sequences are only biologically active, when they are cleaved intact from parent protein without disrupting its native sequence. Bioactive Peptides, commonly known as

"Biopeptides or BAP" are the tiny protein sequences of around 2–20 amino acids cleaved from the whole parent protein sequence (Xu et al., 2019). These are the small isolated protein fragments with unique physiological roles. Whey is the excellent protein source and believed to have many biological properties (Table 7.3). Hence, whey based bioactive peptides can serve to be very effective and efficient in future developments.

Till date, numerous whey based bioactive peptides has been identified which poses certain biological activities. For BAP production various methods are used viz., enzymatic hydrolysis, microbial fermentation, in silico analysis, germination, gastrointestinal digestion, and genetic engineering (Fig. 7.2). However, recent studies indicates that the enzymatic method was predominantly used in whey-based BAP production. Whey being the potent protein source for BAP production from whey. The biological properties associated with whey BAP are anti-microbial, Immunodulatory, cytomodulatory, anti-hypertensive, antioxidant, antithrombotic, and Hypocholesterolemic, (Madureira et al., 2010).

Whey based bioactive peptides can be produced by using various microbial species including *Lactobacillus helveticus*, *Saccharomyces cerevisiae* etc. with different bioactivities (Table 7.3) (Mann et al., 2015). In a study, *Lactobacillus helveticus* was used for BAP production which resulted in the development of tripeptides (VPP & IPP) with ACE-inhibitory activity (Aihara et al., 2005). Hence, they have great application prospects as potential functional ingredients in the food industry.

7.2.3 Bacteriocins

The agro-food industries and regulatory agencies are favouring about the usage of natural additives e.g. color, flavour, preservatives etc. Bacteriocins are the major step in this direction, as bacteriocins are the ribosomal antimicrobial peptides which are active against spoilage microorganisms and may act as preservatives in growing food industry (Sabo et al., 2019). They are basically small peptides with heat resistance property (Guerra et al., 2001). These are proteinaceous in nature and are generally produced using GRAS (Generally recognised as safe) microorganisms such as lactic acid bacteria (Table 7.4). The bacteriocins either kill or inhibit microbial growth, i.e., antagonist in nature. The optimization of pH, temperature and relative humidity is very important, as these will affect the bacteriocin production drastically (Cladera-Olivera et al., 2004). The production of bacteriocin will take place in a complex media having nutritious components. Whey as substrate for the bacteriocin production is the best alternative to other complex proteinaceous compounds because of its composition and utilization. Whey is considered to be the waste of cheese industry but this bacteriocin production makes whey as valuable as cheese. Different microorganisms have been used to produce bacteriocins using whey (Table 7.4).

Table 7.3 Bioactive peptides from whey and their biological activities

Protein source	Production method (enzymatic hydrolysis)	BAP sequence	Biological activity	References
β-lactoglobulin	Corolase PP	W_{19}YSLAMAASDI$_{29}$	Antioxidant activity	Hern'andez-Ledesma et al. (2005)
	Trypsin	V_{15}AGTWY$_{20}$, A_{25}ASDISLLDAQSAPLR$_{40}$, I_{78}PAVFK$_{83}$	Antimicrobial activity	Pellegrini et al. (2001)
	Chymo-trypsin	H_{146}IRL$_{149}$	Opioid activity	Pihlanto-Lepp¨al¨a et al. (1997)
	Alcalasa	KTKIPAVF, IDALNEK, HIRLS	Antioxidant activity	Mann et al.(2015)
	Corollase	MAASDISLL, VEELKPT	Antioxidant activity	Mann et al. (2015)
	Pepsin	KVAGT, IRL, VRT, LMPH, EKF	Antimicrobial activity	Th'eolier et al. (2013)
	Cardunculus	D_{97}KVGINYW$_{104}$ D_{33}AQSAPLRVY$_{42}$	ACE inhibition	Tavares et al. (2011)
Lacto-ferrin	Pepsin	F_{17}KCRRWQWRMKKLGAPSITCVR RAF$_{41}$	Antimicrobial, Antitumoral	Vorland and Rekdal (2002)
α-lactoglobulin	Protease from flower of Cynara	K_{16}GYGGVSLPEW$_{26}$	ACE inhibition	Tavares et al. (2011)
	Synthetic	f(18–20) Tyr-Gly-Tyr, f(50–51) Tyr-Gly	Lymphozyte proliferation	Gauthier et al. (2006)
	Pepsin	KVGIN	Antimicrobial activity	Th'eolier et al. (2013)
	Flavorzyme	VGINYWLAHK	Antioxidant activity	Mann et al.(2015)

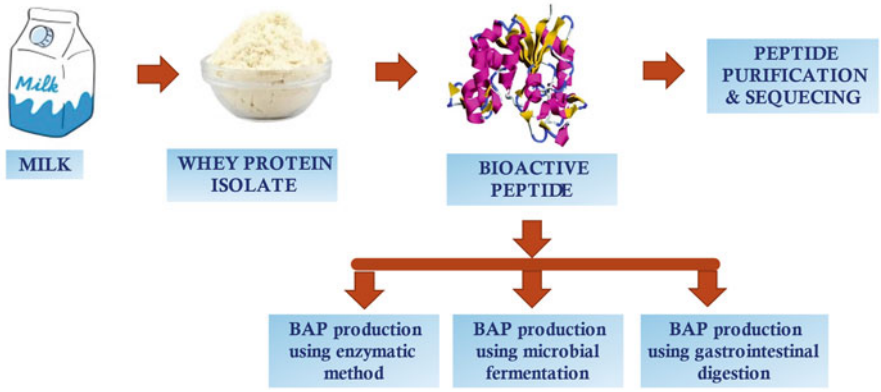

Fig. 7.2 Whey based bioactive peptide production

Table 7.4 Whey derived bacteriocins and their production conditions

Microorganism involved	Substrate	Conditions	Bacteriocin	References
Lactobacillus plantarum ST16Pa	Whey powder formulations	Incubated for 24 h at 30 °C in an orbital shaker (100 rpm)	Bacteriocin ST16Pa	Sabo et al. (2019)
L. lactis subsp. lactis CECT 539 & Carnobacterium piscicola CECT 4020	Whey	Incubation for 20 h at 30 °C	–	Guerra et al. (2001)
B. linens ATCC 9175	Cheese whey medium	–	–	Motta and Brandelli (2003)
Lactococcus lactis	Goat cheese whey	Incubated at 37 °C for 5 h	–	Lima et al. (2017)
Bacillus licheniformis strain P40	Acid whey	Incubated at 30 °C under Shaking for 24 h	–	Cladera-Olivera et al. (2004)
Pediococcus pentosaceus 147	Cheese whey broth	Incubated at 37 °C for 24 h	Pediocin	Gutiérrez-Cortés et al. (2018)

7.2.4 Biofuels (Ethanol, Butanol, Hydrogen Etc.)

The ever-lasting global population needs safer and environmentally friendly energy sources as, fuels are most important in today's era as it the basic necessity of living. Biofuels are the type of energy sources which are safe for our environment and their consumption will not cause any harmful effects. The burning of fossil fuels causes majority of environmental threat, that are, green house gases emission, biodiversity loss, air pollution, global warming, and ozone layer depletion (Franta, 2018, 2021;

Fig. 7.3 Biofuel production flowchart

Table 7.5 Biohydrogen from whey and their production

Substrate	Microbial strain	Hydrogen yield (mol H_2/mol of lactose)	Hydrogen productivity (mL/g/h)	References
Cheese whey powder	*L. acidophilus*	1.02	–	Patel et al. (2016)
Cheese whey (permeate)	*M. consortium*	3.63	141.05	Romão et al. (2019)
Cheese whey (powder)	*M. consortium*	1.15	1082	Lima et al. (2016)
Fresh cheese whey	*Clostridium* sp.	6.37	138	Patel et al. (2016)

Yoro & Daramola, 2022). Whey is the highly produced by-product of dairy industry with the potential of biofuel manufacturing (Fig.7.3). Bioethanol, bio-methanol, biohydrogen are some of the examples. These are renewable sources of energy. Cheese whey has the major potential to be used as biofuel but the limiting factors creates the production system a tedious one. The limitations include the high acid content, dissolved oxygen, biological demand and low solid content in cheese whey (Tsolcha et al., 2016; Lavelli & Beccalli, 2022). There are many technologies developed to treat cheese whey to overcome the above-mentioned limitations, that includes, physical (Filtration), chemical (Catalysis), biological (Aerobic/anaerobic digestion), and physiochemical (Flocculation/coagulation) methods.

Bioethanol is the best alternative for gasoline, with wide application in cosmetic, pharmaceutical, food and beverage industries (Das et al., 2016). The bioethanol production is directly depends on the kind of microbial strain and yield can be increased up to 80%. *Kluyeromyces* sp., *Saccharomyces* sp., *Candida* sp., *Neolentinus* sp. are some the microorganisms that can be used. Anaerobic digestion is the main mechanism through which methane (Biogas) can be produced, with major applications in electricity, heat and renewable gas production. As anaerobic digestion is mainly applied in methane production but there are some complications which can be overcome by using different reactors and other recent technologies (Lisowyj & Wright, 2020; Amani et al., 2010).

Biohydrogen production can be done through fermentation, biophotolysis and bioelectrochemical method (Table 7.5). These are not at all cost-effective method as they are the newest technologies applied in this field with some challenges. Commercially, hydrogen is produced through steam methane, coal gasification and oxide electrolyzer technologies which are not at all environmental friendly methods (Tian et al., 2019; Gopalakrishnan et al., 2019).

7.2.5 *Bioplastics*

The non-biodegradability of plastic waste in the environment is a major concern to human health and ecosystem worldwide. That's why alternatives, including bioplastics, are being promoted to prevent waste. Whey, which is currently used as a base material for the manufacture of bioplastics, has become an area of research (Table 7.6) and world whey production is estimated about 200 million tons per year, with an annual growth rate of 2%, of which 47% is simultaneously discharged into the sewage system. Compounds containing polyhydroxyalkanoates (PHA) and polylactic acid (PLA) can become bioplastic due to the presence of lactose in serum. Because these whey bioplastics are biodegradable, they are useful alternatives to conventional plastics. Polyhydroxyalkanoates are polyesters of different hydroxyalkanoates that can be produced using different microbes, where they act as carbon and energy storage materials. There are three methods of microbial conversion of whey lactose to polyhydroxyalkanoates. The first is the direct conversion of lactose to polyhydroxyalkanoates by microorganisms such as *Hydrogenophaga pseudoflava* and *Escherichia coli* (both of which have beta-galactosidase activity). The second is the enzymatic hydrolysis of lactose, followed by the fermentation of glucose and galactose monomers. The resulting glucose and galactose monomers can be used in *Pseudomonas hydrogenovora* to produce polyhydroxyalkanoates (for strains lacking beta-galactosidase activity). Another way suggested is to convert lactose to lactic acid with the help of Lactobacilli and lactic acid is subsequently used to produce polyhydroxyalkanoates (Koller et al., 2007). The production value of bioplastics from whey may decrease due to new fermentation, processing and purification technologies and the use of

Table 7.6 Bioplastics from whey and their production

Bioplastics	Source	Microorganism	Condition	References
Polyhydroxyalkanoates	Whey	*Hydrogenophaga pseudoflava, pseudomonas hydrogenovora Escherichia coli*	pH 7.0	Bosco and Chiampo (2010)
Polylactic acid (lactic acid)	Whey and whey permeate	*Lactobacillus bulgaricus, Lactobacillus casei, and Lactobacillus acidophilus*	37 °C and pH 6.5, 36 h of incubation	
Poly (3-hydroxybutyrate)	Acid whey	*Lactobacillus* sp. *Rhodovulum sulfidophilum DSM-1374*	–	
Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) with hydroxyvalerate	Ricotta cheese exhausted whey	β-Galactosidase treatment and Haloferax mediterranei DSM1411	pH 6.5–4.5 at 37 °C,5 h	
PHA with a hydroxyvalerate (HV)	Cheese whey	Mixed photosynthetic consortium of bacteria and algae	pH 6.5,at 30 °C	

microorganisms that over concentrate PHA. Bioplastics are used for packaging and insulation of food and disposable cutlery. PLA is used in the form of screws, plates, pins and meshes for scientific textile work. It can also be used as a compost bag, compost food and tableware. Polylactic acid (PLA) is a biodegradable bio polyester made from lactic acid monomers. Its properties are similar to polystyrene (PS) and polypropylene (PP) and have low toxicity. PLA is an excellent alternative to common hydrocarbon-based polymers. Cellulac was one of the leading commercial producers of lactic acid from deproteinized lactose whey. They used a non-GMO (genetically modified organism) Lactobacillus to turn deproteinized lactose whey into a bioplastic. Bioplastics can also be synthesized using whey proteins. Whey protein bioplastic is a desirable packaging material due to its excellent oxygen barrier properties and sufficient solubility. Bioplastic whey protein is obtained using a combination of whey protein with plant latex and egg albumin. When mixing plant latex and egg albumin, the correct presence of amino acids with complementary reactive structures in the polypeptide chain is selected so that the mixture is compatible. Whey protein bioplastics have the thermal process ability to produce large-scale food-like films for the food and packaging industry. The main challenging conditions for an integral bioplastic based on whey protein are heat and water stability. Consistency and reliability with overall internal mechanical performance; price and competitiveness. Some of these difficulties can be overcome by blending, where additional polymers are blended into a single polymer fabric. In the case of, the weakness of an element can be somewhat hidden by the energy inside the end piece. The combination of complex elements in a homogeneous mixture loses its consistency. The last house in the combination follows the "shuffle rules". The nature of the impurities found in the mixture depends on the length of the mold and the adhesion to the interface. Therefore, many studies have been conducted to increase the use of bioplastics in blends. Bioplastics can be made from a combination of starch, rubber, latex, whey protein and biodegradable polymers to improve overall performance and water resistance (Wu et al., 2007).

7.2.6 Organic Acids

Industrial cheese production generates 26% of all food waste so the disposal and proper processing of cheese whey is becoming increasingly important due to increasing waste disposal costs and environmental concerns. Lactose-rich waste from protein separation (whey permeate) is highly contaminated and requires creative waste management. This is because only about 60% of the world's whey production is converted into marketable products such as whey proteins (Panesar & Kennedy, 2012). Whey is an economical and sustainable raw material for the production of organic acids due to its availability, affordability and lack of competition from food sources. In addition to lactic and succinic acids, several studies have shown that whey can be used for the microbial synthesis of carboxylic acids, including acrylic, lactobionic, and succinic acids (Alonso et al., 2013, Wang et al.,

2009). Unlike many cheap raw materials, no pre-recovery is required before submerged fermentation. Currently, there are some microbes that cannot digest lactose, but these are reduced when whey is used as a carbon source for natural acid production. Control of whey uptake by microbial lactose uptake and overexpression of heterologous lactose transporters or metabolic pathways can certainly increase the value of whey as a cheap raw material. Organic acids such as lactic acid, propionic acid, succinic acid, acetic acid and formic acid are used in various food, pharmaceutical, cosmetic and chemical industries. Demand for natural acid production has improved due to the power of microbial methods and the use of cheap natural waste to improve common petroleum-based acid production strategies. As a result, a number of studies have investigated cost-effective and environmentally friendly approaches to enzymes and enzyme production from natural acids and the use of whey as a substrate (Louaste & Eloutassi, 2020). Lactic acid bacteria (LAB) produce the enzyme β-galactosidase to ferment and bio convert whey lactose to lactic acid. LAB produce lactic acid by homolactic fermentation of lactose, and at some stages of lactose fermentation, LAB heterolactic acid fermentation lines produce acetic acid and ethanol along with lactic acid (Kaur et al., 2020). Whey permeates and whey protein hydrolyzate have also been effectively used as carbohydrate rejuvenating agents for lactic acid production, along with *L. plantarum* strain (Sharma et al., 2021). Succinic acid is in high demand in the chemical, pharmaceutical and food processing industries and is currently produced using chemical methods including electrolytic reduction and catalytic hydrogenation of maleic anhydride (Cok et al., 2014). However, the excessive value of greenhouse gases associated with chemical production and fuel technology has led to the search for enzyme production of succinic acid and its use as an agro-commercial commodity (Louaste & Eloutassi, 2020). Batch fermentation of whey by *Actinobacillus succinogenes* was completed with a succinic acid yield of 62.1% (Louaste & Eloutassi, 2020). Similarly, the electroactive fermentation of whey *K. marxianus* has been implicated in the production of several natural acids, including acetic, lactic, citric and propionic acids (Karim & Aider 2022). However, low substrate pH due to wild acid production was associated with a decrease in fermenting spores, ultimately leading to a decrease in wild acid production (Louaste & Eloutassi, 2020). Formic acid production was also associated with a 30% reduction in A. succinogenes mobile wood (Lin et al., 2008). Therefore, pH control during the fermentation or the use of acid-tolerant fermentation spores can ensure long exponential phases, resulting in better yields from natural acid production and the use of whey as wood medium.

7.2.7 Polysachharides (Sugar)

Polysaccharides are like natural, safe, and eco-friendly building blocks providing textural and coating attributes. These are involved in protection from germs, surface adherence, and communicating signals (Kumar et al., 2007; Sutherland, 1998). Most of whey is made up of something called lactose, which is a type of sugar. Some

people have used cheese whey carbon and nitrogen source of xanthan (Silva et al., 2009) and gellan (Fialho et al., 1999). Some strains of bacteria (*campestris* strains) were able to make a substance called xanthan from mozzarella cheese whey, but with lower efficiency (Silva et al., 2009). Another bacterium (*S. paucimobilis*) has been used to produce gellan from sweet cheese whey in high amount due to microbes ability to utilise lactose (Pollock, 1993). Another bacterium (*L. mesenteroides* NRRL B512 cultures) was used to produce dextran from whey too, but they had to take out the proteins first and add something called carob extract to help it work better.

7.2.8 Lipids

The manufacturing of lipids, especially triacyl glycerides (TAG), is crucial for power garage and may be transformed into biodiesel through transesterification reactions (Sharma et al., 2012). Algae are recognized to build up lipids as a method of resisting unfavorable environmental situations, with TAG being produced in most cases strain situations which includes nutrient starvation, radiation, pH, osmotic, temperature stresses, and presence of heavy metals and different chemicals (Wang et al., 2009). Microalgae are a promising supply of lipids, as they may be without problems incorporated right into a bio refinery for acquiring lipids with excessive productiveness the usage of less expensive media consisting of wastewater streams (Menetrez, 2012). *Chlorella protothecoides* is an especially green microalgae lipid manufacturer and may be grown in whey permeate the usage of glucose as a substrate for lipid synthesis. Recent research have proven that a pressure of *C. protothecoides* became capable of production of 42 g of lipids on dry weight bases, cultured in whey permeate in batch culture; at the same time as 20.5 g became acquired the usage of fed-batch cultures (Espinosa-Gonzalez et al., 2014). Productivity turned into even better while simultaneous scarification and fermentation tactics in batch mode with the use of immobilized enzyme. Other oleaginous microorganisms, which includes *Apiotrichum curvatum* and traces from *Cryptococcus*, also are able to generating lipids at some stage in their increase in whey, with *Cryptococcus laurentii* having a most lipid productiveness of 0.00822 g/L/h with excessive content material of 15- and 17-carbon chain with saturated and monosaturated fatty acids, that are taken into consideration appropriate for biodiesel manufacturing.

7.3 Enzymes

Enzymes are maximum treasured and industrially crucial organic molecules the are used to enhanced the chemical reactions. The whey is used as substrate to produce enzymes by yeast, bacteria, algal and fungal lines (Table 7.7). Enzymes include proteases, amylases, poly-galacto-uronase and beta galactosidase etc. Whey is

Table 7.7 Enzyme production from whey using microbial fermentation

Enzyme	Substrate	Microorganisms	Process condition	References
Beta-galactosidase	Whey	*L. paracasei MK852178*	pH 7.0, temperature 37 °C	
Beta-galactosidase	Cheese whey	*Enterobacter ludwigii*	pH 7.3	Alikunju et al. (2018)
Beta-galactosidase	Whey	*K. lactis*	pH 7.0–8.48, temperature 27.6 °C	You et al. (2017)
Beta-galactosidase	Cheese whey permeate	*Saccharomyces fragilis IZ 275*	pH 5.0, temperature 35 °C	Bosso et al. (2019)
Beta-galactosidase	Acid whey permeate	*Tetradesmus obliquus*	pH 6.8, temperature 21 °C	Bentahar et al. (2019)
Pepsin	Whey	*Pythium* sp. *and Staphylococcus sciuri*	pH 3.5 and temperature 35 °C	
Polygalaxturonase	Whey	*Kluveromyces fragilis*	pH 5.2, temperature 37 °C	Donaghy and McKay (1994)
Protease	Cheese whey	*Bacillus* sp.	pH 9.0, temperature 40 °C	

considered to be a raw material for generating diverse forms of industrially great enzymes with bacterial, fungal, yeast and algal lines that have been proven in one-of-a-kind research. Because of the excessive content material of lactose with inside the substrate, whey has been extensively used for generating β-galactosidase (Kaur et al., 2020). It is manufactured using whey, rice straw, and orange peel (*L. Paracasei* MK852178) as starting material which confirmed stepped forward enzyme manufacturing through 5 times (1.784 × 104 U/mL), depicting the opportunities of the use of agricultural waste for generating value-brought merchandise. It can also be produced via means of the psychrotrophic bacterium; cheese is made the use of *Enterobacter ludwigii*. When medium is optimized, whey confirmed a 3.5-fold growth in enzyme manufacturing (33.09 U/mL) (Alikunju et al., 2018). Several research have proven the manufacturing of β-galactosidase using yeasts and whey as a growth medium instead of bacterial lines (Bosso et al., 2019; You et al., 2017). β-galactosidase manufacturing through *K lactics* is an unidentified procedure (You et al., 2017).When the β-galactosidase is produced via means of the fermentation of micro filtrated cheese. Whey is permeated through *Saccharomyces fragilis* IZ 275 that is related to enzyme yield of 14.78 U/mL (Bosso et al., 2019). In the dairy industries, β-galactosidase acquired from fungal traces, which include *A. niger and A. oryzae* are broadly used for generating nutraceuticals and value-brought merchandise and those have excessive industrial value. The fungal traces are used for generating enzymes the use of whey that is a less expensive substrate (Kaur et al., 2020). Whey permeates as substrate while β-galactosidase is constituted of microalgae which include *Tetradesmus obliquus*, the use of acid proven recently (Bentahar et al., 2019). Due to the dietary complexity of whey, it has the capacity to ferment lines to provide quite a few enzymes. Pepsin, an industrially large enzyme,

is made with the aid of using combined inoculum comprising *Pythium* sp. and *Staphylococcus sciuri* the usage of whey as a carbon source. The acquired pepsin indicates a 3.714-fold growth in enzyme hobby at most excellent pH of 3.0 and an most suitable temperature of 35 °C whilst its miles partly purified. The boom of the yeast *Candida antarctica* and the manufacturing of the particular cutinase-like enzyme, PaE, which suggests biodegradable plastic-degrading interest, is because of the cheese whey that's used the carbon source. From all of the research we are able to finish that whey may be utilised as a low-value medium for the manufacturing of industrially substantial enzymes.

7.4 Conclusion

Whey is novel protein source with abundant health benefits and can be used an ingredient, substrate, and fortificant for various value added products. The regulatory bodies guidelines favours and motivates the professionals to work on the whey valorisation and solve the environmental issue also. The advanced technologies in conjugation with physical, chemical, and biological methods can be adopted for extraction of valuable components form whey. Biofuels, bioactive peptides, lipids, polysaccharides, bacteriocins are the broad sectors of whey utilization. The future of whey is highlighted due to its higher biological value and great impact in pharmaceutical industry. Bioethanol production has the major potential in food industry. Overall, whey valorisation will have golden future of food industry.

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Chapter 8
Whey: A Potential Substrate for the Production of Natural Pigments

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Abstract Whey is a spin-off by-product from the paneer, cheese, and casein firms and is regarded as a dairy squander. The production of whey ranges from 180 million tons to 1900 million tons yearly. Whey is a significant contaminant due to the number of raw materials it contains. Due to its high biological and chemical oxygen requirements, it presents a considerable threat to the ecosystem. Whey contains 4.5–5.0% lactose, 0.6–0.7% protein, and 0.4–0.5% lipids, and it is a good carrier of vitamins, and minerals. Because of its rich nutritional extent, it provides valuable

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substrate for the microbes required to make natural colors. This chapter investigates manufacturing processes of several types of pigments from whey, a low-cost substrate, and the applications of such pigments in diverse industries. This chapter focuses on different natural pigment types, stability, ways to increase their production, and their function in the circular economy. Whey is used to create natural colors. Adding it to food products enhances its antioxidant properties, which contribute to health improvement by chelating free groups from the body and enhancing the color of food. The sustainable habituation of whey to produce natural colors may improve the biological foundation of the economies of many industries and, as a result, the entire national and global economy. It may be possible to produce natural colors more effectively and sustainably using whey. The circular economy and sustainability strategy will benefit reliant firms and health-conscious consumers. The main topic of this chapter is the possible use of whey for the synthesis of natural colors employing a range of species.

Keywords Whey · Pigments · Chelating · Biological activities · Health improvements

8.1 Introduction

Whey is commonly regarded as dairy waste and is a raw material for producing cheese, paneer, and casein firms (Poonia & Pandey, 2023). Lactose is the sugar that makes up the majority of whey, accounting for around 50% of the total milk solids, and is followed by proteins, minerals, and other minor substances (Rocha-Mendoza et al., 2021). A sufficient sum of lactose is ubiquitous in whey and enhances the soaking up of zinc and magnesium (Sangeetha et al., 2022). Whey proteins provide significant biological value predominately due to albumins, globulins, vitamins, lactic and citric acids, amino acids, and minerals (Minj & Anand, 2020). The market for whey-derived components is expected to increase at the quickest rate of any other dairy ingredient today, from $53.8 billion in 2019 to $81.4 billion by 2025 (Babar et al., 2021; Tsermoula et al., 2021).

Whey also contains an abundant supply of sugars, which supports its appropriateness for the commercial manufacture of various biotechnological products (Rocha & Guerra, 2020). Although several contemporary methods of whey valorization have been investigated, the bacterial fermentation of whey into affordable agitation broth to synthesize diverse natural colors can offer a long-term solution for whey use (Poonia & Pandey, 2023). Whey contains lactose, which is inexpensive and a useful carbon source for various bacteria, so microorganisms can easily utilize it as a carbon source. Hence, using whey to make pigments is one option to lessen the impact on the environment and the nutritional and financial waste in the dairy business (Ali et al., 2021a), making a whey a good substrate for bioprocessing (Rani et al., 2023). A creative strategy for reducing pollution and maintaining the long-term survival of the circular economy is to use whey to manufacture natural colors (Barone et al.,

2021). Hence, using whey as a cheap agitation broth has elongate been of interest to industry.

8.2 Whey Types

Whey's nutritional value varies depending on the chemical makeup of the milk, which is affected by the animal's age, type, and nutrition schedule (Bekhit et al., 2022). Cheese whey, sweet whey, salty whey, whey (Edam), and caprine cheese whey are all listed as some of the variations of whey (Poonia & Pandey, 2023). Cheese whey contains 35.28 g/L of lactose, 14.8 g/L protein, and 59.76 g/L of total solids (Poonia & Pandey, 2023). Furthermore, sweet and salty whey had 5.8–8.5% and 8.6–12.4% total solids, 0.6–1.0% and 0.6–0.7% protein, and 0.2–0.5% and 0.6–0.8% fat, respectively (Poonia & Pandey, 2023).

8.2.1 Acid Whey

Acid whey is the term used to describe whey produced through isoelectric precipitation or rennet-induced coagulation (Lucey et al., 2022). Calcium, magnesium, phosphate, and citrate are much more abundant in acid whey than in other types of whey. Acid whey is typically manufactured commercially from effectively skimmed milk to produce acid-coagulated cheeses or acid (isoelectric) casein (Kravtsov et al., 2021). It is fat-free but includes some phospholipids and has a pH range between 3.57 and 4.34.

8.2.2 Sweet Whey

Sweet whey is the term used to describe whey produced through isoelectric precipitation or rennet-induced coagulation (Jyry, 2021). Sweet whey is an outcome of the production of rennet-thickened cheese or rennet casein, and the makeup of this by-product varies depending on where it comes from. For example, pH ranges from 6.2 to 6.6 depending on how much acidification occurred before whey detachment (Achaw & Danso-Boateng, 2021).

8.3 Whey Proteins

Whey proteins are produced by removing casein from the serum and causing it to coagulate under the influence of acid or rennet (Li & Zhao, 2019). The major whey proteins include immunoglobulins, bovine serum albumin, lactoglobulin and lactalbumin. Other minor proteins include lactoferrin and lactoperoxidase (Ostertag et al., 2021). The primary serum protein is lactoglobulin, and because of its abundance in whey, it predominates the characteristics of whey protein formulations, particularly heat-induced responses. Its solubility is significantly influenced by pH and ionic persuasiveness, but it is still soluble when milk is made more acidic. The secondary and tertiary geometries of lactoglobulin have been widely studied because of their significance. It has a single free sulfhydryl radical inhaled deep within the molecule in its original state and two—S-S linkages. When the pH or heat changes, the protein experiences significant variations in its tertiary and quaternary shape (Ostertag et al., 2021).

Lactalbumin functions as a coenzyme in lactose production. It is a somewhat spherical, succinctly folded protein that is modest in size (Scholl, 2016). A small protein called bovine albumin or blood serum leaks into milk from the blood serum (Zhang et al., 2022). It has three globular domains, making it a big molecule with an extended structure. It has an unpaired thiol group and 17 disulfide links (Zhang et al., 2022). Antibodies called immunoglobulins are generated in reaction to activation by particular antigens. They are substantial glycoprotein molecules excreted by several secretory cells, giving them a diverse composition (Ventura et al., 2023). The whey obtained following the manufacturing of rennet cheese frequently incorporates glycomacropeptide in complement to these proteins present in milk whey (Aprianita, 2015).

Initially thought to be an iron-tie protein with antimicrobial characteristics, lactoferrin is now known to play several crucial physiological duties, such as controlling iron homeostasis, enhancing parasite defence in opposition to a variety of microbial infections, reducing inflammation, and preventing the growth and metastasis of cancer cells (Singh et al., 2019). Infant formula, dietary meals and additives, sports nutrition ingredients, and pharmaceutical goods all include lactoferrin (Ali et al., 2021b; Mehra et al., 2021; Ahmad et al., 2023). The secretary epithelial cells produce lactoperoxidase, an enzyme that functions as a natural antibacterial. Both dietary supplements and personal care items like shampoo, skin lotion, and mouthwash include lactoperoxidase (Gupta et al., 2022). Little research has been done on the usage of whey in the creation of natural colours. A highly appealing biotechnological method for producing various pigments that have been extensively utilized as food colors in Asian nations is the production of microbial pigments using whey. Several variables, including temperature, pH, carbon supply, forms of fermentation, light intensity, humidity, minerals, nitrogen source, moisture content, and aeration rate influence the generation of microbial pigments. Some desired characteristics of microorganisms employed for commercial manufacture of

pigments include acceptance of a broad range of carbon and nitrogen sources, wide range of pH, and a wide range of temperatures.

8.4 Market Trends in Natural Pigments

Previously, only natural colorants such as saffron, paprika, turmeric, prickly pear, and similar plants were employed (Meena et al., 2022). Synthetic colors were first created in the middle of the nineteenth century and quickly gained popularity as food coloring agents due to their devalued production, eminent tectorial persuasiveness, and chemical constancy (Mohiuddin, 2019). However, employing numerous potentially harmful synthetic chemicals as food pigments has consequently led to the discovery of many health issues in later years, which resulted in the outlawing of numerous such food color additives (Boutillier et al., 2022). Although there are currently strict regulations in place in various countries for the authorization of synthetic pigment used as a food additive, consumers are also more orientated toward their replacements with the increased use of compounds originating from natural origin, mainly due to the awareness about their health benefits (Sarkis et al., 2020). However, synthetic colors still account for a sizable portion of the food coloring market.

Synthetic food colorants are replaced with natural pigments or colored chemicals produced by plants, animals, microbes, or mineral ores. For example, iron oxides (E172), calcium carbonate (E170), and titanium dioxide (E171) are a few mineral pigments that the FDA has approved as food colorants (Silva et al., 2022). Although, in most cases, the monetary value of synthetic pigmentation is less than that of natural colors for similar shades, the large-scale manufacturing of natural pigments may reduce this disparity (Rana et al., 2021). Some of the natural pigments are also related to cytotoxic, antioxidant, antibacterial, anti-malarial, anticancer, antitumor, and antifouling actions and have some potential nutritional benefits (Nabi et al., 2023; Anwar et al., 2021; Ramesh et al., 2019). Furthermore, the documentation procedure prior employed as a food supplement does not apply to identical compounds generated by chemical procedures and natural colorants and pigments (Rana et al., 2021; Ali et al., 2022a). Natural pigments primarily come from plants, but those derived from microbial origin have distinct benefits (Table 8.1).

8.5 Different Types of Microbial Pigments

Microbial pigments appear more reliable, affordable, non-dependent on seasonal variations, amenable to yield enhancement, and easily extractable than plant-based pigments (Thakur & Modi, 2022). The bacterial overuse for pigment production will likely help the environment where the market offers various food ingredients using fermentative processes (Voidarou et al., 2020).

Table 8.1 Few examples of firmly significant pigmented microbes separated from different conditions and their applications

Pigmented microbe	Pigment	Application	Source	References
Bacteria				
Paracoccus carotinifaciens	Astaxanthin	Coloring agent	Soil	Hayashi et al. (2021)
Streptomyces sp.	4,8,13-trihydroxy-6,11-dione-trihydrogranaticins A (TDTA)	Feed additive	Soil	Ramesh et al. (2022)
Bacillus	Carotenoid	Colorant	Different sources	Koyanagi et al. (2021)
Janthinobacterium sp.	Violacein	Antimicrobial	River water	Lyakhovchenko et al. (2021)
Streptomyces cavourensis	Melanin	Antimicrobial	Sea cucumber	Wibowo et al. (2022)
Serratia marcescens	Prodigiosin	Dye, antimicrobial	Soil	Giri et al. (2004)
Planococcus sp.	Carotenoid	Food additive	Wastewater	(Majumdar et al. (2019)
Chromobacterium violaceum	Violacein	Antimicrobial	River water and agricultural waste	Durán et al. (2016)
Fungi				
Talaromyces albobiverticillius	*Monascus*-like	Industrial	Marine	Venkatachalam et al. (2018)
Monascus ruber	*Monascus* red pigment	Food colorant	Soil	Darwesh et al. (2020)
Monascus purpureus	*Monascus* red pigment	Food colorant	Red mold rice	Chen et al. (2021)
Yeast				
Rhodotorula paludigena	Carotenoid	Fish feed	Mangrove	Rekha et al. (2022)
Rhodotorula glutinis	Carotenoid	Food colorant	Soil	Taskin and Erdal (2011)
Xanthophyllomyces dendrorhous (=*Phaffia rhodozyma*)	Astaxanthin	Food additive	Trees	Libkind et al. (2012); Libkind et al.(2018)
Cyanobacteria				
Arthrospira platensis	Phycocyanin	Food and drug	Seawater	Leema et al. (2010)
Arthrospira platensis (=Spirulina platensis)	Phycocyanin	Dye and food additive, fluorescent probe	Freshwater	(Kissoudi et al. (2018); Zheng et al. (2019)
Arthrospira maxima (=*Spirulina maxima*)	Phycocyanin	Food and drug	Freshwater	Ruiz-Domínguez et al. (2019)

(continued)

Table 8.1 (continued)

Pigmented microbe	Pigment	Application	Source	References
Microalgae				
Galdieria sulphuraria	Phycocyanin	Food and drug	Hot and acidic springs	Sørensen et al. (2013)
Cyanidioschyzon merolae	Phycocyanin	Food colorant	Hot sulfuric springsand geysers	Rahman et al. (2017)
Cyanidium caldarium	Phycocyanin	Food colorant	Thermal area	Eisele et al. (2000)
Phaeodactylum tricornutum	Fucoxanthin	Anti-inflammatory	Marine	Lee et al. (2021)
Cyanophora paradoxa, *Dunaliella salina*	Zeaxanthin and β-cryptoxanthin	Anticancer	Freshwater	Baudelet et al. (2013)
Haslea ostrearia	Marennine	Food colorant and drug	Seawater	Francezon et al. (2021)
Haematococcus pluvialis	Astaxanthin	Feed additive	Freshwater	Kang et al. (2006)
Chlorella vulgaris	Carotenoids	Food and drug	Freshwater	Ru et al. (2020)

Fungi, bacteria, and microalgae are among the microorganisms familiar with producing a variety of natural hue substances that differ noticeably in their chemical components, activities, stabilities, and solubility (Medina-López et al., 2022). These naturally present colors reflect secondary metabolites highly valuable commercially in the food and dairy, cosmetics, pharmaceutical, textile, and tinting industries. According to their chemical makeup, functional properties, and natural occurrence, they can be dichotomous into various characteristics. The primary pigments found in microbial sources used as food colorings are riboflavin, carotenoids, canthaxanthin, prodigiosin, phycocyanin, melanin, violacein, astaxanthin, and lycopene (Sen et al., 2019).

Riboflavin, or B_2, is a water-soluble colorant with a yellow tint that is used as a food additive and nutritional supplement in dairy products, sauces, infant foods, fruit, and energy drinks (Malabadi et al., 2022). It enables the body to break down food polymeric substances, including proteins, carbs, and lipids, to produce energy and utilize oxygen. A very abundant source of vitamin A for the human body, beta-carotene, a reddish-orange water-insoluble organic color, enhances immunity, delays aging, and aids in night vision problems (Sarkar, 2019). It is known to be produced by several bacteria, including *Mucor circinelloides*, *Blakeslea trispora*, and *Phycomyces blakesleeanus* (Sarkar, 2019). The lipid-soluble pigment cantha-xanthin is a keto-carotenoid with an orange to dark pink hue. Its synthesis by Bacteriochlorophyll-containing bacteria like *Bradyrhizobium* sp. and *Halobacterium* sp. has been documented (Geron, 2022).

It has been demonstrated that prodigiosin has insecticidal, antibacterial, antifungal, anticancer, and anti-malarial properties (Ramesh et al., 2022). Blue-green algae

that contain chlorophyll-A create the photosynthetic pigment phycocyanin, which is blue in hue (Adams et al., 2022; Ali et al., 2022b). It is a companion pigment to chlorophyll and is water soluble and its presence is recorded in *Spirulina* sp. and *Aphanizomenon flos-aquae*. Currently, this pigment is utilized in desserts, ice creams and as a protein-rich food additive (Khalid et al., 2022). In addition, pyocyanin functions as a biocontrol agent with antibacterial and antifungal activity (Marrez & Mohamad, 2020). Several bacteria, including Colletotrichum lagenarium, Aspergillus fumigatus, Vibrio cholerae, Shewanella colwelliana, and Alteromonas nigrifaciens, are known to create melanin, a natural pigment (Ghattavi et al., 2022). Both plants and animals contain this pigment and are used in food products and other applications, including eyeglasses, cosmetic creams, and medications (Rather et al., 2022; Rasool et al., 2023).

Violacein is a powerful purple tint with a variety of biological functions. This pigment is accounted to be manufactured by several bacteria, including Chromobacterium violaceum, Pseudoalteromonas, Collimonas, Janthinobacterium, and Microbulbifer (Saqr et al., 2021). Large-scale manufacturing of cosmetics, food, pharmaceuticals, and textiles has also been noted to depend on its continuous supply. Many beneficial bioactivities, such as antibacterial, anticancer, antiviral, enzyme modulation, antiulcerogenic, and anti-leishmanial, are linked to this pigment (Rana et al., 2021).

Moreover, several microorganisms, including Paracoccus carotinifaciens, Agrobacterium aurantiacum, and Halobacterium salinarum, have been claimed to make whey (Saini & Keum, 2019). Lycopene is a pigmentation ingredient in foods made from animals and fish and is connected to anti-aging and memory-improving actions. Developing nations accept lycopene as a meat coloring additive (Castro et al., 2021). It is a carotene-related pigment that is insoluble in water. It can be extracted from tomatoes and other red fruit and vegetables. Through genetic engineering techniques, Lycopene has been synthesized in microbial hosts such as Blakeslea trispora, E. coli, and yeasts, although it is comparably more affordable and durable (Madhavan et al., 2022; Manzoor et al., 2023). Arprink red is an extracellular metabolite of the anthraquinone group that Penicillium oxalicum produces that is red in appearance. It is used as a dietary supplement and may also have anticancer benefits (Rana et al., 2021). Based on the recommendations of the Codex Alimentarius Commission, a pink red is employed as a food pigment in various food products at several different concentrations. The filamentous fungi in the genus Monascus of the Ascomycetes group produce a class of secondary metabolites known as azaphilones, which are also referred to as monascus pigments (Kalra et al., 2020). These pigments are orange (rubropunctatin and monascorubrine) and red (monascorubramine and rubropunctamine) in hue (Lima et al., 2023).

8.6 Application of Pigments in Various Sectors

There have been many technical developments in recent years that have successfully produced microbial tints for a variety of industrial firm uses (Fig. 8.1).

8.6.1 Food Industry

Microbial pigments are a better alternative to synthetic food colors than plants due to their availability, non-seasonality, scalability, higher output per hectare, and ease of downstream processing (Rana et al., 2021). The food industry already employs microorganisms to color food, such as Blakeslea trispora's-carotene, Penicillium oxalicum's Monascus, Arpink Red (a natural red used in industry), and Astaxanthin from various bacteria (Christaki et al., 2021). To keep up with the usage of synthetic pigmentation, much research has been done to reduce the costs associated with the manufacture and processing of natural colors, as well as to boost stability and shelf life (Sen et al., 2019; De Corato, 2020; Munekata et al., 2020).

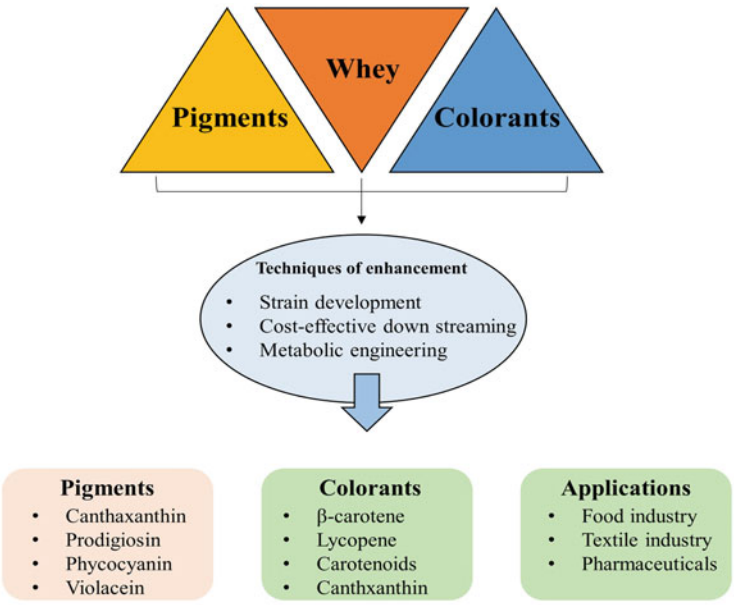

Fig. 8.1 Utilization of whey for the production of natural pigments

8.6.1.1 Antioxidant Activity

Microbial pigments, including violacein, carotenoids and anthocyanins, are potent antioxidants (Sen et al., 2019). Strong antioxidant violacein supports the mucosal defense mechanisms to protect against oxidative damage in stomach ulcers. It is a purple pigment mainly produced by Chromobacter violaceum and Pseudoalteromonas (Anahas et al., 2022). The yellow pigment staphyloxanthin, produced by Staphylococcus aureus, shields Swiss albino mice from the oxidative harm caused by carbon tetrachloride (Mishra et al., 2023).

8.6.1.2 Anticancer Property

The anticancer properties of different pigments and colorants have risen in recent years. These pigments are associated with triggering apoptosis, which results in the death of malignant cells (Safaei et al., 2019). The greenish-yellow pigment scytonemin, manufactured by aquatic cyanobacteria, restricts the protein kinase's activity that controls the cell cycle, having an antiproliferative impact in vitro (Mishra et al., 2023). Serratia marcescens and Pseudomoalteromonas rubra create the red pigment prodigiosin, a potent anticancer agent associated with the anti-human cervical cancer apoptotic impact—synthetic indole derivatives and prodigiosin parallel exhibit anticancer action in vitro (Yu et al., 2019). Violacein exhibited cytotoxic consequences on HL60 leukemia cells via activating Caspase-8 and p38 MAPK through the TNF signaling cascade (Park et al., 2021). Many pigments, including Carotene, Canthaxanthin, Lycopene, Monascorubramin, Riboflavin, Rubropunctatin, and others, have also been demonstrated to have in vitro anticancer properties (Ghosh et al., 2022a).

8.6.1.3 Antimicrobial Activity

Some of the antimicrobial substances produced by numerous microorganisms are currently employed as antibiotics (Hutchings et al., 2019). An endophytic fungal pigment was discovered to be more effective than the widely used antibiotic Streptomycin. Moreover, it demonstrates antiviral, antifungal, and antiprotozoal properties. The recent growth of microbial strains resistant to several drugs and antibiotics has prompted researchers to look for new and unusual substances that can be used as antibiotics (Anyaegbunam et al., 2022). Discovering new microbial pigments that are both pigment-producing and antibacterial is highly beneficial.

8.6.2 Pharma Industry

Despite the health advantages, consumers prefer products made with natural colors since they also have critical therapeutic efficacy in the pharmaceutical sector (Siddiki et al., 2022; Iqra et al., 2023). The pharmaceutical sector in various products uses many microbial pigments. Many studies are being conducted to treat diseases like cancer, leukemia, diabetes mellitus, and other conditions using a variety of colored secondary metabolites produced by the bacteria that have tremendous potential for use in medicine (Ikram et al., 2021; Cheng et al., 2023). Antibiotic, anticancer, antiproliferative, and immunosuppressive chemicals may all be produced by these colorants. Here are some exemplary of this pigment types:

1. *Anthocyanins:* Flavonoid pigments that are water-soluble. They participate in several biological functions, including antioxidant activity, lowering the risk of cancer, and lowering and regulating immune response insult (Manzoor et al., 2022b; Aboonabi & Aboonabi, 2020). Via two key processes, anthocyanins may prevent the formation of tumors and cancer cells: Alteration of the redox state and interference with crucial cellular processes (cell cycle, apoptosis, inflammation, angiogenesis, invasion, and metastasis) (Aboonabi & Aboonabi, 2020). Anthocyanins also have strong antioxidant capacity due to their phenolic hydroxyl groups, which are likely to provide a free radical, a hydrogen atom, or an electron (Speer et al., 2020).
2. *Prodigiosin:* This is a possible pigment with various pharmacological attributes. It demonstrates that certain eubacteria, including Vibrio psychroerythrus, S. marcescens, Pseudomonas magnesiorubra, and others, produce a wide range of cytotoxic activities (Dev & Jayabalan, 2022). A tripyrrole pigment called prodigiosin was initially discovered in S. marcescens (a Gram-negative bacterium). Prodigiosin, a non-diffusible red pigment, is produced by S. marcescens. It is also produced using Streptomyces or Serratia (Soenens & Imperial, 2020). In 60 cell lines of human tumor cells, it exhibits immunosuppressive activity and antiproliferative and cytotoxic properties (derivative from lung, colon liver ovarian, brain cancers, melanoma and leukemia). Moreover, prodigiosin was listed as an effective ingredient for managing diabetes mellitus (Tiwari & Hajoori, 2022).
3. *Violacein:* An indole derivative primarily obtained from the microorganisms Chromobacterium violaceum, violacein is a violet pigment with potent antitumor, antiparasitic, antiprotozoan, anticancer, antiviral, antibacterial, and antioxidant characteristics (Park et al., 2021).
4. *Red yeast rice (RYR):* Traditionally, Red Yeast Rice was produced by fermentation of cooked rice kernels with the yeast *Monascus* spp (Monascusrubur, Monascuspurpureus, Monascusruberand Monascuspilosus) (Zhu et al., 2019). These Monascus species are notable for producing secondary metabolites with polyketide structures and pigments in yellow, orange, and red. To make angkak, a fermented rice product having anti-cholesterol properties, monascusruber was employed. The active ingredients found in this type of rice included substances with statin-like structural similarities, unsaturated fatty acids, sterols, and

B-complex vitamins (Pandey et al., 2019). Furthermore, it has also been shown that consuming Red Yeast Rice may lower blood glucose levels in diabetic patients (Zhu et al., 2019).

8.7 Micro-Encapsulation, Nanoemulsions, and the Formation of Nano-Formulations

Micro-encapsulation and nano-expression can maintain, increase solubility, and distribute natural colors to the food used. Natural pigments such as anthocyanins and carotenoids have problems with solubility in some media and environmental stability (Sen et al., 2019). The packing matter, in this example, the microbial colorant and the core or active ingredient, is referred to as the wall or shell substantial. The chosen wall matter should have emulsifying abilities, down viscosity, biodegradability, film-forming abilities, resistance to the gastrointestinal tract (GIT), low cost, and low hygroscopicity (Dailin et al., 2019). Maltodextrins, adapted starches, inulin, furcellaran, and other fence materials, among others, are now employed to encapsulate microbial colorants for use as food color (Mishra et al., 2023).

Encapsulated pigments have a longer shelf life because they are more manageable, soluble, and stable in ambient circumstances (Mishra et al., 2023). The wall substantially shields the active core matter from matrices, oxygen, light, temperature, and humidity. Increased shelf life, protection of the core material from unfavorable environmental circumstances, relieve and flexibility of addressing control of the pigment-free time, and suppression of any aroma or flavor are the main goals of capsulized microbial colorants and their application in the food firm (Costa et al., 2021). Numerous techniques for micro-encapsulation and food business frequently use prominent examples like drying spray, concertation, drying freeze, and emulsion creation (Ahmed et al., 2022; Massicotte & Cranston, 2022). Several studies have used spray-drying to create micro-encapsulations of encapsulated microbial pigments, including anthocyanin, utilizing maltodextrin as the wall material (Kumar Vivekanandhan et al., 2016; Sen et al., 2019; Mazumder & Ranganathan, 2020; Schlindweinn et al., 2022). Modified starch was employed as the wall substantial to encapsulate beta-carotene utilizing freeze drying (Eun et al., 2020; Riaz et al., 2022). Furthermore, these encapsulated colors have proven reliable and efficient in food and beverage arrangements, including yogurt, soft drinks, cake, and others (Ghosh et al., 2022a).

Microbial pigments can be enclosed in nano-encapsulation or nanoemulsions, which can be created in droplet sizes of 100 nm or less (Aslam et al., 2020). Water, oil, and an emulsifier are the three components that makeup nanoemulsions. The primary measure in creating a nanoemulsion is adding an emulsifier since it assists in reducing the stress between the water and oil stages of the emulsion (Nishad et al., 2021; Manzoor et al., 2022a). It also maintains the nanoemulsion by reducing steric

resistance and repellent electrostatic interactions. Surfactants, as well as proteins and lipids, are the most common types of emulsifiers employed. Nanoemulsions have better applicability, a higher surface region per unit, more excellent kinetic maintenance, and opposition to any chemical or physical variation than micro- and macro-emulsions (Kumar et al., 2021). The dispersion of unsatisfactorily water-soluble pigments in solutions can be facilitated by using nanoemulsions and nano-capsules since they are sufficiently little to be undetectable in solutions. The nano-sized droplets created during the creation of nanoemulsions have a significantly larger surface region, which results in increased soaking up (Sarheed et al., 2020).

Moreover, these nanoemulsion products can be made in various formulas, including creams, liquids, sprays, and foams. Nanoemulsions maintain the emulsion's pigmentation from all environmental circumstances and do not impart undesirable flavors to the food particle (Sen et al., 2019). Food colorant nanoemulsions can significantly reduce the pigmentation required to get the appropriate stain in the food substance, resulting in a cost-effective product (Soliman et al., 2021). The creation of β-carotene nanoemulsions has been the subject of numerous research; a study examined the size and stability of these nanoemulsions about temperature, pH, and surfactant type. To stabilize their nanoemulsions, Qian et al. combined beta-lactoglobulin, a biocompatible emulsifier, with b-carotene (Geng et al., 2023).

8.8 Metabolic Engineering

The replication of pigment biosynthesis genes and the ability to manipulate genes to produce more of these pigments result from recent advances in molecular biology and metabolic engineering (Butnariu, 2023). Streptomyces coelicolor's Actinorhodin, a blue pigment, a genetic mechanism has been altered to provide kalafungin, a similar dark yellow polyketide that is utilized to create antraquinone, a reddish-yellow pigment (Rather et al., 2022). By conveying biosynthetic tracts from unknown or well-known colorant manufacturers, different attempts were made to create cell factories that produce stains quickly and effectively (Cardoso, 2022).

Knowing the biosynthetic processes by which microbial pigments are produced is a crucial first step. The pigment-producing genes then follow this and gene cascades identification as these genes must be engineered to produce too much color (Sen et al., 2019). Cloning the color biosynthetic congenital traits into microbial carriers, such as yeast or bacterial cells, has emerged as a different affordable and efficient commercial manufacturing system (Eun et al., 2020).

(Joshi et al., 2023) increased carotenoid synthesis in R.mucilaginosa KC8, which primarily generates—carotene and torularhodin, using metabolic engineering and mutagenesis. Using glucose as a substrate, (Grewal et al., 2022) described the manufacture of betaine in Saccharomyces cerevisiae, a heterologous microbial host. They also showed that ailments might produce new betalain derivatives in the culture of various amines.

The manufacturing of the red stain has been increased while the generation of the mycotoxin citrinin has decreased (Wen et al., 2023). Citrinin has been decreased by employing diverse strategies involving dissolved oxygen, pH variations, and genetic modifications. In Monascus purpureus, the polyketide synthase gene included in citrinin production has been investigated. The polyketide synthase gene pksCT has been successfully cloned in the muscular strain M. purpureus SM001 to stop citrinin synthesis (Poonia & Pandey, 2023).

8.9 Strain Development and Fermentation

Advancement in the production of microbial colorants presents several hurdles (carbon, nitrogen sources, and minerals), including pH, temperature, incubation time, moisture content, and aeration rate. However, recent technological advancements have helped to overcome these obstacles somewhat. A cost-effective and firmly possible production method for pigments and other natural elements has been developed by employing agitation tanks for wider-scale pigment generation, strain-improving ways, strain production through arbitrary mutagenesis, and numerous preference series (Korma et al., 2022; Brar et al., 2021). Because the colors produced by wild-type strains are often too few in number and need longer agitation times, the procedure is typically unprofitable and strain development is crucial. Common mutagens that can boost pigment production by a factor of several, such as 1-methyl-3-nitro-1-nitrosoguanidine (NTG), are used to improve strains (Kamath et al., 2008).

The technique of medium adjustment is crucial for increasing the yield of the fermented product. Controlling operating factors, including temperature, pH, aeration, fermentation, and media elements, is part of optimizing the culture (Wang et al., 2020). Response surface methodology is an efficient way to improve and optimize pigment manufacturing. It decreases the quantity of experimental assessment needed to evaluate several variants by solving the multivariate information acquired to solve multivariate equations. To cultivate *Serratia marcescens* to produce prodigiosin, a study created the ideal medium composition. Adding glycine and sucrose as an energy and carbohydrate source boosted the prodigiosin synthesis by 2.12–2.15 times (Poonia & Pandey, 2023). Inorganic mono-potassium phosphate addition stimulated cell growth and boosted prodigiosin synthesis. The standardization of the medium and effective fermentation design are key components of developing an affordable producing process (Pillaca-Pullo et al., 2023) (Table 8.2). Therefore, the use of statistical methodologies and predictive modeling implementation may be used as a way that can enhance output response, lower variability, and lower overall costs.

Table 8.2 Growing environment set along with different agro-waste to generate various microbial pigments in inundated fermentation

Pigment	Substrate	pH	Temperature (°C)	References
Carotenoid	Cheese whey	7.3	26	Roukas et al. (2015)
	Rice powder	7.0	35	Korumilli and Mishra (2014)
	Slaughterhouse wastewater	7.6	26	Rodrigues et al. (2014)
	Coffee husk media	5.7	28	Moreira et al. (2018)
	Corn steep liquor and parboiled rice water	4.0	25	Valduga et al. (2014)
	Corn maceration and rice parboiling water	4.0	25	Colet et al. (2017)
	Cassava bagasse	6.0	25	Manimala and Murugesan (2017)
	Primary-treated piggery wastewater	7.5	23	Kang et al. (2006)
	Mesquite pods and corn steep liquor	5.5	20	Villegas-Méndez et al. (2019)
	Wheat straw hydrolysate	5.3	30	Liu et al. (2020b)
Flexirubin	Liquid pineapple waste	7.0	30	Aruldass et al. (2018)
Melanin	Vegetable waste	7.0	25	Tarangini and Mishra (2013)
	Fruit waste	6.8	30.7	Tarangini and Mishra (2014)
	Fruit pulp	6.5	25	Bezirhan Arikan et al. (2020)
Monascus	Grape waste	6.5	30	Lopes et al. (2013)
	Glucose fermentation media	5.5	30	Liu et al. (2020a)
	Whey medium	6.0	30	Mehri et al. (2021)
	Brewer's spent grain media	5.5–7.5	30	Silbir and Goksungur (2019)
	Potato pomace	5.0	28	Chen et al. (2021)
	Rice powder	3.5	32	Lian et al. (2007)
Monascus-like	Orange peels	5.0	24	Kantifedaki et al. (2018)
	Potato dextrose broth	6.4	24	Venkatachalam et al. (2020)
Prodigiosin	Wheat bran medium	–	30	Luti et al. (2018)
	Peanut oil cake	7.0	30	Naik et al. (2012)
	Peanut powder and olive oil	7.0	26	Lin et al. (2019)
	Peanut oil	–	28	Hernández et al.(2020)
	Cassava wastewater	7.0	28	de Araújo et al. (2010)
	Powdered peanut broth	7.0	28	Giri et al. (2004)
	Brown sugar	7.0	25	Aruldass et al. (2014)
Pyocyanin	Cotton seed meal media	–	37	El-Fouly et al. (2014)
Riboflavin	Corn steep liquor	6.8	28	Park et al. (2011)
	Corn steep liquor	7.2	37	Lee et al. (2007)
Violacein	Sugarcane bagasse	7.0	30	Ahmad et al. (2012)
	Liquid pineapple waste	7.0	30	Aruldass et al. (2015)

8.10 Cost-Effective Down Streaming

Employing standard techniques to isolate and regain pigments on a wide scale is relatively costly (de Souza Mesquita et al., 2021) hence, there is an increased need for the development of affordable isolation methods for microbial colorants. While a significant number of organic solvents is employed throughout the lengthy and challenging process of organic solvent extraction, the relent of the excessively detoxified product may be incredibly low (Khan et al., 2021). However, since most organic solvents are synthetic, utilizing solvents other than water and ethanol can undermine the aim of obtaining a natural color for regulatory objectives (Pagels et al., 2023). Many nucleic acids, organic acids, peptides, and other elements have been effectively separated and purified by employing non-ionic adsorption resins. Due to their excellent loading capacity, these resins aid in regaining several chemicals. Also, these resins can be utilized immediately to absorb substances from the culture broth. Using fewer extraction solvents and enhancing their reusability aids in reducing the cost of separation (Rao & Rathod, 2019). (Zhang et al., 2016; Aziz et al., 2022; Maqbool et al., 2022) they demonstrated a good way for prodigiosin isolation and detoxification in which non-ionic resins were utilized directly from the growth medium, omitting the requirement for cell isolation and producing a strenuous and semi-detoxified product (Jatoi et al., 2021).

8.11 Production Cost

Using agro-industrial waste and by-products as a carbon source to produce various value-added items decreases manufacturing costs and encourages environmental sustainability. (Manikandan et al., 2022). Transport, handling, and pre-treatment expenses are the key things considered when choosing garbage as a carbon source. Cellulosic agricultural wastes must undergo pricey pre-treatments, which limits the sustainability of the environment (Kwikima et al., 2021).

8.12 Stability of Natural Pigments

The greatest obstacle to storing and using natural pigments is their stabilization over a prolonged time. Despite showing great promise in conditions of applications, original pigments have restricted markets because of their low stability (Rather et al., 2022). There are numerous techniques for producing and maintaining original colors with a longer shelf life and economic potential, as demonstrated by Sen et al. (2019). The stability of colorants must be ensured through encapsulation to increase their shelf life. Natural pigments for food applications are chosen based on the substance's physical and chemical characteristics, rectification maintenance, and

commanding requirements (Ghosh et al., 2022b). In addition, the value of anthocyanin's durability may give it a prospect in various food colorings. The utilization of -carotene as a food coloring is restricted by its solubility, maintenance, melting end, and down bioavailability, which has a nutraceutical activity (Kharat & McClements, 2019). Antioxidant beta-carotene is extremely unstable and demeans both during food manufacturing and reserve. In conclusion, carotene is encapsulated to identify stability problems and boost its stability in the food sector. Bacterial pigments are highly prone to instability when exposed to high heat, light, or oxygen, and they have a hard time retaining their properties under specific environmental circumstances (Jurić et al., 2022).

8.13 Conclusion

For decades, synthetic pigments have been used almost routinely, posing concerns to human health and the environment. Initially disregarded, the potential of bacterial colorants for the large production of an excessive range of coloring properties is now attracting considerable interest from academia and business. When creating the latest pigments for the food firm, pigments must be safe, have a high nutritional value, and have little influence on the product's cost. Additionally, natural rather than synthetic food pigments should be chosen. Yet, bacterial pigment production is negligible compared to the expanding global market demands. Using various biotechnological technologies, they can raise their output volume to meet the market's demands better. Among other things, agitation ways for scaling production to firm levels, genome shuffling for strain enhancement, and genetic engineering are vital in increasing bacterial stain outcomes cheaply with higher maintenance.

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Chapter 9
Whey: As a Fermentation Substrate for the Production of Exopolysaccharides

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Abstract The dairy and food processing sectors as well as researchers have been attempting to find suitability to utilize by-products of the dairy industry in recent years. The effectiveness of the final product and the conversion process are key factors in whether this attempt is successful. Whey is the main source of lactose but isolation of lactose from whey is a challenging task. Exopolysaccharides, including xanthan gum and alginate, are widely valued because they can enhance the texture and processability of food. The building elements for these polymers are particular microbes and simple sugars, which are synthesized naturally during fermentation processes. Due to the specificity of the involved enzymes and the controllability of the bioprocess, the bioconversion of whey lactose into exopolysaccharides is the most optimal application for it. This chapter provides an outline of the bioconversion of whey lactose into microbial exopolysaccharides.

Keywords Exopolysaccharide · Whey · Lactose · Biopolymers · *Bifidobacteria*

9.1 Introduction

In dairy industry, whey is one of the major by-product which is produced during the cheese or casein-making process. It is a translucent green-yellow liquid that left and this yellowish colour is due to the presence of riboflavin (Vitamin B2) in higher quantity (Sáenz-Hidalgo et al., 2021). Furthermore, it stands with 80–90% of total

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Table 9.1 Composition of acid and sweet whey obtained from different animal sources

Components	Cow		Goat		Sheep	
	Sweet whey	Acid whey	Sweet whey	Acid whey	Sweet whey	Acid whey
Water (%)	93.36	93.40	93.88	93.87	92.06	91.81
Fat (%)	0.05	0.07	0.07	0.08	0.10	0.13
Lactose (%)	5.08	4.72	4.18	3.93	5.30	4.72
Total protein (%)	0.87	0.88	1.00	0.99	1.71	1.76
α–Lactalbumin	23.96	23.24	25.54	24.77	13.75	13.79
β–Lactoglobulin	50.33	49.76	42.25	41.58	56.22	57.87
Immunoglobulins	15.81	16.32	25.32	26.61	19.32	18.68
Serum albumin	5.94	6.48	4.19	3.92	6.33	6.30
Ash (%)	0.60	0.70	0.67	0.83	0.56	0.79
Total solid (%)	6.64	6.76	6.12	6.22	7.94	8.19
pH	6.01	4.86	5.90	4.80	6.09	4.92

Source: Karimidastjerd and Gulsunoglu-Konuskan (2021)

milk and retains around 50% of original milk's constituents, including lactose (~70% greatly influenced by whey acidity), whey proteins (~10%), minerals (~12%), water-soluble vitamins and minute proportion of fat (Echegaray et al., 2019). Minerals such as calcium, sodium, magnesium, and potassium are most abundant in whey. Additionally, calcium represents the majority of these minerals (including >50% NaCl and KCI), whereas tiny amounts of metals like zinc and copper are present (Ryan & Walsh, 2016). Whey contains around 6–6.5% of total solids on a dry matter basis.

Whey can be classified into two major varieties i.e., sweet whey and acid whey which are broadly depended by the processing technique which shown in Table 9.1. Sweet whey comprised with a pH range of 5.9–6.6 and generated during cheese and rennet casein making, whereas acid whey showed a pH range of 4.3–4.6 and produced during mineral acid-precipitated casein and bacterial culture-produced cheese (Barukčić et al., 2019). The composition and sensory properties of whey differ due to the factors such as types of whey (acidic or sweet), milk source (cow, sheep, bovine milk, etc.), method used for cheese production, season, feed for the animal during milking and lactation stage. However, the chemical composition of whey varied widely due to the production method i.e., acid whey or sweet whey. In comparison to conventional whey, acid whey has increased quantities of calcium, phosphate, lactic acid, and lactate. Although in much smaller quantities, it also contains lactic acid, urea, uric acid, vitamin B and citric and. Whey protein contains all nine essential amino acids and has high biological value as compared to other sources of dietary proteins. It can be used in the production of industrialized foods, different beverages and protein supplement (Poonia & Pandey, 2023).

Due to its high levels of biological oxygen demand (BOD) and chemical oxygen demand (COD), whey is regarded as dairy waste and pollutes the environment., ranging from 40,000 mg/L to 60,000 mg/L and 50,000 mg/L to 80,000 mg/L,

respectively (Papademas & Kotsaki, 2019; Poonia, 2020 Shikha & Singh, 2019). This is primarily attributed to the large quantity of lactose, which constitutes 70–72% of the total solids in whey. The waste load of whey is considerably greater than that of domestic wastewater, with estimates suggesting it can be 100–175 times higher (Ryan & Walsh, 2016). Additionally, this waste volume is equal to the volume to processed milk during cheese production and is estimated ~190 million tons annually, and in a nutshell, the volume of whey is growing at an average rate of 2% per globally.

Although, traditionally it was used as animal feed or fertilizer, these applications are becoming increasingly impractical due to high volumes and transportation costs. Land spreading of whey can also lead to soil fertility issues. Therefore, alternative processing methods are being investigated to increase the economic value of whey. These include whey protein isolate (WPI) production and whey protein concentrate (WPC) as well as fractionation of specific protein components such as α-lactalbumin and peptides (Božanić et al., 2014). Drying whey into powder or processing it into bio ethanol is also possible, but these methods are capital-intensive and require significant investment (Zhou et al., 2019a). Production of whey-based beverages, whether fermented or unfermented, may be a more practical and economical choice for small and medium volumes of whey.

Biopolymers are either biodegradable or bio-based polymers or can be both. These may be synthetic, natural, or microbial substances (Asgher et al., 2020; George et al., 2020). Among them, microbial extracellular polysaccharides (EPS) are microorganism generated such as yeast, bacteria, and fungi, and their potential applications in the food, cosmetic, and pharmaceutical industries have garnered significant interest (Qamar et al., 2022). Lactic acid bacteria (LAB), including *Fructilactobacillus, Lactobacillus, Lactiplantibacillus, Lactococcus, Latilactobacillus, Leuconostoc, Lentilactobacillus, Limosilactobacillus, Streptococcus*, *Pediococcus,* and *Weissella* species are generally used to produce EPS for in the food industry (Harutoshi, 2013; Korcz & Varga, 2021). *Weissella* spp. possesses a wide range of functional and technological properties that can increase the safety and flavour of food products. EPS with various structures serve a diversity of functions in EPS-producing cells. In general, the producer microorganism does not use these polymers as a source of energy, but they do provide effective protection of microbial cell integrity in ecosystems with harsh environmental conditions such as osmotic stress, dehydration, and pathogenic microorganisms (Jurášková et al., 2022). EPS is involved in bacterial colonisation of natural habitats, the formation of biofilms, and cell recognition. Cell walls may be physically guarded by EPS from obnoxious compounds and boost cellular resistance to antibiotics, bacteriophages, and phagocytosis. The preponderance of commercially available probiotics produces EPS. EPS can be used as a natural substitute for synthetic food additives, resulting in desired rheological modifications, such as enhanced viscosity in the food matrix, decreased syneresis, and improved texture (Korcz & Varga, 2021; Tiwari et al., 2021). EPS offers desirable body texture, firmness, opulence, and mouthfeel, and could also be used in food packaging (de Souza et al., 2022). Contrary to customer expectations that foods should have fewer additives, the use of EPS generated in situ by LAB is

not needed to be labelled on food packaging. It has been shown that EPS, a different class of bio-thickeners that is frequently employed in the food sector, has a number of other technologically useful qualities (Cruz et al., 2022; Dong & Karboune, 2021).

Polysaccharides are ubiquitous biomolecules found in a wide range of organisms, including plants, animals, and microorganisms, and exhibit a variety of chemical and biological properties that are frequently related to their structure or source (Thapa et al., 2020). Capsule polysaccharides, lipopolysaccharides, and EPS can be categorised based on their location (Jurášková et al., 2022). EPS, which are extracellularly generated or released into the extracellular environment during microbial development, range in branching degree and monosaccharide composition from linear to highly branched molecules (Zhou et al., 2019b). On the basis of the presence of identical or distinct monosaccharides, they can be further subdivided into homopolysaccharides (HoPS) and heteropolysaccharides (HePS), resulting in a wide range of structural diversity (Jurášková et al., 2022; Liao et al., 2020; Zhou et al., 2019b). Despite this diversity, the biosynthetic pathways for EPS in various microbial species exhibit striking similarities. HePS genetic sequences typically encode regulatory proteins, polysaccharide length regulation proteins, multiple glycosyltransferases, and polymerization and export proteins, whereas HoPSs are synthesised by a single enzyme encoded by a single gene (Oleksy & Klewicka, 2018). Unique physicochemical properties of EPSs, such as advanced viscosity and rheology, have been exploited for a variety of industrial applications in the dairy industry. EPS produced by LAB during fermentation has been utilised to extend shelf life and preserve flavour by influencing the viscosity, syneresis, and sensory properties of the resulting product (Korcz & Varga, 2021; Xu et al., 2019). In addition, EPSs exhibit numerous physiological and biological activities, such as anti-oxidation (Zhou et al., 2019b), anti-bacterial (Zhong et al., 2020), cholesterol-lowering (Nguyen et al., 2020; Yildirim-Elikoglu & Erdem, 2018) and immunoregulatory (Rajoka et al., 2020).

This chapter provides a detailed overview of the characteristic of whey, fermentation of whey, and functions of EPS's production from whey. The chapter also emphasizes the potential functional processes as well as the interactions between structures and functions. The current challenges and approaches in LAB and EPS research, as well as promising applications will be discussed in brief. The aim of this chapter is to provide a valuable resource for academics and professionals who are currently working in the fields of food science, microbiology, and biotechnology.

9.2 Characteristic of Cheese Whey

Whey is mostly made up of organic compounds, primarily sugar and milk proteins. Other components include nitrogen, phosphorus, and suspended particles, with a carbon/nitrogen/phosphorus ratio of around 200/3.5/1 (de Ramos et al., 2021). In terms of its overall composition, whey is roughly 0.1% lipids, 1.0% protein, 5.0%

lactose, 1.0% minerals and 92% water as shown in Table 9.1. Additionally, whey contains minor fractions of compounds such as citric acid and B vitamins. Approximately 50% of the total solids, 20% of the proteins, and all of the lactose from the milk remain in the whey after the cheese is made, with lactose accounting for about 75% of the total solids composition (Macwan et al., 2016). The contents of total solids, ash, and fat are lower in whey than in milk, with respective levels of approximately 6.3%, 0.5%, and 0.1%, while the lactose and whey protein contents remain similar (Barukčić et al., 2019; Božanić et al., 2014). Whey contains only a negligible amount of casein, with levels below 0.1%, in contrast to the 2.8% found in milk. Mineral salts, particularly NaCl and potassium chloride, which make up more than 50% of the total inorganic content are responsible for the inorganic material found in whey and are a by-product of the salting process used to make cheese (Moatsou et al., 2019; Nishanthi et al., 2017).

Depending on the source and production method, sweet whey protein can have varying chemical compositions. When utilising whey protein as a supplement, it is especially crucial to comprehend each fraction's role in both nutrition and wellness. β-lactoglobulin, α-lactoglobulin, bovine serum albumin (BSA), lactoperoxidase, lactoferrin, and glycomacropeptides make up the majority of sweet whey protein, which has typical values of 3.5 kg/m^3, 1.4 kg/m^3, 0.4 kg/m^3, 0.06 kg/m^3, 0.05 kg/m^3, and 1.4 kg/m^3, respectively. The estimated concentration of immunoglobulins (IgG, IgA, IgM) is 0.6 kg/m^3 (Nath et al., 2014; de Ramos et al., 2021).

It has been discovered that whey proteins and peptides have bioactive qualities that can improve physical performance and promote post-exercise recuperation, aid in weight management, exhibit anticancer effects, improve child nutrition, and promote healthy aging, among other benefits (Bull et al., 2022). Additionally, whey peptides have been shown to have a positive effect on the treatment of ulcers by inactivating *Helicobacter pylori* (Masood et al., 2011; Sachdeva et al., 2014).

β-Lactoglobulin, one of the main whey proteins, is considered an excellent supply of vital amino acids and has additional functions such as acting as a carrier for cholesterol, retinol and vitamin Das well as having gelling properties. While immunoglobulins have antimicrobial properties, bovine serum albumin is known to possess antimutagenic and anti-cancer properties (Gupta & Prakash, 2017). Lactoperoxidase is often used in oral health products due to its ability to fight against microorganisms associated with oral irritation and gingivitis, as well as having anti-inflammatory activity in the oral cavity.

9.3 Exopolysaccharides (EPS)

Microbial EPS are secreted by bacteria to form a barrier against dehydration and harmful compounds. They could also prevent immune cells or other microbes from phagocytosis, neutralising bacteriophages (Abdalla et al., 2021). Capsular EPS are highly immunogenic, with their diversity possibly evolving to evade antibody responses (Abdalla et al., 2021). In addition to their protective functions, secreted

EPS aid in adhering to and penetrating host organisms, contributing potentially to pathogenicity. *Xanthomonas campestris* attaches to its host cabbage using viscous xanthan gum., whereas Alginate, which is produced by *Pseudomonas aeruginosa,* obstructs the respiratory tract and spreads infection (Lei & Edmund, 2017; Lo et al., 2007). In contrast to the polysaccharides found within the cell, the function of EPS differs from that of higher plants.

EPS from microorganisms exhibit varying rheological properties due to differences in their molecular weights and compositions which can range from HoPS containing a single type of monosaccharide to heteropolysaccharides HePS consisting of repeating units containing various monosaccharides and non-sugar molecules (Baruah et al., 2016). Species of bacteria i.e. *Agrobacterium* and *Rhizobium*, are capable of synthesising multiple types of EPS. Despite the potential availability of EPS from numerous genotypes, only a few have been commercialized, including xanthan, gellan, curdlan, dextran, and bacterial cellulose. These microbiological EPS are hydrocolloids and perform the customary thickening, gelling, and suspending tasks in the food sector. Both Gram-positive and Gram-negative microorganisms are capable of producing EPS (Lo et al., 2007).

9.3.1 EPS Generated by Acidifying, Dairy-Associated Microbes

Additionally, dairy-associated acidifying microbes are able to produce EPS directly via processing the lactose which is generally associated with dairy-based products. So, it has been attracted an increasing amount of research and attention in recent years. There is a substantial possibility that these bacteria will have applications broadened to include non-dairy products because it has been revealed that some of them may offer probiotic benefits to humans.

9.3.2 EPS from Lactic Acid Bacteria

There are a great number of distinct strains of LAB, each of which is capable of producing EPS with a unique structure and range of dimensions. It is possible to classify the EPS produced by LAB into two distinct categories—HoPS and HePS. HoPS. The HoPS may be further categorised down into the following four groups: fructans (including levan produced by *S. salivarius*), α-D-glucans (including dextrans, alternans, and mutans), β-D-glucans and other groups like polygalactans. Different mesophilic and thermophilic LAB are responsible for HePS production (Garcia-Ochoa et al., 2000; Lo et al., 2007). The molecular weight of EPS that are derived from LAB can be a range from 1.0×10^4 to 6.0×10^6, which is generally the molecular weight of other EPS that are typically put to use.

Although generation of EPS on an industrial scale from LAB is not particularly frequent because to its poor yield, it has been reported that certain strains, such as *S. thermophilus LY03* (1.5 g/L), *Lactobacillus returi* strains (4.1 g/L and 4.8 g/L), and *L. sakei* 0–1 (1.4 g/L), generate greater amounts (Lo et al., 2007; van Geel-Schutten et al., 1998). The reduced amount of energy produced by anaerobic LAB places a restriction on the synthesis of EPS and ultimately leads to poorer product yields than those produced by aerobic strains like *X. campestris*. Nevertheless, *Leuconostoc mesenteroides* produce bacterial dextrans that are widely used in the medical industry as a blood extender, in gel filtration columns, and as polysaccharides standard in molecular mass calibrations. Even though the majority of the ropy strains only produce <1 g/L of EPS, the fact that the resulting rheologically active biopolymers are biocompatible makes it more likely that they will be used in the food business (Torino et al., 2015). Increasing the amount of EPS-producing bacteria in dairy products, such as *Lactobacillus casei* and *L. delbrueckii* ssp. *bulgaricus RR*, improves the product's viscosity, texture, and mouthfeel, making the goods more palatable without the need for costly commercial EPS (Shegaw & Kurtu, 2017). EPS derived from LAB can also make it possible to produce novel goods, such as yoghurts with a reduced amount of milk solids and fat (Khurana & Kanawjia, 2007; Lo et al., 2007).

The rheological characteristics of fermented dairy products can be negatively impacted by the development of EPS-producing bacteria (Tiwari et al., 2021). It is the primary reason for the increased interest in EPS that is bacteria produced that have been isolated from dairy products. EPS produced by ropy bacteria, some of which are produced by probiotic strains, show a great deal of promise for use in the production of healthful products. The value of EPS produced by ropy strains may be greatly improved if more is understood about the physical, chemical, and rheological characteristics of ropy strains and whether those characteristics could be utilised in non-dairy food applications (Lo et al., 2007). *S. thermophilus* exhibited a higher production of EPS when both the carbon and nitrogen contents were elevated, whereas bacteria like *P. acidipropionici* benefited from a higher carbon to nitrogen ratio in the medium (Lo et al., 2007; Nicolaus et al., 2010). This was true even though a higher carbon-to-nitrogen ratio in the medium encourages bacterial synthesis of EPS. Therefore, it is necessary to conduct detailed research into how the carbon-to-nitrogen ratio of the substrate affects the pace of cell growth and the production of EPS by bacteria associated to dairy products.

9.3.3 EPS Produced by Bifidobacteria

Health benefits have been increasingly incorporated into dairy products in recent years, especially yoghurt-like products. They are supplemented with microorganisms like *bifidobacteria* to achieve this. It has been demonstrated that this species, which is the most prevalent in the intestinal microbiota of healthy individuals, possesses probiotic, nutritional, and therapeutic properties. *L. delbrueckii* ssp.

bulgaricus RR is a common microorganism utilised for EPS production in yoghurt (Lo et al., 2007; Ryan et al., 2015).

The biosynthesis of EPS in *Bifidobacteria* is a difficult process that involves numerous enzymes and genetic regulators. The biosynthesis pathway can vary depending on the specific strain of *Bifidobacteria* and the conditions of growth. Glycosyltransferases (GTs) serve as crucial enzymes in the manufacture of EPS in *Bifidobacteria*. GTs are responsible for transferring sugar molecules from donor molecules to growing chains, thereby creating the repeating units that make up EPS (Kaur & Dey, 2022). Different GTs have different substrate specificities, which allows for the production of EPS with varying chemical structures.

Another important enzyme in the biosynthesis of EPS in *Bifidobacteria* is a glycosidase. This enzyme is responsible for trimming the growing EPS chains to achieve the desired length and branching pattern (Hidalgo-Cantabrana et al., 2014). Furthermore, *Bifidobacteria* depend substantially on enzymes and genetic regulators for the manufacture of EPS. These regulators directly control gene expression and involved in EPS biosynthesis, as well as the timing and quantity of EPS production.

The availability of nutrients, pH, temperature, and oxygen concentrations have an impact on the generation of EPS in *bifidobacteria*. Studies have shown that the production of EPS in *Bifidobacteria* can be enhanced by providing certain carbon sources, such as fructose or lactose, and by optimizing the fermentation conditions.

The EPS produced by *Bifidobacteria* have numerous health benefits, including their ability to act as prebiotics. Prebiotics are non-digestible dietary components that stimulate the development and function of beneficial gut flora. EPS produced by *Bifidobacteria* have been shown to selectively stimulate the growth of other beneficial gut bacteria, such as Lactobacillus, while inhibiting the growth of harmful bacteria such as *Clostridium perfringens* and *Escherichia coli*.

Bifidobacteria are strictly anaerobic gram positive rods, despite certain strains' ability to tolerate oxygen in the presence of carbon dioxide (Lo et al., 2007). The most effective promoters for *bifidobacteria* growing in a synthetic medium were found to be yeast extract and bovine casein digest (Lo et al., 2007; Robitaille, 2013). Other growth boosters, such as hog gastric mucin, bovine serum albumin digest, human and bovine milk whey, were useful for certain species but unsuccessful for others. While the nature of additional growth factors is yet unknown, bovine casein digest has been identified as the nitrogen source for the growth of *bifidobacteria*.

Bifidobacterium longum is a strain with a reputation for inhibiting liver tumours in rodents and improving diarrhoea by reducing stool frequency. However, It took until the last 10 years to characterise the EPS production in the species *Bifidobacterium* (Prasanna et al., 2014). Roberts et al. (1995) found first report on composition of an acidic EPS with a molecular weight greater than 200 kDa generated by *B. longum BB-79*, where The principal carbon source of the culture medium has an impact on EPS synthesis. The corrosive nature of EPS, comparable to that of other microbial EPS, has made pH an important variable in determining EPS production during fermentation. (Lo et al., 2007). With an initial pH of 6.0, EPS production decreased in media during a 10-day period, while the pH range of 6.0–9.0 had minimal impact on EPS yields. According to pertinent studies, the incubation

period is crucial for the generation of microbial EPS, but it appears to have little influence on EPS yields.

9.3.4 Implications of Carbon Source

Nevertheless, several researchers believe that the substrate's characteristics have no effect on the composition of the EPS produced. While, a large number of researchers have discovered that variations in carbon source can affect composition of sugar, molecular weight, and yield of EPS biosynthesis. Many ropy isolates utilise sucrose, skim milk and glucose as carbon sources for EPS production, whereas *bifidobacteria* prefer lactose over other carbohydrates (Parhi et al., 2022; Ryan et al., 2015). Microorganisms that produce EPS typically use carbohydrates as both their energy source and carbon source. In defined media, restricting nutrients like nitrogen and supplying surplus carbohydrates stimulate EPS production, whereas limiting the carbon source results in negligible EPS production (Lo et al., 2007). Nevertheless, Different bacteria have different responses to the carbon source in terms of EPS synthesis and sucrose content. Different *Lactobacillus* strains produce different amounts of EPS with different sugar compositions when cultured with different hexose sugars. The sugar composition of EPS generated by *L. bulgaricus* was altered by introducing glucose to both milk and milk ultrafiltrate retentate (De Vuyst & Degeest, 1999). *Lactobacillus rhamnosus strain* 9595 M produced the same quantity of EPS regardless of whether glucose or lactose was used as the carbon source (Polak-Berecka et al., 2015).

Higher initial carbon source concentrations result in greater EPS yields during *L. rhamnosus C83* fermentation (Dupont et al., 2000). Additionally, *P. acidipropionici* produced the most EPS in a whey-based medium that also contained 60 g/L of lactose. However, lactose content increase did not enhance EPS production or carbon source utilisation. *Bacillus longum BB-79* produced the most EPS by weight in liquid media containing lactose as the principal carbon source (Lo et al., 2007). This suggests that EPS can be produced in substrates containing lactose which is abundant in cheese whey. Additionally, the optimal conditions for *B. longum BB-79* growth and EPS production must be identified to economically maximise EPS productivity (Lo et al., 2007; Sheth et al., 2022).

9.3.5 Fermentation Conditions and Scale-Up

Conditions related to fermentation have a substantial impact on EPS production. The optimal conditions for EPS production by bacteria may differ from their optimal growth conditions. Factors such as medium composition, pH and temperature on EPS production have been identified by researchers. EPS generated by *L. mesenteroides* and *S. thermophilus*, for example, can decay after being

synthesised, whose concentrations decreased during the later phases of fermentation (Badel et al., 2011). *L. casei* produced EPS degraded when the pH was maintained at 5.0, probably due to the activation of polymer-degrading enzymes. According to the strain and culture conditions, the decline in EPS production during prolonged fermentation appears to be dependent (Saha et al., 2020). It may, however, be avoided by extracting EPS at the correct time and under the proper pH and temperature conditions.

Prior research on the kinetics of xanthan fermentation has mostly concentrated on the nutritional requirements for xanthan production, as well as the specific impacts of carbon and nitrogen sources on cell growth and xanthan biosynthesis. According to studies, rapid cell multiplication and high cell density require high nitrogen concentrations, but xanthan biosynthesis is encouraged by high carbon to nitrogen sources (Bhat et al., 2022). Based on various nutritional requirements for cell development and xanthan biosynthesis, numerous kinetic models have been developed. However, precise mechanisms that emphasize synergistic effects of carbon and nitrogen substrates are not yet completely understood.

There have been numerous findings in different scientific literature concerning microorganisms that produce EPS and produce acid. In a study conducted by Cerning (1990) revealed that the EPS generated by *L. bulgaricus* had more galactose than glucose, and that the carbon source substantially influenced the composition of the EPS (Cerning, 1990). The composition of sucrose in EPS generated by *L. casei* CG11 was affected by the medium (Lo et al., 2007; Mozzi et al., 2001). However, (Lo et al., 2007) noted that the carbon source had no effect on sucrose composition of EPS that was produced by *L. sake* 0–1. Thus, it makes sense to infer that the EPS's composition has a significant impact on how well the microorganisms use carbon sources.

The design of a bioreactor and its successful scalability are crucial elements in the creation of an efficient bioprocess. The cost- and time-effectiveness of large-scale production motivates the scale-up technique. The "six-tenth factor" was developed by analysing statistical data for both individual pieces of equipment and the entire plant's construction (Lo et al., 2007), which indicates that doubling the plant's capacity will result in a cost increase of only $2^{0.6}$ times. In addition, operational costs typically decline with an exponent in the range of 0.3–0.5, bolstering the need for scale-up. Consequently, scale-up process is crucial in determining capacity of production and efficacy of a bioprocess, and it has a direct bearing on operational expenses.

9.3.6 Biosynthesis of EPS Via Lactose Fermentation

Microorganisms can convert lactose into other substances with the aid of lactase, an enzyme that breaks lactose down into glucose and galactose (Ugidos-Rodríguez et al., 2018). However, only a few strains are capable of directly fermenting lactose into the required end product. Most yeasts, for example, lack the lactose permease

system and galactosidase, which makes lactose fermentation into ethanol difficult (Lane et al., 2011; Lo et al., 2007).

Numerous microbes associated with dairy use lactose as their primary source of energy and for polysaccharide synthesis. EPS is produced by a variety of genotypes, including *Lactobacillus* and *Pseudomonas*, *B. longum*, and dairy *Propionibacteria*. Some strains perform well at lactose bioconversion, while others prefer alternative carbon sources such as glucose and sucrose. Unlike *L. delbrueckii* subsp. *bulgaricus* NCFB 2483, which generated more EPS when grown on lactose, *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 produced more EPS when grown on glucose or lactose than fructose. Nonetheless, certain Lactobacillus strains i.e., *L. reuteri*, generate less EPS when cultivated on lactose as opposed to sucrose.

Lactose fermentation produces exopolysaccharides (EPS) with prospective applications, making them viable alternatives to glucose-derived EPS (Tan et al., 2020). Dairy *propionibacteria* and LAB have been identified as being capable of directly utilising whey through lactose fermentation to generate beneficial polymers for both food and non-food applications (Lo et al., 2007; Mazzoli et al., 2014). *Rahnella aquatilis* was notably active in lactose metabolism under conditions of adequate aeration (Lo et al., 2007). However, modest concentrations of some exopolysaccharides produced via the fermentative pathway render them unsuitable for industrial use. To address this issue, lac genes were introduced into the wild-type strain to create genetically modified genotypes (Bosma et al., 2017; Lo et al., 2007).

9.3.7 EPS Biosynthesis in Dairy Associated Bacteria Via Lactose Fermentation

EPS production by LAB is a complex process involving the biosynthesis mechanism and necessary precursors (Lo et al., 2007). The formation of extracellular polysaccharides (EPS) by LAB is a complicated process that involves the biosynthesis mechanism as well as the required precursors. The EPS biosynthesis pathway comprises sugar import into the cytoplasm, sugar-1-phosphate formation, sugar activation and coupling, and EPS export (Korcz & Varga, 2021; Patel et al., 2012). Extracellular glycosyltransferases are responsible for the production of certain EPS, such as dextran. These enzymes add a monosaccharide to a polysaccharide chain that is developing. However, the majority of other EPS are produced intracellularly by glycolysis from sugar nucleotide precursors before they are discharged into the environment (Lo et al., 2007).

The Phosphoenolpyruvate-phosphotransferase system (PEP-PTS) is responsible for transporting carbohydrates from the periplasm to the cytoplasm in LAB (Douglas et al., 2014). Phosphorylation is the state that sucrose is in once it has entered a cell; this determines what role it will do next. Lactose can enter the cell unphosphorylated or phosphorylated, as well as in the released sugar form, depending on the method of transport (Fig. 9.1). After entering a cell, lactose is converted into glucose and

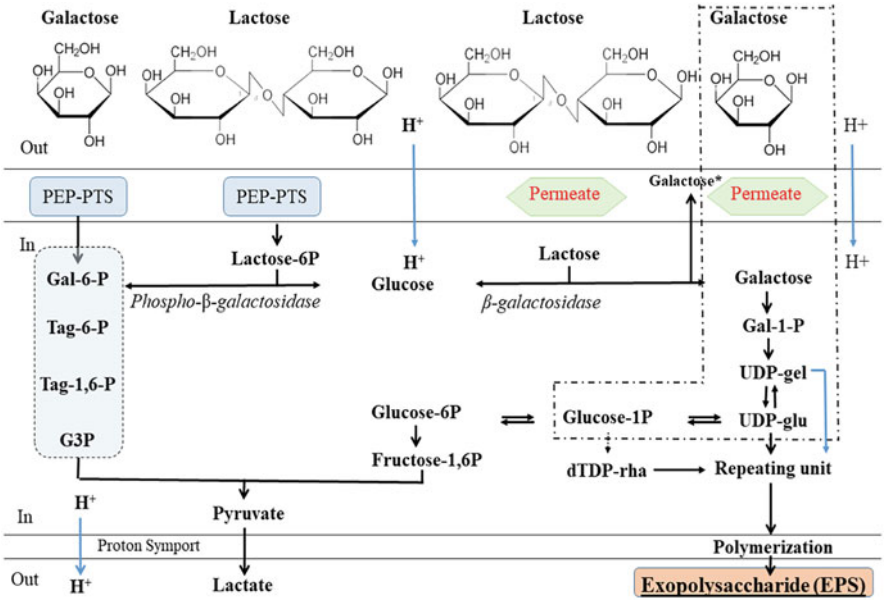

Fig. 9.1 Schematic diagram of lactose and galactose absorption and dissimilation processes, as well as exopolysaccharide (EPS) synthesis in lactic acid bacteria. Tagatose-6-phosphate pathway (-------) and Leloir pathway (.-.-.-.-.) are shown respectively as grouped. (galactose*: in the case of galactose-negative strains, galactose is exported) (adapted and modified from (Lo et al., 2007)

galactose through a process called glycolysis. The glycolytic and phosphoketolase routes are responsible for the metabolism of glucose in homofermentative and heterofermentative LAB strains, respectively, whilst tagatose-6-phosphate (galactose-6-phosphate) and Leloir pathways are responsible for the degradation of galactose as shown in Fig. 9.1(Lo et al., 2007; Wu et al., 2015).

Lactose-specific PEP-PTS sugar transport systems are found in *L. lactis* strains. These systems are responsible for the importation of extracellular lactose and the generation of intracellular lactose-6-phosphate. Phospho-galactosidase is the enzyme that converts lactose-6-phosphate to galactose-6-phosphate and glucose. Following this, the enzyme glucokinase transforms glucose into glucose-6-phosphate. Lactose is transported into galactose-negative cells of *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* strains by lactose permease that also functions as a lactose-galactose antiporter (Cui et al., 2017). Following this, the galactose component is removed from the medium, leaving glucose as the only source of energy and carbon (Lo et al., 2007).

The generation of EPS in LAB is a complex process, which results in a wide variety of EPS based on biosynthesis mechanism and precursors that are required. EPS can also be produced in a variety of other ways. Although the Phosphoenolpyruvate-phosphotransferase system is good at transporting a wide

variety of carbs and uses little energy in the process, lactose can only be carried by processes that are particular to LAB strains.

Precursors such dTDP-rhamnose, UDP-galactose and UDP-glucose are polymerized in the cytoplasm to generate HePS. These precursors are formed at the membrane by adding activated sugars in a sequence to an expanding repeating unit. Following the completion of the cycle of the repeating unit, the HePS is then polymerized after being exported through the cell membrane (Lo et al., 2007; Thapa et al., 2020). Polymerization, the process of determining chain length, and export from plasma membrane are all processes that continue to be poorly understood.

Bifidobacteria use a mechanism known as proton symport to import lactose into their cells. This process connects substrate translocation to proton incorporation. Lactose is processed intracellularly by β-galactosidase into glucose and galactose. Through a process known only to bifidus bacteria, the breakdown of two glucose molecules results in the production of three acetic acids, two lactic acids, and five ATP molecules. During the exponential phase of growth, certain enzymes follow the Leloir pathway to convert galactose into glucose-1-phosphate. During the stationary phase, other enzymes follow the pyrophosphorylase pathway (Fig. 9.1). The glucose-1-phosphate that is generated from galactose must first be transformed to glucose-6-phosphate (Gitzelmann, 1995; Lo et al., 2007). Only then can the bifidus pathway be accessed. Even though study has been done on the energy mechanism of bifidobacteria, more studies are required to establish the way of lactose use that occurs during the synthesis of EPS by *B. longum*.

9.3.8 Key Challenge in Bioconversion of Whey Lactose

Microbial cultures are frequently thought to be possible alternatives for converting whey lactose. However, in order to build a sustainable and cost-effective operation, multiple factors such as waste reduction, production scale, and product yields must be carefully analysed (González-González et al., 2022; Karim & Aider, 2022). Although the conventional use of EPS as a thickening agent is not profitable, there is the opportunity to uncover and characterise EPS's unique and/or synergistic capabilities. *B. longum* BB-79 is one interesting choice, as it provides additional health advantages due to its probiotic nature and has proven good lactose digestion when compared to other fermentable sugars (Pyclik et al., 2020). However, data on the culture conditions that influence the organism's capacity to make a polymer is limited (Lo et al., 2007). As a result, more optimisation of *B. longum*'s growing environment is required to obtain maximum EPS production.

While some strains of interest may not be able to use lactose directly, one option is to break it down into glucose and galactose, which may be easily fermented (Harper et al., 2022). Galactosidase enzymes can be used to hydrolyze the disaccharide enzymatically; however, they are too expensive to produce a product unless their economic worth justifies the expense.

However, acid-catalyzed hydrolysis is a less expensive option. This method has been validated for pure glycoside solutions as well as various dairy effluents. Although acid hydrolysis employs simple chemicals and heating, the process is complicated and can result in undesirable consequences from further breakdown of monosaccharide molecules. Because the number of probable side reactions vary depending on the permeate composition, acid hydrolysis as a technique of creating monosaccharides from lactose in whey permeate must be considered in conjunction with the planned application of the hydrolysis products in mind (de Albuquerque et al., 2021; Lo et al., 2007). Factors influencing the physiological condition of the fermenting organisms must also be considered, and adding inorganic acids followed by neutralising agents may increase ionic strength and osmotic pressure, decreasing the activity of the microbe.

The medium used to create EPS requires a nitrogen source in addition to lactose. When nitrogen level of feedstock is considered, using whey directly would be a cost-effective option. Whey proteins can be hydrolysed to boost nitrogen availability by stimulating the development of specific bacteria. Whey permeate, which is produced in large amounts during the synthesis of whey protein concentrates, might alternatively be utilised as an alternate feedstock for the production of exopolysaccharides.

Plain whey and whey permeate, have low quantities of peptides and free amino acids, which may hinder lactose absorption. Exopolysaccharide synthesis can also be inhibited by a shortage of molecular oxygen and the presence of salt (Wang et al., 2019). It has been proposed that the quality and quantity of the nitrogen fraction in the media influence lactose metabolism as well as the generation of exopolysaccharides and organic acids. Furthermore, anaerobic growth of *Propionibacterium acidipropionici* on milk permeate was only possible with the addition of yeast extract (Bücher et al., 2021; Pais-Chanfrau et al., 2020). With additional increases in the amount of supplied yeast extract, the strain's fermentation capabilities were greatly increased.

Previous research on lactose bioconversion into ethanol has revealed that using mixed cultures of bacteria and yeast in a two-stage fermentation process is a viable strategy (Patria et al., 2022). *Lactobacilli* bacteria made conversion of lactose into lactic acid during first step at a pH of 4.5–5.0. The pH of the solution can be reduced to the ideal level of 4 for yeast growth by adding inorganic acids such as sulfuric acid. Yeast consumes the lactic acid in the second stage, increasing the pH to 6.5 and supplying additional nutrients to the yeast cells. However, when a combination of glucose and galactose is used as a carbon source, yeast cells undergo diauxic development, resulting in decreased ethanol yields, even for galactose-adapted strains. Furthermore, due to the high cost of β-galactosidase and its inability to hydrolyze all lactose contained in the medium, the problem of effluent disposal remains unsolved (Lo et al., 2007; Zhou et al., 2019a).

9.4 Current Applications and Limitations

Because of developments in separation techniques such as chromatography, ultracentrifugation, sterile filtration, ultrafiltration, reverse osmosis, electrophoresis and enzymatic hydrolysis, the usage of whey in many applications has risen. Although these uses are generally focused on decreasing waste rather than generating high-value goods, whey and whey permeate are used to make dry whey powder and refined lactose. Purified lactose from cheese whey or permeate is utilised in newborn formulas and therapeutic applications due to its flexibility, mild taste, and reduced sweetening power. It can also be used as an excipient in tablet form. Only a small portion of the available whey is used for lactose synthesis, despite the fact that the generation of lactose from whey is on the rise. As a result, different applications for lactose are being investigated, such as its conversion into lactitol, a non-digestible sweetener used in low-calorie diet goods. Another disaccharide, lactulose, is utilised in pharmacology and as a Bifidus factor in nutrition. Lactose is isomerized in an alkaline solution to produce it.

Lactose has been examined for usage in a range of applications due to advancements in separation technologies. To keep excess whey out of sewers, one of the main applications of whey and whey permeate is in the production of refined lactose and dry whey powder. The dairy industry's ultimate goal should be to employ whey lactose as a lucrative feedstock for high-value products.

Due to its flexibility, mild flavor, and diminished sweetening power, purified lactose developed from cheese whey or permeate is utilised as an excipient for medicinal goods as well as a supplement in new-born formulae. Lactose may be converted to lactitol, a non-digestible sweetener having somewhat more sweetening capacity than lactose. Lactitol-palmitate an ester, is also employed in human nutrition and has an emulsifying effect. Lactose may be isomerized in an alkaline solution to produce lactulose. Lactulose is a highly valued disaccharide with international pharmacological markets. It is used in nutrition as a Bifidus factor and has a sweetness that is 48–62% that of sucrose.

Direct reaction and lactose hydrolysis may also be used to generate galactose, which has been used to replace sorbitol, and lactosylurea, a non-protein nitrogen source in ruminant feed with ammonia levels below the danger limit. In place of saccharose or starch syrup, hydrolyzed lactose solutions are used in ice cream and confectionery industries because they have a higher sweetening capacity than lactose. Immobilised glucose isomerase can be used to convert the glucose in lactose-hydrolyzed whey permeate to fructose, resulting in increased sweetness.

Furthermore, lactose from whey has been used in yeast fermentation, where lactose serves as a food supply for the microorganisms involved. CO2, ethanol, and single-cell protein are produced as biomass as a result of the microbial metabolic activity. It is possible to give enriched whey straight to livestock or to isolate and dry the biomass to create a feed concentrate. In addition to these other uses, ethanol and CO_2 are produced on a big commercial basis from whey in some nations. Direct fermentation by yeast does not make a lot of technological or economical sense since

just a few yeasts can use lactose as a substrate for fermentation. Because the fermentation process cannot be improved to deliver an expected yield of 0.5 g yeast cells per g lactose, the distillation procedure is too expensive. Furthermore, it is challenging to generate the intense aeration needed for yeast development.

9.5 Conclusions

Using whey as a fermentation medium allows manufacturers to generate value-added products. Extracellular polysaccharides (EPS) generated by specific microorganisms are being employed as stabilisers and binding agents in the food industry to raise viscosity and improve the rheological qualities of food products. Based on the rheological properties of the EPS in dairy-based systems, it is feasible to manufacture whey-derived components with modified or increased functional capabilities. This would allow for the use of a nutrient-rich waste stream while also increasing the prospects for the usage of whey-derived components. A fermentation medium appropriate for the development of *Lactobacillus delbrueckii* ssp. *bulgaricus* RR and the formation of EPS may be made from whey using limited protein hydrolysis. Unhydrolyzed whey fermentation result in lower lactose intake, lactic acid generation, viable cell counts, and net cell dry weight than whey hydrolyzed to varied degrees.

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Chapter 10
Whey Protein Based Edible Coatings: Recent Trends

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Abstract To replace non-renewable packaging resources with renewable and biodegradable ones, interest has grown in the development of edible coatings and films. By minimising gas and mass fluxes throughout the packaging, these materials have proven successful in preserving the quality and shelf life of food goods. Even though several natural biopolymers, including polysaccharides, proteins, lipids, and their composites, have been investigated for their potential as sources of palatable coatings and films. Therefore, the whey protein is a by-product of the dairy industry that makes up 20% of the total protein in milk and is recovered through ultrafiltration, diafiltration, electrodialysis, gel filtration, ion-exchange chromatography, and reverse osmosis. It contains high concentrations of amino acids, immunoglobulin, and their functional properties, such as antioxidants, among others. It is highly desirable in the pharmaceutical and food industries due to its properties such as replacer of fat and emulsifier, film forming ability, gelling properties, and others. The whey protein-based edible coating are odourless and transparent in nature and possess barrier properties against water and gas transmission, which make it fit to utilize for development for coating formulations for extending the shelf life of perishable food products. Furthermore, the whey protein is potential carrier of active ingredients such as antioxidants, antimicrobials, nutrients, vitamins, and colorant and flavour agents. In the present chapter authors explored briefly the potential of whey proteins in development of edible coating, their properties and functions in the extending the shelf life of perishable food and food products such as fruits, vegetables, meat, dairy and bakery based products.

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10.1 Introduction

In recent decade, the consumers are more consciousness about edible and eco-friendly packaging substance due to sustainable approach. Edible coatings are semi permeable, protective thin layer that have thickness less than 0.3 mm; protecting food products from microbial contamination and physiological disorders. Initially, in the twelfth century and thirteenth century a wax coating for extending shelf life of lemons and oranges by Chinese scientist was developed (Kumar & Neeraj, 2019; Suhag et al., 2020). In the food processing sector, edible films and coatings are being used increasingly to preserve the nutritional value of a wide range of foods, notably fruits, vegetables, meat and meat products, and dairy-based food products (Pérez-Gago et al., 2006; Huber & Embuscado, 2009; Aguirre-Joya et al., 2018; Parreidt et al., 2018). In 1992, the first coating material was developed for commercial usage as a wax coating for applications on fruits and vegetables (CPMA, 2014). The edible coating is an excellent carrier of bioactive agents (antioxidant, antimicrobial, probiotics, vitamins, nutrients etc.) and can be used as alternative of plastics and synthetics polymers (Campos-Requena et al., 2015; Jaramillo et al., 2016; Bashir et al., 2017; Moghadam et al., 2020; Kumar et al., 2021a). The application of edible coating may help in the reduction of postharvest losses in horticulture commodities by retarding the oxidation, moisture loss and reducing the microbial load; these may result in shelf life extension of fruits and vegetables (Siracusa et al., 2008; Chaurasia et al., 2010; Sapper & Chiralt, 2018; Grosso et al., 2020; Hasan et al., 2020; Dong et al., 2020; Kazemian-Bazkiaee et al., 2020; De Pilli, 2020). Additionally, there are numerous substances that are readily available that could be used for this purpose, including polysaccharides like starch and its derivatives, cellulose, gums (arabic gum, guar gum, and xanthan gum), agar, alginate, carrageenan, chitosan/chitin, pectin, and pullulan (Kumar et al., 2022; Liu, 2005; Lacroix & Cooksey, 2005, The barrier and functional qualities of the packaging system may be improved by the composite edible coating, such as binary and ternirary systems. Many researchers have improved the mechanical, functional and barrier properties of composite edible packaging with combination of different types of polysaccharide and proteins (Li & Chen, 2000; Gennadios, 2002; Erdohan & Turhan, 2005; Gounga et al., 2007; Henriques et al., 2016). Despite that, the plasticizers such as glycerol (glycerin), sorbitol, propylene glycol, polyethylene glycols (PEG) use to improve the flexibility and barrier properties of coating formulations (Laohakunjit, and Noomhorm, 2004; Ahmad et al., 2023).

The proteins such as collagen, gelatin, myofibrillar proteins, keratin, egg white protein, casein, and whey proteins may derive from natural sources. Furthermore, the fluid by-product of milk's proteins precipitating out has been considered "whey." Growth of microbes (such as cheese whey), the incorporation of acid (the formation of acid casein), or the combining of enzymes can all decrease the precipitation of

rennet casein production. Whey can be categorized as acidic or sweet as a result. The production of cottage cheese and acid casein leads the manufacture of acid whey. Other than that, they termed it "sweet whey." The most of the whey production worldwide is sweet whey. Whey has unique nutritional value and hence must be processed judiciously into the edible as well as non-edible value added products. The disposal of these by-products has now become a challenge and danger to the environment (Poonia, 2020).

Nowadays, the whey is widely used in the manufacturing of infant formulas, food supplements, sports bars, and beverages to help people of all ages accomplish a variety of wellness benefits. In addition, the whey protein commonly accepted as the proteins that persist in milk serum when the casein has curdled at pH 4.6 and 20 °C (Eigel et al., 1984). In industrial applications, the manufacturing of caseinate or cottage cheese produces acid whey as a by-product. It is produced by preparing the curd, evacuating the following whey, and bringing the pH of skim milk to 4.6 by incorporating acid, glucono-delta-lactone, or lactic acid bacteria culture. Cheddar and other rennet-type cheeses were produced by adding the coagulating enzyme rennet (chymosin, EC 3.4.23.4) to milk following its being inoculated with a lactic acid bacteria culture to decrease the pH to 6.2–6.4. Besides that, the whey protein possesses good film forming ability and can be used to developing edible coating and films for food applications to extending their shelf life for a longer period (Ozdemir & Floros, 2008; Ghanbarzadeh & Oromiehi, 2008). The whey protein based edible coating possesses good barrier properties against water and gas transpiration and act as carrier of active ingredients (Zinoviadou et al., 2009; Kandasamy et al., 2021). However, whey proteins are the derived by-products from the largest by-product of dairy world which is 'whey' (Mishra et al., 2022). The 70% of the total whey protein components such as β-lactoglobulin and α-lactalbumin contribute to its gelling and film making properties. Whey proteins possess excellent film forming ability due to its conformational denaturation, amphiphilic nature, and evenly distributed negative charge (Umaraw & Verma, 2017).

Badr et al. (2014) was incorporated different essential oils such as cinnamon, cumin and thymes as antimicrobial agents in whey protein based edible coating and found effective to preserving the shelf life of fresh red meat up to 12 days at 5 °C by retarded the microbial load. The application of the whey protein based edible coating is potential to extending the shelf life of the food products by retarding the lipid oxidation and moisture loss throughout the storage period and may retained the higher consumer acceptability (Khwaldia et al., 2004). Several researchers preserved the shelf life of different types of food products such as fruits and vegetables, dairy based products and meat & meat products (Perez-Gago et al., 2006; Belgheisi et al., 2016; Feng et al., 2019; Galus et al., 2021; Mileriene et al., 2021) with maintained their higher consumer acceptability by retarding the browning index, loss of moisture and oxidation as well. The properties of the whey protein based edible coating/films are interconnected and have to be exploring in food packaging categories. However, there is limited information about the whey protein applications. Therefore, the present chapter congregates the information about the whey protein and their application for the formulations of edible coating and films for food packaging

applications. In addition, the effects of whey protein based edible coating on the different food products and their key findings have been also discussed.

10.2 Edible Coating

Edible coatings are primary packaging can be applied on the surface of food and food products. It is non-toxic, biodegradable, and semi permeable in nature have thickness less than 0.3 mm on the surface of food products (Kumar & Neeraj, 2019; Suhag et al., 2020; Trajkovska Petkoska et al., 2021a). Besides, that the edible films are wrapping materials can be obtained through casting and extrusion methods for food applications (Kumar et al., 2019: Suhag et al., 2020). The edible coating and films are generally recognized as safe for consumptions and can be used as alternatives of plastic and synthetic based packaging. Numerous plant and animal derived biopolymers such as polysaccharide, proteins and their combinations have been used for the development of edible coating and films for food applications (Lisitsyn et al., 2021). Figure 10.1 summarizes different types of biopolymers used in the development of edible packaging. The application of edible coating and films potential to extending the shelf life the food products such as fruits, vegetables, dairy, bakery, meat & meat based food products by reducing the oxidation, loss of moisture, microbial load, browning index etc. along with maintained their higher consumer

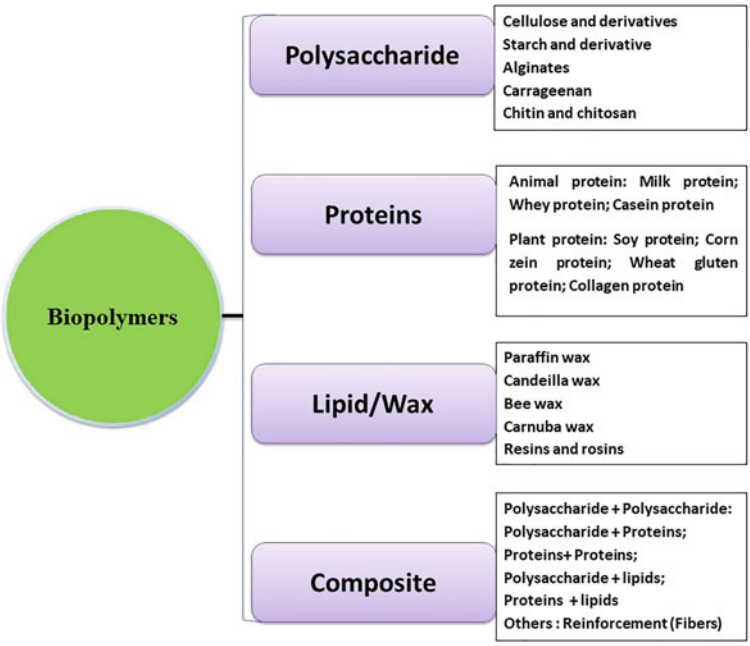

Fig. 10.1 Natural derived biopolymer components for development of edible coating/films

acceptability due to barrier properties of edible packaging (Lin & Zhao, 2007). In addition, these biopolymers also act as a carrier for antimicrobial, antibrowning, and antioxidant agents etc. to scavenge or delay deteriorative actions of microorganisms on the food product (Kumar et al., 2019; Trajkovska Petkoska et al., 2021b).

10.3 Functions and Advantages of Edible Coatings and Films

10.3.1 Edibility and Biodegradability

Biodegradability of a material means the capacity of the material to be decomposed over time as a result of microbiological activity and may reduce the negative impacts on the environment. The terms edibility refer to material that is fit to be eaten and generally recognized as safe for consumption without negative impacts on the health.

10.3.2 Physical and Mechanical Protection

The use of edible coatings and films on food items is acknowledged for offering food and food products across supply chains mechanical and physical protection. Tensile strength, elongation-at-break, elastic modulus, compression strength, puncture strength, stiffness, tearing strength, burst strength, abrasion resistance, adhesion force, folding endurance, etc. are some examples of the factors that contribute to the mechanical strength of the packaging material (Šuput et al., 2015; Petkoska et al., 2021).

10.3.3 Migration, Permeation, and Barrier Properties

Permeation of a packaging material refers to rate of molecular diffusion of gases, vapor, and fluids through the packaging material. The permeability of packaging material is crucial for the food product's safety. The permeability of food packaging materials is characterized generally by light transmission, gas and water vapor permeability of the material (Siracusa, 2012). All barrier properties of a packaging material are altered by composition of the film composition and environmental factors (relative humidity and temperature).

10.3.4 Convenience and Quality Preservation

Since edible coatings and films form a part of the food product, their application could prevent microbiological deterioration, ripening/aging, aroma loss, moisture absorption, oxidation of ingredients, and surface dehydration of ingredients (Avramescu et al., 2020). Additionally, they support qualities with marketing-related consequences including aesthetic quality, surface smoothness, flavour conveyance, edible colour printing, and other quality factors.

10.3.5 Shelf-Life Extension and Safety Enhancement

Food preservation occurs across the whole distribution chain thanks in large part to food packaging. Without packing, food quality could be impaired because it might become contaminated by direct contact with physical, chemical, or biological pollutants. Increased protection provided by packing materials could prolong food products' shelf lives and lessen the risk of foreign matter contaminating them (Alamri et al., 2021).

10.3.6 Carrier for Active Ingredients

Edible film may transport a variety of food additives, such as flavouring and colouring agents, antioxidants, antibacterial agents, anti-browning agents, texture enhancers, and nutraceuticals, probiotics, and vitamins in addition to functioning as a barrier against moisture and gases. The addition of these active ingredients improved the functional attributes of the packaging as well as food products by reducing the microbial load and free radical scavenging activity (Daniloski et al., 2021; Singh et al., 2022; Trajkovska Petkoska et al., 2021b). In addition, the natural plant based extract and essential oils are also used as an antimicrobial and antioxidant agents to improve the antimicrobial activity of the edible coating/films and help in the reducing microbial load on the food products (Kumar et al., 2019; Kumar et al., 2021a: Yadav et al., 2022). The previous researchers were incorporated pomegranate peel as antioxidant agents in chitosan: pullulan composited edible coating to evaluate their effects on the shelf life of different fruits and vegetables such as bell pepper (Kumar et al., 2021a), tomato (Kumar et al., 2021b), mango (Kumar et al., 2021c), and litchi (Kumar et al., 2020). De Bruno et al. (2023) was improved the shelf life of strawberries fruits by application of gum arabic edible coating formulations with addition of different antioxidant agents such as (bergamot pomace extract, bergamot essential oils). In addition, the synthetic antioxidant (butylated hydroxytoluene) was also added in coating formulations for comparative study. In comparison to edible coating supplemented with synthetic antioxidant

agents, their findings showed that edible coating enriched with natural extract and essential oil had a greater ability to sustain the strawberry fruits' postharvest shelf life during the storage duration of up to 14 days. When cinnamon essential oil plus oregano, rosemary, and garlic essential oils were added to a whey protein-based edible film, the barrier, antibacterial, and functional qualities were improved (Bahram et al., 2014; Seydim & Sarikus, 2006).

10.4 Whey Protein

Nowadays, the demands of the protein have been increased for consumption and it is expected that the demands of the animal based protein will be double by the year of 2050 (Henchion et al., 2017; Westhoek and Colleagues, 2017). Whey protein is also in high demand because of its useful properties and widespread usage in the food industry for the creation of various food items. The global market size of the whey protein is valued US $5.33 billion in 2021 and it is expected to grow with 10.48% of CAGR from 2022 to 2030 (Whey Protein Market, 2022–2030). Whey protein is dairy by-products comprised 20% of the total milk protein and recovered through ultrafiltration, diafiltration, electrodialysis, gel filtration, ion-exchange chromatography, and reverse osmosis process (Milani & Tirgarian, 2020). It is health beneficial against several types of disease and disorders such as cardiovascular, skin allergy, obesity, hypertension, diabetes, phenylketonuria and cancer due to presence excellent amounts of amino acids, immunoglobulin and their functional attributes such as antioxidant, etc. Whey protein valued higher as compared to other protein sources (egg, casein, soy protein) and β-lactoglobulins and α-lactalbulins are the main components of whey protein (Sindayikengera & Xia, 2006; Patel, 2015). It is highly desirable in the pharmaceutical and food industries due to its properties such as replacer of fat and emulsifier, film forming ability, gelling properties, and others (Patel, 2015). The effects of whey protein-based edible coating on the shelf life of various fruits, vegetables, and other food products have been studied by a number of researchers (Rossi-Márquez et al., 2021). The whey protein-based polymers are generally clear, odourless, and have barrier qualities that prevent the passage of water and gases, making them suitable for use in the creation of coating formulations for increasing the shelf life of perishable food goods (Galus & Kadzi´Nska, 2016; Galus et al., 2021). Additionally, whey protein has the capacity to transport active substances including antioxidants, antimicrobials, minerals, vitamins, colourants, and flavouring agents (Daniloski et al., 2021; Kandasamy et al., 2021).

10.4.1 Properties of Whey Proteins

Whey proteins are mostly soluble over a wide pH range, especially an acidic pH that can generate firm gels on heating and denaturation, act as efficient aerating agents

and exhibit good fat and water-binding characteristics. The proteins' capacity of binding water and other characteristics such as swelling, gelation, and rheology are important for maintaining texture in a wide range of food items. Therefore, whey proteins are utilised for maintaining the texture of many food products. The applying heat to whey protein under appropriate conditions (pH, ions, protein level etc.) resulted in retain water greatly affects the rheology and texture of several processed foods.

10.5 Important Properties and Functions of Whey Proteins as Packaging Materials

In general, all plastics with good moisture barrier properties have higher oxygen permeability. Therefore, whey protein-based coatings with good oxygen barrier could be layered in between the plastic and food to provide a strong oxygen barrier (Nedović et al., 2016). Whey protein-based coatings and films have been successfully applied by researchers across the world. Foods, which are prone to oxidation, require protection against rancidity. The quality attributes of the products such as fast foods, chocolates and snack peanuts were improved by the application of whey protein based edible coating (Chan & Krochta, 2001; Lee & Krochta, 2002; Lee et al., 2002).

10.5.1 Barrier Properties

Barrier properties in biopolymers are important to prevent undesirable changes in physical properties of foods such as textural (softening, hardening), physical (caking, aggregation, swelling, shrinkage, and breakage), and loss in solubility or water holding capacity. Various parameters such as appropriate packaging materials, storage conditions, and methods of distribution could affect the extent of physical deterioration in food products (Petersen et al., 1999). The barrier properties play an important role in estimation of shelf life of food products to be kept in a package. The food properties and the intended end-use application influence the selection of package materials with desirable barrier properties. Permeability is a measure of degree of gas, vapor, or liquid transmission through a resisting material. Henry's law and Fick's laws are used to describe the diffusion rate of gas through the packaging material (Siracusa, 2012). Fick's first law indicating the permeate (gas or vapour) flux in stationary phase is given by Eq. (10.1) (Siracusa, 2012):

$$J = -D\,(\Delta c/l) \tag{10.1}$$

where J = diffusion flux (expressed in mol cm^{-2} s^{-1}), D = diffusion coefficient or diffusivity (cm^2/s), l = membrane thickness (cm), Δc = concentration difference (mol/cm^3) across the membrane.

Henry's law applies to the diffusion of gas or vapor in the steady state, when there is an equilibrium between concentration and partial pressure of gas at the surface. It is expressed by the following Eq. (10.2) (Siracusa, 2012):

$$J = -D\left(\frac{S\Delta p}{l}\right) \tag{10.2}$$

where p = vapour pressure (atm), S = solubility coefficient (mol/cm^3 atm) indicating the amount of permeate in the polymer, Δp = pressure difference across the membrane.

The barrier properties of polymers such as Oxygen permeability and Water vapour permeability are the most important pertaining to use in food packages.

10.5.2 Moisture Barrier

Water activity plays a major role in maintaining the shelf life and sensory attributes of foods. In addition to impacting the extent of microbial growth, chemical and enzymatic reactions during storage, it also influences the sensory aspects of the material, such as appearance and texture. Water permeability is a crucial quality to consider when choosing packaging materials. Because whey proteins are hydrophilic, films and coatings made from them have a comparatively high water vapour permeability. Denser film networks typically result in lower water permeability, depending on the extent of cross-linking or film alteration. Plasticizer and the relative humidity of the films and coatings loosen the network by reducing the protein chain interactions and generating free volume, resulting in a significant impact on the moisture permeation properties (Khwaldia et al. 2004; Pérez-Gago & Krochta, 2002). Hydrophobic compounds could be included in the film formation process to decrease water vapor transmission rate. Lipids or waxes could be added directly to the film solutions or films coated with a lipid layer (Ramos et al., 2012a). In addition, the utilization of an edible coating to reduce the adverse consequences of minimal processing has been described. By acting as a part of the barrier to the exchange between moisture and gas, they could help in reducing the amount of fruit and vegetable spoilage (CO_2 and O_2). According to Quintavalla and Vicini, (2002) the use of edible films could protect meat, fruits, and vegetables from toxic and harmful microbes. Other functions are provided by barriers to fats and oils, oxygen, other gases, and moisture. This barrier can be used both for fresh produce, such as fruits and vegetables, and ready-to-eat food (Rossman, 2009). The quality of barrier properties has been impacted by the chemical component's material (Pascall & Lin, 2013). Film properties such as viscosity, composition, chemical structure, the concentration of solids, degree of cross-linking, polymer morphology, and type of

plasticizer are affected by external conditions such as relative humidity, temperature, and commodity characteristics such as maturity, variety, water activity, and type of product (Olivas & Barbosa-Cánovas, 2005). Apple slices' oxidative browning and moisture loss were significantly reduced by milk protein-based edible coatings created with derivatives of vegetable oils, according to Krochta et al. (1990).

10.5.3 Gas Barrier

The decomposition in foods due to oxygen comprise of physicochemical reactions like vitamin loss, microbial growth, enzymatic browning, and fat rancidity. To prevent these reactions, most products require packaging, which provides protection against oxygen. While oxygen and carbon dioxide are needed for respiration during storage period for fresh fruit & vegetable products, there is a need for packaging material providing barrier against oxygen, which is offered by a whey-based coating. The synthetic polymers provide excellent barrier against oxygen and carbon dioxide degradation, but they are non-biodegradable and non-recyclable. The whey protein-based coatings and films provide very low oxygen permeability and found suitable for use as coating substance for improved oxygen barrier properties of food package (Jooyandeh, 2011). The relative humidity, temperature, water vapour permeability, and thickness are the important factors for optimum efficiency of edible films as gas barriers. An increase in relative humidity could substantially increase the oxygen permeability of the films (Kandasamy et al., 2021).

10.5.4 Aroma Barrier Properties

A defence against flavour and fragrance deterioration might be provided by edible films. A poor oxygen barrier or oxidation-induced taste property losses point to a decline in sensory characteristics. Thus, edible films might help preserve food's distinctive flavour and stop food quality from declining owing to oxidation thanks to their ideal barrier qualities (Miller & Krochta, 1997). To prevent the adsorption and loss of aromatic/flavor molecules and to maintain the sensory qualities of food, edible coatings and films must have flavour and aroma barrier capabilities. It has been claimed that whey protein isolate is a particularly effective -limonene barrier. WPI films mixed with 25% glycerol transported -limonene similarly to ethylene vinyl alcohol copolymer films (Kandasamy et al., 2021).

10.5.5 Mechanical Protection

Given that physical protection is a package's primary goal, the mechanical characteristics of packaging materials are vital for usage in meals. When being stored, transported, and handled by customers, mechanical stress must be able to be placed on the packing material. High tensile qualities are required, including elongation at break and tensile strength. The greatest force that can be applied to a film per unit of cross-sectional area before breaking is known as its tensile strength. The stretched distance in the film before the break is calculated by dividing it by the total length of the film. Resiliency is calculated by multiplying the tensile strength by the % elongation and shows the overall toughness of the film. Tensile strength of whey protein-based films could be modified by various factors such as addition of plasticizers, change in the state of protein, and cross-linking of protein chains (Jooyandeh, 2011).

10.5.6 Surface Properties

Cohesion (particle-particle stickiness) and adhesion are two concepts that could be used to characterise a substance's stickiness (particle-wall surface stickiness). Adhesion is a measurement of the forces binding the particles to the surface of another material, whereas cohesion is a measure of the forces holding the particles together (Boonyai et al., 2004).

Cohesion and adhesion on surfaces are important, especially for coatings and multilayer systems. The degree of cohesiveness affects the film's characteristics including resistance, flexibility, and permeability. Cohesion is influenced by factors such as biopolymer thickness, plasticizers, cross-linking agents, temperature, pressure, solvents, manufacturing techniques, and structure. For better film cohesion, high chain order polymers are beneficial (Guilbert et al., 1996). Whey protein films have less adherence than most edible hydrophilic coatings. In order to improve substrate and coating compatibility, surfactants are utilised (Lin & Krochta, 2005; Ramos et al., 2012b).

10.5.7 Optical Properties

The optical properties like transparency, colour, ultraviolet or light barrier properties of a material play a significant role in terms of packaging applications. Organic coloured molecules are usually present in polymers made from natural ingredients such as whey proteins. To accurately describe colours, they can be measured with three distinct hues in a three-dimensional space that use the CIE L* a* b* system, Hunter Lab, CIELCH, or CIEXYZ. The instruments like colorimeters could

calculate the differences in colour (ΔE*) connecting chrominance points in the space (Klein, 2010). Whey protein-based films and coatings show similar transparency to synthetic polymers which are frequently used, with only slight colour differences (Ramos et al., 2012c; Schmid et al., 2012).

10.6 Composite Coating of Whey Protein

Due to improved barrier and functional qualities of edible coating and films, biopolymer-based composite coatings may have a considerable impact on food product quality when compared to polymers used alone (Perez-Gago et al., 2005). According to this theory, the use of a composite edible covering made of whey protein and other biopolymers extended the shelf life of food products while maintaining their superior quality and sensory qualities (Kandasamy et al., 2021). Researchers in the past have made several attempts to create an edible coating made of whey protein that would increase the shelf life of fruits and vegetables. For instance, in Pérez-Gago et al. (2005).'s application of coatings to chopped apples, the hydrophilic phase was whey protein isolate (WPI), whey protein concentrate (WPC), or hydroxypropyl methylcellulose (HPMC), while the lipid phase was beeswax (BW) or carnauba wax (CarW). Their findings demonstrated that whey proteins support an anti-browning impact as cut apples coated with whey protein-based coatings had good L* and lower b*-, a*-, and BI- values than HPMC coated and uncoated cut apples. On the other hand, Reinoso et al. (2008) studied WPI and WPI composite coatings containing 5 or 10% (w/w) flaxseed oil combined with beeswax on plums (*Prunus domestica* L.). During the 15 days of storage at 5oC, WPI and 10% lipid composite coatings were less prone to crack, flake, and blister issues than the 5% lipid formulation. The coated samples also exhibit noticeably better penetration due to the WPI sheets' smaller thickness. Due to coating, plum mass loss was also minimised, and the addition of flaxseed and beeswax significantly decreased water permeability. The oxygen barrier and mechanical characteristics are also decreased by the introduction of the lipid phase into WPI.

10.7 Effects of Whey Protein Based Edible Coating on Quality of Food and Food Products

By delaying moisture loss, oxidation, and microbial load from the food and food product surface, whey protein-based edible coatings-with or without the inclusion of active agents-have been demonstrated to aid in food preservation. When employed as coatings on food goods, whey protein films have durability because of their mechanical characteristics. It serves as a layer separator in heterogeneous foods or food ingredients in pouches when it is in the shape of films. Table 10.1 shows the

Table 10.1 Application of edible whey coating on various products

Composition of coatings/films	Edible coating/ films	Application	Key findings	References
Beeswax (BW), 4-hexylresorcinol (4-hexyl), ascorbic acid (AA) (0.5% and 1%), cysteine (Cys) (0.1%, 0.3%, and 0.5%), and WPC are all combined (0.005% and 0.02%)	Coatings	Fresh-cut apples	Reduced weight loss, Enhanced sensory attributes of the fruit	Pérez-Gago et al. (2006)
WPI + beeswax (BW), WPC + beeswax (BW), WPI + carnauba wax (CarW), WPC + carnauba wax (CarW)	Coatings	Fresh cut apples	Anti-browning effect reduced weight loss	Pérez-Gago et al. (2005)
WPC + olive oil composite films	Film	Dried Peanut	Reduced peroxide value Reduced rancidity at 50% RH Improved sensory attributes	Javanmard (2008a)
Whey protein + rice bran oil + Zataria multiflora extract	Coating	Chicken egg	Minimize oxidation and water losses, enhanced shelf life of the eggs	Safavi, and Javanmard (2016)
WPI + pullulan (WPI–Pul)		Fresh roasted chestnuts	Reduced moisture loss	Gounga et al. (2008)
WPC + glycerol	Coatings	Pistachio kernel	Excellent oxygen barrier, extended shelf-life Reduced lipid oxidation	Javanmard (2008b)
WPC + glycerol (1:1)	Coatings	Frozen Atlantic salmon (*Salmo salar*)	Modified color, delayed lipid oxidation	Rodríguez-Turienzo et al. (2011)
Ultrasound treated WPC	Coatings	Frozen Atlantic salmon (Salmo salar)	Delayed lipid oxidation	Rodríguez-Turienzo et al. (2012)
WPC + microbial transglutaminase	Coatings	Frozen Atlantic salmon (*Salmo salar*)	Delayed lipid oxidation	Rodriguez-Turienzo et al. (2013)

(continued)

Table 10.1 (continued)

Composition of coatings/films	Edible coating/ films	Application	Key findings	References
WPI + glycerol + antimicrobial compounds {lactic acid, natamycin, or chitooligosaccharides (COS)}	Coatings	Cheese	Decreased water loss, hardness, color changes, and microbial development	Ramos et al. (2012b)
WPC + lactic acid bacteria (*Lactobacillus buchneri* UTAD104)	Films	Cheese	Prevention of fungal (P. nordicum) contamination	Guimarães et al. (2020)
WPC + 0.3% (w/w, solution basis) Chinese cinnamon bark (*Cinnamomum cassia*) CO_2 extract	Coating	Fresh unripened curd cheese	Preserved moisture Decreased growth of yeasts and molds	Mileriene et al. (2021)
Liquid acid WPC + *Lactobacillus helveticus*	Coating	Acid-curd cheese	Slowed down discolouration of cheese preserved moisture Impart antimicrobial effect	Vasiliauskaite et al. (2022)
Whey protein (12%) + sodium alginate (0.5%)	Film	Frozen gutted kilka	Decrease in Total bacteria count, *Staphylococcus aureus* count, increased shelf-life	Seyfzadeh et al. (2013)
Whey protein and WPI	Films	Potato pellets chips	Reduce oil uptake, good sensory features	Angor (2014)
Whey protein nanofibrils (WPNFs) + glycerol (Gly) + trehalose (Tre)	Coatings	Fresh-cut apples	Increased antioxidant properties, increased surface smoothness, retarding total phenol content	Feng et al. (2018)
Whey protein isolates nanofibers (WPNFs) + carvacrol (CA) + glycerol (Gly) + antimicrobial agent	Coatings	Fresh-cut Cheddar cheese	Higher antimicrobial activity, smooth and continuous surface, good moisture content, better textural properties	Wang et al. (2019)
WPI + essential oils (lemon and lemongrass)	Coatings	Fresh—Cut pears	Good transparency, excellent oxygen and carbon dioxide permeability, increased	Galus et al. (2021)

(continued)

Table 10.1 (continued)

Composition of coatings/films	Edible coating/ films	Application	Key findings	References
WPI + jojoba oil	Coatings	Fresh-cut root parsley	Decrease hardness, increased polyphenols and flavonoids	Galus et al. (2022)
WPC + aloe vera gel + tamarind starch +	Coatings	Ber fruit (*Ziziphus mauritiana*)	Delay in physiochemical changes	Bhadu et al. (2022)
Blended with beeswax and whey protein isolate (WPI) and 5 or 10% (w/w) flaxseed oil	Coatings	Plums	Reduced mass loss Provided glossy surface	Reinoso et al. (2008)
Potassium sorbate (PS), sodium benzoate (SB), potassium sorbate (PS), thyme, coriander, pimento, rosemary, and basil essential oils, together with Nisin, are all combined to create WPI (10.000 IU)	Coatings	Sliced bologna-type sausage (4 ± 1 °C)	Antimicrobial activity against *L. innocua,* preservation of sensory characteristics of coated food product	Kalkan and Erginkaya (2019)
WPC + apple pomace extract (APE)	Coatings	Fresh cut apples	Decreased weight loss Anti-browning and antimicrobial effect	Hammad et al. (2021)
Clove oil, glycerol monosterate, xanthan gum, and whey protein isolate	Coatings	Tomatoes	Improved firmness and color An improved retention of titrable acidity, ascorbic acid concentration, total phenolics, total sugars, and reducing sugars	Kumar and Saini (2021)
Fresh whey + lemongrass essential oil	Film	Halloumi cheese	Shelf-life extension Decreased hardness	Kafiya et al. (2023)

important findings from prior whey protein-based edible coating applications on various food product types. Galus et al. (2022) studied the effects of coatings made of whey protein isolate and enhanced with jojoba oil at different concentrations (1% and 2%) on the qualitative traits of freshly cut root parsley. The outcomes showed that applying an edible coating improved fruit hardness and preserved the colour

characteristics of freshly cut fruits throughout storage. The number of polyphenols and flavonoids in freshly cut root parsley increases when edible coating is applied. On the other hand, Mileriene et al. (2021) applied a whey protein-based edible covering along with cinnamon bark essential oil as antimicrobial agents to enhance the shelf life of cheese. The whey protein isolates and pullulan composite edible coating was found effective in improving the shelf life of chestnut during storage (Gounga et al., 2008).

10.8 Conclusion

Whey protein coatings and films make a great substitute for artificial packaging materials. Their use tackles two environmental issues: the disposal of whey and the dangerous environmental effects of packaging debris because they are edible and biodegradable. They offer excellent oil and oxygen barrier properties as well as very appealing visual characteristics. Their poor moisture barrier properties due to hydrophilic nature could be enhanced by using additives which improve their barrier properties. Moreover, whey proteins could be vehicles to carry out a variety of nutrients, vitamins, minerals, antimicrobials that will provide additional functions that will declare them as active edible materials. The whey protein could be utilized to develop mechanically versatile and environmentally stable edible packaging films, which would be better equipped to resist the physicochemical and microbial spoilage of foods and open a variety of new commercial applications.

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Chapter 11
Valorisation of Whey for Development of Different Types of Food Products Including Fermented Beverages

Rekha Chawla, Swarup Roy, and Bhawna Malik

Abstract Whey has been the most significant part of dairy waste till date and accounts for immense attention from researchers, academicians and scientists for its meaningful utilization. Whey obtained from cheese and paneer industry can be used in different manner to bring healthier and wonderful tasting products on the shelves of upcoming modern supermarkets. Nutritionally dense with vital minerals, whey can be utilized as a major base material to add economy. Prudent choice of operations, technical throughput required and expanding market size to this entity would trigger the use of whey towards value-added products including a range of cheeses like Ricotta, Manouri, Mato etc. and whey powders, which are in increasing weights in the modern times. Not only this, considering enormous availability of lactose content and other benefits such as presence of minerals and organic acids, whey can also be successfully used to prepare thirst quenching beverages to offer a great opportunity in harnessing its nutritional benefits. These products with special reference to the cheeses and powders highlighted, hold a promising future in the years to come at a global platform owing to its product portfolio, nutritional benefits, easy availability and low cost. This chapter confers various aspects of whey from its origin to utilization and production of products employing novel technologies.

Keywords Whey · Valorisation · Food waste · Dairy · Beverages

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11.1 Introduction

Worldwide, milk production and processing have grown at a tremendous high rate, achieving a value of more than 10% every 4 years (FAOSTAT, 2017). Compared to 2018–2019, it has increased by 5.70% according to the survey done by GOI (Anonymous, 2021). A major chunk of total milk produced globally (more than 801 million tonnes), a hefty per cent is diverted (37%) to make cheese and other coagulated products, whereas a share of 30% is contributed by butter to meet the consumers' demand. The figures also illustrates that only 10–20% of the milk fraction in terms of total solids is processed as the desired end-result during processing of various dairy-based commodities, while 80–90% of the liquid portion emerges is a liquid, referred as whey (Panghal et al., 2018), which is a yellow-green colored liquid by-product. Riboflavin, often known as vitamin B_2, is responsible for conferring whey its characteristic yellowish hue (De Wit, 2001). In other words, whey, a greenish yellow coloured, salty liquid obtained from curd during the production of chhana, paneer, and cheese, is nutritional reservoir of many elements. It is a nourishing by-product including beneficial components counting lactose, proteins, minerals, and vitamins that are vital for human nutrition. In addition to making up 45–50% of the total milk solids, 70% of the milk sugar (lactose), 20% of milk proteins, and 70–90% of minerals, and most significantly, nearly all of the water-soluble vitamins that were previously present in milk, goes in the whey. Additionally, whey contains beneficial proteins such as α-lactalbumin, β-lactoglobulin serum albumin (SA), immunoglobulins (IG's), lactose, milk salts, and so on (Bankar et al., 2021). Since the benefits of using whey for humankind were so great, it was first used in the 1970s for whey baths to nourish the skin. Owing to rich nutritional competency, whey could be used in production of various food products mentioning whey-based cheeses, whey-based unfermented and fermented beverages, protein concentrates and powders of various ranges. Valorisation of whey is a prudent manner not only will bring economic fringe benefits over main products but also helps in utilizing the enormous production of whey from the dairy industries, leading to create its own commercial worth in billion dollars. The whey utilization is not only challenging due to its high Biological oxygen demand (BOD) and chemical oxygen demand (COD), but also a herculean task, visualising its enormous production, which also included its restricted direct discharge into rivers and use as animal feed (Divya & Kumari, 2009; Papademas & Kotsaki, 2019). Literature states the production of 9–10 L of whey, against 1 kg of cheese, (Jelen, 2003) which not only poses an alarming situation for the environment but also look for sustainable solutions in this field.

Though the recent trend has identified many novel products to be placed in the shelves of supermarkets, many strategic interventions are still to be seen to check its viability model. Although owing to its health advantages, it targets to people of all ages, including children and teenagers. It also aids in the treatment of certain illnesses, such as digestive tract conditions and bears a long history of treating urinary tract scaling, intoxication, and other skin-related illnesses with the finest

results. Furthermore, significant concentration of amino acids (a.a) in the beverage makes it extremely beneficial for athletes. Known primarily as branched chain amino acids (BCAAs) naming isoleucine, leucine, and valine, when performing resistance training, these are the first amino acids used that are metabolised right into muscle tissues. Among them are lactoferrin, GSH, and glycomacropeptide (GMP). These elements, especially the beverage, can be used to test the iron content's absorption capacity and, possibly, improve the GIT's capacity to absorb iron (more helpful in infants and neonates). In addition to being anti-inflammatory and antioxidant, absorption of various vital minerals benefits older people who suffer from osteoporosis. Envisaging the limitless opportunities, whey has become the vital entity for its exploration in valorisation wherein endless products like cheeses (Bhatti, 2021), beverages (Otte et al., 2007), lactose powders, infant foods (Bozanić et al., 2014), food supplements (Marshall, 2004; Morris & FitzGerald, 2008), soups and drinks (Marshall, 2004) hold a promising future to cater the needs of modern consumer. Though valorisation of whey has been taken up many researchers in the recent past emphasising sustainable biorefining (Goyal et al., 2023); industrial high-value added products (Arshad et al., 2023); non-ionic biosurfactants (Semproli et al., 2023), biomass to vitamins and many more to mention. However, to limit the length of the chapter, herein we precisely limit our discussion to food and related products.

11.2 Types of Whey: Origin and Nature

The whey can further be separated into two kinds depending upon nature and origin associated, process opted for the production of main product and acidulants added. Acid whey results from acidification of milk at pH levels lower than 5.0 (paneer whey) while sweet whey is the resultant product of chymosin-induced milk coagulation which occurs at pH 6–7 (cheese whey) (Papademas & Kotsaki, 2019). Characteristically, sweet whey is the waste product resulting from the production of cheeses including both hard and semi-hard cheeses, such as Cheddar cheese whereas acid-whey is produced as an end- product of various fermentation processes during fresh acid-coagulation processes, counting fresh cheeses such as Cottage cheese or direct acidification of milk which is undertaken during production of casein and caseinates. These type of products leads to a whey having a pH of about 4.6–5.0 (Tunick, 2008). Although both kinds of whey contains an equal amount of protein i.e. 11–13.5%. However, the former contains more amount of lactose content mentioning almost 63–75%. Therefore, presently cheese whey is most commonly used commodity in the food industry having a pH around 5.8–6.3 (Pouliot, 2008). The total composition of both kinds of whey has been compared and shown in Table 11.1. Whey contains beneficial proteins such as α-lactalbumin (LA), β-lactoglobulin (Lg) serum albumin (SA), immunoglobulins (Ig's), lactose, milk salts, and so forth (Bankar et al., 2021). Apart from this, whey produced or released after the production of whey-based cheeses such as Ricotta cheese are termed as second cheese whey (SCW), having low fat and protein content compared to primary whey.

Table 11.1 Typical composition (g/L) of sweet and acid whey

Component	Sweet whey	Acid whey
Total solids	63.0–70.0	63.0–70.0
Total protein	6.5–6.6	6.10–6.2
Lactose	46–52	44–47
Milk fat	0.20–0.50	0.3
Minerals	5.00–5.2	7.5–7.9
Lactic acid	2	6.4
pH	5.9–6.4	4.6–4.7
Calcium	0.4–0.6	6.0–8.0
Phosphates	1.0–3.0	2.0–4.5
Lactate	2	6.4
Chlorides	1.1	1.1
Free amino acids	0.133	0.45

Source: Papademas & Kotsaki (2019)

11.3 Therapeutic Value of Whey

As mentioned earlier, whey has also been successfully applied for diarrhoea, bile disease, skin problems, urinary tract scales and some intoxication treatments. Such beverages are also often regarded as an excellent source of energy and vital nutrients for athletes because of the high amount of quality proteins and a high nutritional value. Additionally, Whey proteins are rich source of branched chain amino acid (BCAA's), such as isoleucine, leucine, and valine. Unlike other essential amino acids (EAA), BCAA's are metabolized directly into the muscle tissue and are claimed to stimulate muscle protein synthesis in combination with resistance physical exercises (Santos & Nascimento, 2019) and many such claims have also been verified.

Whey being rich source of various vital minerals exerts its critical role in specific functions including transmission of nerve impulses and muscular contractions owing to presence of potassium, depolarization of nerve or muscle causing relaxation and consequently its role in lowering blood pressure due to magnesium. Apart from this, calcium present in whey helps in maintaining alkaline tissue pH, with maintaining bone density, integrity of the cell wall and nerve impulses, whereas lactoperoxidase prevents the growth of iron-dependent bacteria. Presence of lactoferrin inhibits bacterial growth (including pathogenic bacteria) and fungi. Many vital vitamins are included in whey, including vitamins D, B_2, A, B_3, C, B_5, B_1 E, and B_6 (Anirudh et al., 2022). These have also a proven role against obesity and muscle-protection during dieting process by inducing increased rate of thermogenesis and helps in preserving lean mass (Pal & Radavelli-Bagatini, 2013). Therefore, whey proteins are conferred to bestow many therapeutic values (Fig. 11.1) (Gupta & Prakash, 2017; Ranganathan et al., 2020, Mehra et al., 2021).

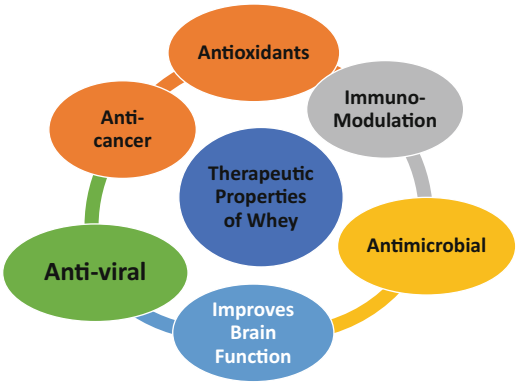

Fig. 11.1 Therapeutic and health properties of whey

11.4 Present Status of Whey Production

Whey production is directly correlated with the production of cheese and paneer (Indian cottage cheese) in the global market and recently, the Indian cheese market had a worth around 71.3 billion in 2022 and is expected to rise by about 24.06% CAGR with a worth around 262.6 billion by 2028 (Anonymous, 2023). Between the 5 years of 2015–2020 only, it has grown nearly to 18% in the CAGR and it is anticipated to grow by 31% during the mentioned years. Globally almost 3000 cheese varieties are available in the commercial market, whilst only few i.e. around 40 to 45 cheese varieties are marketed in India (Anonymous, 2020). The cheese market in India developed at a CAGR of about 26% during the years 2014–2019 and this can be attributed to numbers of factors including addition of new variants like pepper, garlic, red chili flakes, and oregano to meet the different tastes and customer preferences in India (Anonymous, 2020). In the next 6 years, i.e., from 2017–2018 to 2022–2023, the overall cheese market in India is anticipated to reach a growth rate of around 20%. By the end of 2022–2023, it is predicted to attain over 100,000 metric tons of production registering CAGR of approximately 12%. The prevailing conditions of cheese market has demonstrated saturation in Europe and North America, forcing to explore new markets of developing countries such as Japan and Russia, including India.

Generally, cheese whey has a high nutritional value with 45–50% total milk solids (TMS), abundant amounts of lactose i.e. 70–80%, 9–20% proteins (particularly whey protein), 8–20% minerals, almost all of water–soluble vitamins which are found in milk, including minor components like hydrolyzed peptides of *k*-casein, lipids and bacteria on dry matter basis (Kinsella & Morr, 1984; Horton, 1995). Whey protein consists of four major protein fractions and six minor protein fractions. The major protein fractions including beta-lactoglobulin (about 65%), alpha-lactalbumin (about 25%), bovine serum albumin (about 8%) and immunoglobulin (about 2%), while the minor fractions include lactoferrin, lysozyme, lactoperoxidase and glycol

macro peptides (Walzem et al., 2002; Marshall, 2004) and has a high Biological Oxygen Demand (BOD) value, ranging between 39,000 ppm and 48,000 ppm.

According to FAO (2020), there was 117 million tonnes of fresh whey produced worldwide in 2019 in which the European Union and the United States contributed a total of about 69% of this amount. The entire amount of whey produced increases by 1–2% annually. However, only less than 50% of this amount is consumed or utilized using various methods (Panghal et al., 2018). Currently, to valorize the whey, the commercial industrial scaling up the manufacturing process of products including production of lactose, dry whey, and whey paste (FAO, 2022). India produced over 1.3 million metric tonnes of whey in 2021, data validated by the Food Safety and Standards Authority of India's (FSSAI). However, currently not a precise information could be fetched on whey production and wastage in India for 2022. Although the Indian government has started a number of programmes recently to encourage whey utilisation and decrease waste. For instance, the FSSAI introduced the "Eat Right Creativity Challenge" programme in 2020 to encourage the usage of whey in various food and beverage items. In addition, the government has given dairy producers and processors a financial aid to set up whey processing facilities and create infrastructure for its storage and transportation.

11.5 Impact on Environment and Economy

Since whey is regarded as a major pollutant and a by-product of dairy industry, carrying high BOD (40,000–60,000 mg/L) and COD (50,000 mg/L and 80,000 mg/L), the dairy industries are under pressure from the society to re-evaluate how this could be managed successfully without posing any serious threats to the environment. Also, a data illustrated that about 120 million tonnes of whey is produced each year all over the world, but only about half of that quantity is put to use in the manufacturing of products that are consumed by humans and animals alike (Nikodinovic-Runic et al., 2013). Lactose, the primary component of whey and accounting for 70–72% of the total solids, is the key factor contributing to the high BOD and COD values. According to estimates, 4000 L of whey might harm the environment in a similar way to 1900 people producing faeces (Papademas & Kotsaki, 2019). After an accident in 2008 where spilled acidic whey in water body in Ohio, caused the mortality of more than 5400 wild animals, most of which were fish. This established the fact of decline in the concentration of dissolved oxygen led to eutrophication, which ultimately led to the death of the animals. Dumping excess whey to the soil can also lead to a reduction in the redox potential of the soil and leads to the impaired soil nutrition, affecting decreased the productivity of the crops (Ghaly et al., 2007).

Whey is regarded as the dairy industry's leading environmental contaminant in the absence of sustainable practises since it is frequently disposed of as wastewater and is connected to significant perils to the environmental. Henceforth, it is crucial to direct whey management towards a cost-effective and sustainable way of utilisation

to direct its production into novel valuable products. This has two sides of the coin, firstly because the disposal of whey corresponds to a significant loss of prospective nutrients and energy, making it necessary to exploit and harness nutritional value of whey, while also mitigating the negative effects of disposal on the environment. Whey utilisation breakthroughs are contributing to the field of applied technology. The use of whey streams has been improved by membrane processes like ultrafiltration and nanofiltration, and fermentation techniques for turning whey into high-value added products, which have shown a promising route to the establishment of biorefineries. A great economical and sustainable alternative to employing whey for the production of more valuable goods is the simultaneous integration of many work units in one process, which lessens the environmental impact of whey (Zandona et al., 2021).

11.6 Way Towards Valorization: Green Era

It is noteworthy that over the course of the past several years, the dairy sector has introduced a range of technologies and procedures in order to process whey, and researchers and scientists are currently searching for a novel product portfolio that can be created utilising the by-product. Furthermore, it is possible to recover solid components from cheese whey employing a broad variety of separation processes, including hydrolysis, electrodialysis, ion exchange, nanofiltration (NF), microfiltration (MF), reverse osmosis (RO), and ultrafiltration (UF), amongst many others. However, when it comes to the processing of whey in dairy establishments, filtration is the foremost liked choice of manufactures (Pal & Nayak, 2016). Apart from this, simple physical and thermal treatments are not only cost effective but also acceptable for small-scale manufactures yielding acceptable results.

Data depicts that 45% of whey is used in its liquid form, whereas 30% as dried whey powder, 15% as lactose and various by-products and residual as whey protein concentrates (Kosseva et al., 2009; Panesar et al., 2007). Transformation of whey into valuable products derives its caption of “from gutter to gold” (Smithers, 2008). The extracted components are of high biological value and therefore carry a significant importance in healthcare supplements, confectionary and bakery and infant products (Minj & Anand, 2020).

Production of high protein products and recent innovation of these products for the synthesis of bioplastics have gained researchers attention. Many researchers have pointed out valorization of whey using microbial sources involving bacteria’s, yeasts and fungi (bioprocesses) for bioremediation processes, production of bioactive peptides, bacteriocins, including enzymes, bio-alcohol, and many more (Chourasia et al., 2022).

11.7 Value-Added Dairy Based Products

11.7.1 Whey-Based Beverages

Given its benefits, whey protein (WP) has gained popularity as a source of nourishment in number of products, including whey protein beverages. These can be divided into four categories (Chavan et al., 2015) namely—Mixtures of whey (processed or unprocessed, including permeates utilized from the ultrafiltration processes) with fruit or (rarely) juices derived from vegetables; thick or viscous beverages of dairy origin (fermented or unfermented); Thirst-quenching beverages with impinged carbonation; and alcoholic beverages or also termed as fermented beverages (Chavan et al., 2015).

However, for the convenience, these can be broadly classified as fermented or unfermented beverages and their nutritional comparison and sensory acceptability can be assessed.

11.7.1.1 Whey-Based Fermented Beverages

Presence of enormous amounts of lactose makes the prepared whey beverages perishable and vulnerable for microbial attack and proliferation, owing to which some fermented techniques could be opted to extend the life of such beverages. For this range of yeasts and lactic acid bacteria (LAB) could be harnessed to prepare innovative and shelf-stable drinks. Some strains are also reported to degrade β-lactoglobulin, which is otherwise present in whey-based products and is known for its allergenic behaviours in some people (Pescuma et al., 2011). Various reasearchers have explored the possibility of using *L. acidophilus* CRL 636, *L. delbrueckii* subsp. *bulgaricus* CRL 656, and *S. thermophilus* CRL 804, as single or mixed (SLaB) cultures in conjugation with WPC 35, to limit available lactose or protein using microbial fermentations in preparation of functional drinks (Pescuma et al., 2010). The fermentation not only exhibited decreasing β-Lg, but also demonstrated increased amounts of BCAA during storage.

Not only this, whey can also be successfully converted into low alcohol or acetic acid beverages, producing novel food commodity utilizing the bioconversion ways to tackle waste management. Since lactose is the fermentable sugar present in abundance quantities in whey, it can be used to prepare alcoholic beverages with varying alcohol content ranging with low ($\leq 1.5\%$) alcohol content, to name—whey beer and whey wine. Production of beverages involves the technology of deproteinization of whey, concentration of whey, or fermentation of lactose employing various strains including some mentioned strains of yeast such as *K. fragilis* and *S. lactis* (Jelicic et al., 2008). Also, whey permeate from UF has been employed to prepare alcoholic beverage using *Kluyveromyces fragilis* (Parrondo et al., 2000).

Tofu whey has also been evaluated in this context, bringing 6–7% (V/V) ethanol in the final product utilizing yeasts for the same (Chua et al., 2017). In many researches natural juice along with source of fermentable whey were used together to bring out a novel product. For instance, apple juice with whey were used in combined format to prepare fermented beverage using kefir grains. Different ratios of kefir grains with varying temperature were studied and it's on beverage properties was determined, demonstrating increasing high acidity, an increased kefiran concentration, lactic acid amount, viscosity, a higher lactobacilli and yeast population resulting from increasing concentration of kefir grains (Sabokbar et al., 2015). Herein new category i.e. carbonated whey drinks also emerged as a wonderful option to supplement taste with health wherein hydrolyzed lactose whey was utilized with herbs and sugar additives. Adding Fizz in the drinks not only attracts kids but also gives a tingling sensation, which is liked by one by all. In addition, carbonation is least expensive, safe apparently has no effects on dairy products (Paula, 2005). To add carbonation effect, Carbon dioxide was introduced and resulted in more acceptability. However, in few of the tested trials, whey beverages with incorporated lemon, green tea and peppermint extracts were found more acceptable (Mabrouk & Gemiel, 2020). Some other studies involving carbonation includes use of orange juice and whey (Pareek et al., 2014); guava whey beverage (Singh et al., 1999); probiotic functional carbonated beverage (Silva E Alves et al. 2018), carbonated feta cheese whey beverage (Jairath et al., 2012); carbonated flavoured Mozzarella-whey drink (Sameen et al., 2013). In some cases naturally developed carbonated drinks aided with the help of bacterial and yeast cultures (Kadyan et al., 2021) could be employed to prepare desirable carbonated drinks (Chilana et al., 2015; Kaur et al., 2018).

Another wonderful category in this classification stands apart with the remarkable feature of fermented though probiotic beverage based on acid-whey (Skryplonek et al., 2019). Probiotic strains of *L. acidophilus* LA-5 or *B. animalis* ssp. *lactis* BB-12 were tested in pasteurized acidic whey with UHT milk, unsweetened milk and SMP. Results proved the efficacy of beverage made with whey, condensed milk and L. *acidophilus* in sensory results over other tested combinations. Similarly, various functional fermented whey beverages were reported using different strains (Jitpakdee et al., 2022) and probiotic in combination with different fruit juices or milk (AbdulAlim et al., 2018; Bulatovic et al., 2014; Nursiwi et al., 2017; Rosa et al., 2023). Such beverages have been clinically proven to confer anti-obesity effect in animal model (Hong et al., 2015) and a source of biological active peptides (Rosa et al., 2023).

11.7.1.2 Whey-Based Unfermented Beverages

Various researchers have proven the fact that it is utmost necessary to optimize beverage formulation to get the best sensory profile of the drink (Djuric et al., 2004). Therefore, for the utilization of acid and sweet rennet whey-based fruit beverages, the side effect of precipitation of proteins during thermal treatment, which renders it

unacceptable to the consumer, must be compromised or taken into attention (Ryan & Foegeding, 2015). However, in few cases, like Ricotta Cheese Whey (RCW), the secondary cheese whey, being low in protein content was demonstrated to be successfully used for the preparation of clear whey-based beverages (Rizzolo & Cortellino, 2018). Such RCW-based fruit beverages would have many health benefits such as muscle health, helps to burn fat, maintain blood sugar levels, energy production, strengthen immune system, and maintains a healthy heart (Anoymous, 2019).

The storage stability of such beverages stands at prime position for its wider acceptability wherein Cortellino and Rizzolo (2018) also conducted a study to analyze storage stability of novel functional beverages prepared from RCW with addition of fruit juices. The researchers concluded that storage days influenced the stability of total monomeric anthocyanins and a life of 150 days has been reported by the authors at −30 °C while pasteurized drink carries a life of 15 days at ambient temperature. Although whey can be added with variety of juices and purees at varying ratios, studies have demonstrated that it does not go well with much acidic options like oranges comparing with milder options such as pear, apple and peach (Djuric et al., 2004). Other references related to whey-based beverages could be cited at Singh et al., 1999; Rohit et al., 2020).

Whey-based beverages can also be a wonderful source of electrolytes and served as sports drink. According to Resolution no. 18 (09/27/2010), an electrolyte drink is a product designed to help hydration and must have minimal prescribed levels of sodium, potassium, and carbohydrates (Valadao et al., 2016). Various researchers have reviewed the importance of whey in formulation of sports drink carrying specific electrolyte balance, necessary to keep an optimal balance of salts (Anirudh et al., 2022). In addition, adding juice to prepare so, not only improves the sensory acceptability but also is a source of antioxidants.

Whey has also been used in conjugation with various herbs to prepare functional beverages carrying benefits of thirst-quenching properties and aiding health benefits of herbs side by side in one go. Various such reported studies signified the use of whey for the people who wish to manage their weight (Kanchana et al., 2020).

Maya and Ritu (2016) developed a herbal beverage based on guava juice, flavoured with various herbs including basil, mint, ginger, aloe vera, lemon grass, etc., using stevia as a natural sweetener. According to the study, the beverage with 74%, 20%, and 6% of whey, guava juice, and ginger, respectively had the highest sensory rating. Fruits including papaya leaf extract based therapeutic whey beverage was prepared using natural setvia (Singh et al., 2021). Similarly, whey-based banana herbal beverage was developed conferring 15 days shelf-life (Yadav et al., 2010). Different fruits such as apple with jaljeera extract (Sharma et al., 2019); mango with ginger extract (Alane et al., 2017); kokum-honey whey beverage (Terde et al., 2022) etc. In some studies, mixed herbal whey combining benefits of two entities was also taken into consideration, for instance, beverage containing pineapple and bottle gourd in whey (WPBH) had been formulated (Singh et al., 2013). A comprehensive list of few studies on varied areas has been made in Table 11.2, demonstrating that

Table 11.2 Use of whey in various forms and its implied effect in various product portfolios

Type of whey or whey derivative	Beverage/product kind	Salient features	References
Goat cheese whey	Unfermented beverage	• Flavoured with strawberry and peach pulp • Low caloric value • Strawberry flavour had more sensory acceptability than peach flavour • Beverages showed commercial potential, serving an alternative product from goat milk, with minimal cost investment	Tranjan et al. (2009)
Ricotta cheese whey (RCW)	Sports drink	• β-D-galactoside galactohydrolase was added to the RCW • The mixture was kept for 24 h at 8 °C • Post treatment, RCW was pasteurized • Various flavour, certain additives were added to prepare sports drink • Shelf-stable sports drink can be prepared using RCW	Valadaoe et al. (2016)
UHT-processed whey-Banana beverage	Unfermented beverage	• Blend of banana puree to acidified water was combined with sucrose and pectin • Mixture was UHT processed and stored in clear glass bottles • Not much flavour changes were recorded at 4 °C for 60 days • At low temperature, banana beverage can be prepared	
Whey powder	Gluten-free pasta with added grape peel	• Adding whey powder into dough resulted in lower loss while cooking • Resulted in less firmness of pasta and low dough with smooth surface of pasta • Whey powder upto 15% exhibited acceptable range in pasta formulation	Ungureanu-Iuga et al. (2020)
Paneer whey (lemon beverage)	With artificial sweeteners (aspartame and saccharin)	• Binary sweeteners were found appropriate in sensory response compared to individual sweetener • Binary blend not only	Meena et al. (2012)

(continued)

Table 11.2 (continued)

Type of whey or whey derivative	Beverage/product kind	Salient features	References
		eliminated the load over one sweetener but also helped in minimizing flavour or after taste issues related to non-caloric sweeteners	
Paneer whey (fermented)	Rabadi (fermented pearl millet product)	• Rabadi, prepared by fermenting pearl millet (*Pennisetum typhoideum* L.) (PM)flour with fermented whey • Standardized Rabadi was packed in indigenous pouches and stored at 4 °C and 10 °C. the shelf life of the product was 8 days at 4 °C and 5 days at 10 °C, respectively	Poonia and Kumari (2018)
RCW	Synbiotic fermented dairy beverage	• RCW concentrate along with powdered milk in combination with pre and probiotics were used to prepare novel beverage • *Lactobacillus acidophilus, Bifidobacterium and Streptococcus thermophilus* were used to ferment the necessary components • Product yielded acceptable whey beverage with a shelf-life of 45 days and a successful aim to utilize effluent of dairy waste	Schlabitz et al. (2015)
Whey protein (WP)	Yogurt	• Addition of serum whey modified the rheological properties of the product inducing cfeaminess • Had positive effect on aroma and goat flavour	Mazzaglia et al.(2020)
Whey protein (WP)	Edible films	• Edible films containing nisin, natamycin and malic acid were prepared • Films exhibited improved effect against *L. monocytogenes, P. commune and P. chrysogenum* • Film was tested as a surface protective sheath for cheese and proved a potential hurdle	Pintado et al. (2010)

(continued)

Table 11.2 (continued)

Type of whey or whey derivative	Beverage/product kind	Salient features	References
RCW	Growth intended for *Rhodotorula glutinis* for production of carotenoids	• The Scotta was evaluated for its potential as a substrate to produce caroteniods • Comparing with semi-synthetic substrate, similar results were obtained with the RCW • However, promoting the lactose hydrolysis and its effect on microorganisms yet to be studied and could be studied further	Ribeiro et al. (2017)
Cheese whey	Cultivation of LAB	• Bread-whey medium has been tried as an alternative to conventionally available MRS medium • It helped the proliferation of *Lactiplantibacillus plantarum* UMCC 2996, *Furfurilactobacillus rossiae* UMCC 3002, and *Pediococcus pentosaceus* UMCC 3010 • The medium has been fund particularly optimal for the strain *F. rossiae* UMCC 3002, exhibiting an increased growth by 114% compared to conventional medium	Iosca et al. (2023)
Cow milk, sheep milk, goat milk whey protein concentrate (WPC 15%)	Probiotic whey—based beverages with kiwi powder	• WPC resulted in higher protein content and per cent acidity in the beverages than in control samples • It also improved the viability of *S. thermophilus* and probiotic bacteria during the entire storage period • It also led to the increase in free amino acids, essential amino acids and branched chain amino acid contents as well as total phenolic content and the antioxidant capacity of beverages	Dinkçi et al. (2023)
Cheese whey	Alcoholic fermentation on cellulosic material	• A novel system of whey fermentation was ascribed • Yeast (*K. marxianus* IMB3) was immobilized on lignified free cellulosic material (DCM)	Kourkoutas et al. (2002)

(continued)

Table 11.2 (continued)

Type of whey or whey derivative	Beverage/product kind	Salient features	References
		• Fermentation exhibited improved aroma, • Production of novel yet with low alcohol content drink was suggested	
Cheese whey	WPI and anthocyanin extract	• Color stability and astringency of the beverage model was studied involving WPI • Results indicated successful insight for the production of protein-anthocyanin beverages	Wang et al. (2023)

whey can be used in various modes and its implied usage could be harnessed at many places.

11.7.2 Whey-Based Fermented Dairy Products

Whey based fermented dairy products majorly includes cheeses of wide ranges including, Ricotta, Manouri, Mato, Urda, Anari and many more to mention including 28 total various types. According to the Codex Alimentarius (2010), whey cheeses are solid, semi-solid, or soft products which are principally obtained through either of the following processes: (1) the concentration of whey and the molding of the concentrated product or (2) the coagulation of whey by heat with or without the addition of acid'. Due to large amounts of lactose in the whey, the resultant cheese often exhibits yellowish to cookie colour accompanied by sweet or sometimes cooked or caramelized flavour (Bozanic et al., 2014). However, inclusion of milk, cream, or whey while heat precipitation or acidification lowers the overall lactose content and results in white to yellowish cheeses.

Depending upon the whey type, whey cheeses can be prepared wherein further membrane technology plays a pivotal role. The inclusion of membranes enables the fractionalization of whey components required to be retained in the resultant cheese. The general methodology includes heating to 88–92 °C while stirring, wherein rate of stirring and time of attaining the required temperature are critical factors mostly dependent upon the variety to be produced (fresh or dried whey cheese). In most of the fresh whey cheese production, generally lower temperature is employed whereas higher is often desirable for dried whey categories (Bintsis & Papademas, 2023).

Similarly, Ricotta cheese (RC) has also been utilized in production of functional product to combat various nutritional deficiencies as a source of fortification. Nzekoue et al. 2021 analysed the suitability of RC as a base to supplement vitamin D, exhibiting it a wonderful source of proteins (7.8 g/100 g) with good

amount of BCAA's (1.8 g/100 g). It indicated that 50 mg of supplemented vitamin D_3 to a total cheese quantity of 95 kg resulted in a mean fortification level of 41.4 ± 4.0 μg/100 g of ricotta cheese, indicating an ideal alternative for vitamin D3 fortification.

Cheese whey or deproteinised cheese whey (DCW) has been identified as a substratum for production of acids or kefir-like beverages or traditional milk kefir. Changes in lactose amounts, consequent production of ethanol accompanied by various biochemical and physico-chemical changes, including production of volatile compounds have been noticed analyzing this potential dairy waste (Magalhaes et al., 2011).

Not only nutritionally superior whey cheeses can be prepared from this valuable entity, but also the whey cheeses have been recognized as source of Glycomacropeptide (GMP) in management of PKU (Ney et al., 2009). Authors reported low Phenyl alanine (2.5–5 mg/g protein) in cheese whey (CW) indicating it as an excellent palatable source of protein, improving necessary dietary compliance required for Phenylketonuria (PKU) patients. Another breakthrough involves fermentation of cheese whey in production of organic acids, single cells proteins and oils and bacteriocins (Mollea et al., 2013). Synthesis of galactooligosaccharides (GOS) using enzymes offers another wonderful opportunity of using whey as a substrate (Fisher & Kleinschmidt, 2015), which have critical role to mimic infant foods.

11.7.3 Whey-Based Protein Rich Products

Whey proteins are generally highly-valued commodity and available in a variety of forms, comprising whey powder containing 8–12% protein and more than 70% lactose; Whey Protein Concentrate (WPC), a product with between 30% and 89% protein, and Whey Protein Isolate (WPI), having over 90% protein and nearly no lactose (about 3%) (Boscaini et al., 2023).

While preparing WPC and WPI, whey proteins might get denaturized, which tends to impair their functionality. The most widely used methods for pre-concentrating whey in the manufacturing of WPC and WPI are UF and/or DF, along with a few other membrane processes including Nanofiltration (NF), Reverse Osmosis (RO), Electrodialysis (ED), and Microfiltration (MF). The concentrated whey using the UF/DF process, is then pasteurised, evaporated, and dehydrated to create WPC or WPI (Panghal et al., 2018). Currently, production of WPC and WPI has been considered best amongst available approaches to valorize whey into fruitful value-added product. The quality characteristics of the prepared products depend upon many variables, counting physic-chemical composition type of milk used, and the environment where cheese is manufactured. The functional qualities of these products are significantly influenced by processing conditions, such as thermal treatments, pH, and the type and amount of salts present (Pelegrine & Gasparetto, 2005). Methods like use of freeze concentration for enhanced recovery of protein and lactose has been advocated by Lamkaddam et al., 2023.

11.7.3.1 Whey Protein Concentrates (WPC)

Whey protein concentrates derived from the first generation had protein content as low as 30–40% with high quantities of lactose, fat, and undenatured proteins. It is the most concentrated type of protein supplement available, loaded with calories, containing every macro- and micronutrient obtained during production. It can, however, come in a variety of forms depending on the protein concentration, such as a WPC of 35, 50, 65, or 80% (w/w) protein (Minj & Anand, 2020). Nowadays concentrates include between 70% and 80% more protein and less lactose. Ultrafiltration processing is the method employed to accomplish the elimination of lactose to improve the final product's protein and fat contents. Such equipment is expensive, and prevents small- and medium-sized dairy firms from adopting the technology (Carter & Drake, 2018). The final drying phase involved in the manufacture of WPC is carried out by employing freeze or spray drying (Carter et al., 2018). WPC incorporation improves the nutritional value of healthy drinks, meal bars, supplements, baby foods, processed cheese, meat or fish products, and feed rations (Arab et al., 2023). WPC can also be used in salad dressings as an emulsifier. Addition of WPC has demonstrated high firmness and stability in the salad dressings, along with bestowing good emulsifying capabilities (Kotoulas et al., 2019). Modernization in technology to produce instant powder enables this protein to have increased functional capabilities that offer certain performance qualities such as instantized WPC 80% may be quickly dissolved in liquids with hand stirring or shanking, unlike high protein powders that require an electric blender to dissolve (Guo & Wang, 2019).

11.7.3.2 Whey Protein Isolates (WPIs)

Whey protein isolate (WPI) is referred as when the majority of the components have been eliminated, or when the whey has undergone an additional purification step to eliminate or reduce the unnecessary carbohydrates and lipids to achieve a protein threshold of 90% (w/w). It is also the purest form of whey protein currently accessible and& contain over 90% protein, little to no milk sugar (lactose), and nearly no fat, making it a beneficial source of protein for those who cannot consume lactose due to any underlying reasons (Carter & Drake, 2018). Despite being a high-quality protein, whey protein isolate has the drawback of losing some essential micronutrients and protein fractions such as lactoferrins, lactoglobulins, and immune-globulins during the purifying process (Minj & Anand, 2020). Due to the product's purity and higher protein content, WPI cost a little more than a WPC. Adsorption techniques like ion exchange chromatography, which offers an supplementary level of selectivity on the utilisation of membranes, can also be employed for WPI manufacturing. The final product prepared so carries more economic worth and it has better functional qualities than WPC (Foegeding et al., 2002).

Concentrates and isolates have a wide array of applications in many sectors especially predominate in food supplies, due to their extraordinary high protein

content and can also function as water-binding, gelling, emulsifying and foaming agents. It is also believed to be more compatible with other associated ingredients keeping in mind its origin and perception of being natural (Solak & Akin, 2012). Due to its low lactose and fat content, WPI is appreciated by bodybuilders and athletes who follow a rigorous diet. WPC, on the other hand, is more affordable and frequently utilised in food products. WPI is easily digested and absorbed by the body, but WPC is a good source of BCAAs (Sharma et al., 2022).

11.7.4 Lactose Powder

The main source of calories in milk is lactose (O-β-d-galactopyranosyl-(1–4)-β-d-glucopyranose), which is also the primary sugar present in milk. While most mammals produce milk containing lactose, there are a few exceptions such as sea lions and walruses that produce lactose-free milk. Lactose is also the primary source of nutrition for new-borns (Dominici et al., 2022). The udder of cow synthesizes lactose by absorbing blood glucose through the basal membrane of mammary epithelial cells. During lactation, a dairy cow converts around 20% of its circulating blood glucose into lactose (Costa et al., 2019). Lactose is characteristically found in dairy products such as yoghurt, milk, butter, ice cream, and cheese. Some breads and baked foods, ready-to-eat breakfast cereals, instant soups, confectionery, biscuits, salad dressings, sausages, gravy, drink mixes, and margarine may also contain lactose, which is often referred to as 'hidden lactose' (Facioni et al., 2020). Additionally, over 90% of the lactose is found in the whey and it must be separated from whey. Filtration is the most widely used technology in the milk processing sector for removing lactose from whey due to its high quality-to-cost ratio. Lactose separation is carried out using several membranes with varying retention efficiencies: ultrafiltration (40%) and nanofiltration (>90% lactose retention) (Costa et al., 2019). In contrast to the permeate, which contains the bulk of the lactose and mineral salts, whey protein concentrate (WPC), the retentate following membrane filtering, contains the majority of the protein part (Illanes et al., 2016). Crystalline α-lactose monohydrate and β-crystalline anhydrous lactose, which is largely made of beta-lactose, are the two different forms of lactose. Other lactose forms include different polymorphic variations of the alphalactose molecule, as well as amorphous forms created by either quickly drying a lactose solution or grinding lactose. (Hebbink & Dickhoff, 2019).

11.7.4.1 Production of Lactose

Concentration

The permeate is generally pre-concentrated using reverse osmosis, and preconcentration combined with partial demineralization can be accomplished by

the use of nanofiltration in some circumstances, leading to the development of a super saturated solution, this stage is typically carried out after evaporation (Cassano et al., 2019).

Crystallisation

Lactose crystallisation is an extremely complex procedure. Diffusion of lactose molecules to a crystal's surface takes place during this process. When whey is supersaturated or nuclei are added with the intention of inducing crystallisation, the crystallisation process takes place spontaneously. The primary objective of crystallisation is to create a lot of crystals that are proportionately similar in size (average diameter of 0.2 mm), allowing for efficient separation (Sunkesula, 2020).

Recovery

Decantation or continuous centrifugation are two methods for recovering lactose crystals. Crystals are only separated while utilising continuous centrifuges; however, when employing decanters, crystals are additionally cleaned with new, clean water. Separation and rinsing are consequently carried out concurrently. Since lactose recovery from the mother liquor, which contains the majority of the minerals and around 20% of the lactose, is undesirable in the absence of preceding demineralization, it is discarded (Kravtsov et al., 2021).

Drying

Lactose crystals that initially contained 5–12% moisture are finally reduced to 0.1–0.5 g/100 g of water, and the dried powder is then referred to as "crude" lactose after being treated to drying in fluidized-bed, vibration or flash dryers at a temperature of 70 °C. It is undesirable to have a thin layer of amorphous lactose grow on top of the crystals of α-hydrate if the drying procedure is carried out too quickly (Portnoy & Barbano, 2021). The rest of the technological operations, involed in lactose powder such as milling, sieving and/or packaging resembles skim milk powder manufacturing.

Purification

Additional refinement may be required depending on its intended purpose. Pharmaceutical-grade lactose must be refined, whereas food-grade lactose doesn't require polishing. It involves the re-dissolution of the lactose crystals, recrystallization of the lactose, and treatment of the solution with activated carbon to remove various solutes from the combination, including riboflavin. In addition, adding acids

(such HCl) can occasionally be necessary to regulate the acidity, dissolve salts, or denature any leftover proteins. After obtaining a clear lactose solution, neutralisation, sedimentation, and filtration are performed (Durham, 2009).

11.7.4.2 Applications

There are numerous potential uses and applications of lactose. The inclusion of lactose in products for the diabetic population is one of these applications because lactose is a source of energy which is absorbed more quite slowly than sucrose (i.e., has a moderate glycemic index and lesser than the one of sucrose; 45 versus 65) and allows the concentration of blood sugar to rise gradually (Hebbink & Dickhoff, 2019). It is often utilised as a food ingredient in the food industry for dairy, bread, snacks, confectionery, and other foods and dietary products. In the field of food and beverages, lactose performs a number of functions, such as acting as a free-flowing agent, a non-hygroscopic transporter in dry blends, an absorbent and booster of flavours and colours, an ingredient that contributes to the development of colour and flavour through the maillard browning reaction, an alternative for other low calorie sweeteners as a source carrying low sweetness and a natural source of carbohydrate and energy in infant formulas (Rocha & Guerra, 2020).

Due to its physical and chemical characteristics, including chemical inertia, stability, and non-toxicity, as well as its reasonable pricing, lactose is one of the most widely used ingredient in the pharmaceutical business (Dominici et al., 2022). Given the lactose powder's organoleptic properties—namely, that it is white, odourless, and pleasant tasting—its acceptability as a component in medicinal formulations is noteworthy. Lactose is a versatile excipient that can be used in a variety of pharmaceutical formulations. It is present in 6% of over-the-counter drugs and 20% of prescription medications. In tablets, lozenges, capsules, and powder for intravenous injections, for example, lactose can be employed as a diluent (Zdrojewicz et al., 2018).

11.7.5 Infant Foods

Mother's milk is the best natural food available for nourishing infants. However, alternatives are occasionally required when the milk from the mother is inadequate or inaccessible. Infant foods are taken into consideration in such situations so as to fulfil the neonatal body care. They are segmented into five stages.

Infant formula (IF) is the name given to stage one meal, which is intended for new-borns between the age of birth and 6 months. Stage 2, or follow-on formula (FOF), is advised for infants between the ages of 6 months and 12 months. Due to the fact that newborns start eating other things (which include cereals, vegetables and fruits etc.), stage 2 formulae have a somewhat lower energy density than stage 1 formulae. Products which are for stages 3–5 are aimed at young children and

babies between the ages of 1 and 3; 3–6; and older than 6 years old whereas Growing-Up Milk (GUM) is the principal product category for the higher phases (Happe & Gambelli, 2015). In this chapter, authors will be focussing on dairy-based infant formula.

The use of infant milk formula products is now considered indispensable towards ensuring adequate nourishment for young children today. Typically found in powdered form varieties, these formulas aim to recreate the nutritional profile which includes macro as well as micro-nutrients found in human breast-milk upon reconstitution (Masum et al., 2021). Infant formula refers specifically to a specially engineered food item that primarily uses milk as its main ingredient. Infants and young children under the age of one are its intended consumers. Key ingredients used during formulation include lactose, whey protein, casein powder, whole/non-fat milk powder supplements along with plant derived vegetable oils (Ackerman et al., 2017).

A key aspect of manufacturing ideal infant formula is achieving a balanced combination of approximately whey/casein ratio of 60:40. The preferred method involves incorporating demineralized whey alongside WPI or WPC with cow milk/milk powder blends. Finalizing this stage requires introducing carefully calculated amounts of both lactose and vegetable oils to address any variances in fat content. Sticking to this fundamental approach ensures that the resulting baby formula offers consistent quality, mimicking the composition of human breast milk (Guo & Wang, 2019). In infant feeding, lactose is also employed as a carbon source to help prevent the formation of pathogenic and dangerous bacteria while promoting the development of beneficial microbiota (Rocha & Guerra, 2020). Beyond their nutritional advantages, lactose-containing carbohydrates aid in the drying process, improve the physical and chemical characteristics, and enhance the handling attributes of dairy powders, which include fat and protein. Crystalline and amorphous lactose are both possible components of IF powders, with amorphous lactose being more common (Saxena et al., 2021). In some formulations, maltodextrin may occasionally be used to replace lactose (partially), and IF products may additionally contain galacto- or fructo-oligosaccharides (GOS) or fructose-oligosaccharides (FOS) (Masum et al., 2020). Differences may be seen depending on the amount of whey protein hydrolysis when working with IMF powders which include either partially or completely hydrolyzed whey protein. However, IMF powders usually have from 10% to 15% of their entire composition as protein (Kelly et al., 2016). In addition to this, presence of β-Lg could be a concern for infant foods wherein use of High pressure processing (HHP) could as a medium to enrich whey protein in α-La from bovine native whey concentrate (Romo et al., 2023). Authors documented the use of 600 Mega Pascal for 4 min at 23 °C for enrichment with α-La for this membrane- mediated separation.

11.8 Future Trends and Challenges

The unique nutraceutical properties of some whey components hold the key to the future of whey beverages and would bridge the gaps between circular economy. In addition, in the quest of sustainability whey valorisation is definitely necessary to bring novel products into the market and gearing with management issues. Although number of processes and approaches are in-line to valorization, many newer innovative and novel commercial processes are to be sought, visualizing ever-increasing production of this waste, alongside the economical and sustainability challenges. Integration of two or more flexible systems (physical, chemical, and/or biological processes) could be exploited to harness better recovery of desirable components and consequently fiscal benefits and to attain zero-waste approach within the milk industry (Asunis et al., 2020, Barba, 2021). Furthermore, the untapped potential of this waste in production of biofuels, organic acids, edible films and bioplastics as packaging materials is another area, which requires considerable attention to explore its hidden treasure. Although whey based beverages of various genre presents a considerable area of consumer acceptance, techniques like use of hydrolyzed whey (for calorie conscious segment), carbonation is adding more avenues for its further liking.

The new waste framework Directive (WFD, Directive/2008/98/EC), which is the basis for the current trend set forth by the European Commission, emphasises primarily on the prevention of waste creation, with a later focus on reuse, recycling, and recovery, and disposal given the least priority (Buchanan et al., 2023). It is essential that waste management approaches continue to be productive, profitable, and sustainable because the current processes present numerous problems with regard to waste management and the recovery of various profitable components (Roufou et al., 2021). Although much research has been done on the beverage made from whey, it has not yet been implemented or made widely available. Whey-based goods encounter a number of obstacles, including the availability of a variety of products in the market, consumer awareness, tiny industries, low levels of production at units, and competitiveness with other products (Panghal et al., 2018), which needs immediate scientific interventions.

11.9 Conclusions

The expected increase in dairy products by 2050 directly indicates the production of whey from the dairy industries, leading to a thought to valorize it prudently. Although such products have good expected acceptance in the market, still a careful research has to made for widely acceptable products. Also, modern value added whey-based products not only carries enhanced sensory properties but also impart nutrition compared to conventional sugary carbonated beverages, which further has a scope to expand the area employing advanced techniques such as carbonation,

freeze concentration, deproteinization of whey etc. Production of such products not only adds variety to the table, but also helps in meeting the goals of sustainability and bridging the gaps of circular economy.

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Chapter 12
Whey: Source of Bioactive Peptides, Probiotics, Organic Acids, Aromatic Compounds and Enzymes

Dushica Santa and Sonja Srbinovska

Abstract There was scientific evidence in the late twentieth century that whey had biological, nutritional and technological value. In the past, whey was considered as a waste byproduct. Nowadays, there is growing recognition that it is a valuable raw material that can be exploited in various industries. Protein components and elements related to biological functions are present in large quantities in whey, making them the most nutritionally significant part. They consist of heat-sensitive fractions like β -lactoglobulin (β-LG), α-lactalbumin (α-LA), blood serum albumin, and immunoglobulin, as well as heat-stable proteose-peptone. Other whey components, such as lactoferrin and various enzymes like lysozyme, oxidoreductases, phosphatases, lactoperoxidase, lipolytic enzymes, and proteinases, also exist. These components play a crucial role in the human immune response system. At present, extensive research is being conducted on peptides derived from whey proteins. Bioactive peptides can be easily generated through enzymatic hydrolysis, fermentation, and gastrointestinal digestion. These bioactive peptides serve as signaling molecules and have diverse physiological impacts on the immune, digestive, cardiovascular, and nervous systems once released. Dairy-derived bioactive peptides are linked to a broad spectrum of biological actions, such as immunomodulation, antimicrobial activity, antihypertensive effects, antioxidant properties, opioid characteristics, and anti-obesity functions. The development of probiotic whey beverages has been receiving attention recently because of their beneficial effects. Whey is an excellent medium for growing probiotic bacteria of the *Lactobacillus*, *Bifidobacterium*, *Propionibacterium* genera and other good bacteria. These microorganisms impart unique flavor profiles and textures to dairy products making it functional food. Whey flavor is affected by many factors like the quality of the milk used, the type of cheese made, how whey is handled after curd draining and other factors. As a result, we have different chemical compositions of whey. During cheese making, lactic acid bacteria, which are added to milk, are responsible for producing aroma volatile compounds such as aldehydes, ketones, lactones, sulfur

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compounds and other compounds in whey. The aroma of liquid whey may be influenced by a variety of volatile acids with short chains and contribute to the overall flavor of whey. This chapter summarizes the literature on the characteristics, bioactive properties, and factors influencing the viability and flavor of sweet and acid whey. Our focus will be on whey's potential as a source of bioactive peptides, probiotics, organic acids, aromatic compounds and enzymes.

Keywords Probiotics · Bioactive properties · Whey · Immunoglobulins · Bovine serum albumin

12.1 Introduction

In the late twentieth century, whey was proven to have biological, nutritional, and technological benefits. Historically, whey was considered a waste byproduct. There is growing recognition that it is a valuable raw material that can be exploited in various industries. A significant components of whey is its nutritive factors, immunological protection, and bioactive components.

The composition of whey can differ based on its production method. Sweet whey is derived during the creation of dairy products that mainly use rennet-type enzymes, with a pH of around 5.6. Conversely, acid whey is a byproduct of dairy product manufacturing in which the curd forms through acidification at a pH of roughly 5.1 or lower. Specifically, acid whey is a byproduct of producing acid-coagulated cheeses (such as cottage cheese, ricotta cheese, etc.) and Greek yogurt. Acid whey is difficult to dry due to its low pH, high mineral content, and lactic acid content. As a result, a considerable amount of whey is still treated as waste, creating an environmental issue and additional expenses for manufacturers. To address the large volumes of whey that still require processing in emerging sectors like biochemicals, biofuels, and bioplastics, more effective and cost-efficient integrated processes and systems must be developed (Zandona et al., 2021). Generally, whey contains about 50% of milk components, including lactose (typically around 70%, though it varies based on the acidity of the whey), whey proteins (approximately 14%), minerals, and some fat. In addition to it, whey proteins contain high level of essential amino acids and considered as a source of high quality proteins (Arya & Poonia, 2019). The main distinctions between sweet whey and acid whey lie in their calcium, phosphate, lactic acid, and lactate concentrations, with acid whey containing higher levels (Božanic et al., 2014; Panesar et al., 2007).

Cheese whey in liquid form can be transformed into a variety of types, such as condensed whey, acid or sweet whey powders, demineralized whey powder, and delactosed whey powder. These distinct forms provide numerous advantages in terms of preservation, handling, and transportation. Whey protein concentrate (WPC) and whey protein isolate (WPI) are both products of whey processing and boast a high protein content due to the extraction and concentration of the protein portion from liquid whey (Kosikowski, 1979). WPC powders containing 35–65% protein can be created through ultrafiltration by removing lactose, minerals, and

Table 12.1 The major properties of whey proteins

Protein	Molecular mass (kDa)	Isoelectric point	Concentration (g/L)	References
α-lactalbumin	14,147–14,175	4.2–4.5	1.2	Modler (2009), Gerberding and Byers (1998) and Etzel (2004)
β-lactoglobulin	18,205–18,363	5.35–5.49	1.3	Modler (2009) and Gerberding and Byers (1998)
Bovine serum albumin	66,000	5.13	0.4	Gerberding and Byers (1998) and Peters (1985)
Immunoglobulin G	150,000–1,000,000	5.5–8.3	0.7	Gerberding and Byers (1998), Wong et al.(1996) and Etzel (2004)

non-protein nitrogen, leaving only the whey proteins. Using diafiltration, nearly all lactose and minerals are removed from WPC, yielding powders with protein concentrations up to 90% (Early, 2012).

Two approaches exist for handling cheese whey: the first focuses on recovering valuable components like lactose and proteins. Processes of valorization are the favored method for addressing this by-product, surpassing only the production of powdered cheese whey. In the second approach, fermentation processes generate products with added value such as organic acids (for example, lactic, succinic, and propionic), proteins, oils, and biopolymers (enzymes, polyhydroxyalkanoates, exopolysaccharides). Both management strategies have been successfully employed as a fermentation medium when using whey permeate obtained through ultrafiltration (Mollea et al., 2013). Over the past 50 years, researchers have extensively studied whey to optimize its utilization, with a particular emphasis on its high-quality proteins due to their nutritional value and abundance (Božanic et al., 2014). Whey is progressively acknowledged as a valuable resource with numerous potential applications, making it crucial to investigate its constituents and their use in functional foods. Whey is known for its antioxidant, immune-boosting, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating properties (Marshall, 2004).

Whey consists of several primary components, including β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), and immunoglobulin (IG), which constitute 50%, 20%, 10%, and 10% of the whey fraction, respectively. All of these major proteins, except for BSA, are synthesized by the epithelial cells in the mammary gland. Furthermore, whey contains several low-abundance proteins, known as minor proteins, such as lactoferrin (LF), lactoperoxidase (LP), proteose peptone (PP), osteopontin, and lysozyme, among others. LF and LP are the most prevalent minor proteins in whey (Mollea et al., 2013). Apart from glycomacropeptide, all other protein components naturally occur in whey, while glycomacropeptide is generated from casein during the initial step of enzymatic cheese processing (Madureira et al., 2010). Table 12.1 summarizes the main characteristics of whey proteins.

12.1.1 β-Lactoglobulin

Beta-lactoglobulin constitutes around half of the total protein content in bovine whey and holds significant value in terms of functionality and nutritional properties (Marshall, 2004). It makes up approximately 64% of heat-coagulable protein or 51% of total whey protein. β-Lactoglobulin primarily exists as a dimer comprising two identical subunits, with each monomer containing one sulfhydryl group and two disulfide bonds. Its solubility largely depends on pH and ionic strength. With a denaturation temperature of 74 °C, β-Lactoglobulin precipitates before α-LA. Its inherent function remains unclear, but it is known as a source of amino acids, including essential and branched-chain amino acids, and a retinol-binding protein has been identified within its structure (Walstra et al., 2005; Marshall, 2004). Overexpressed in the lactating mammary glands of various species, β-LG primarily serves as an essential amino acid source for the offspring of animals that produce it, promoting muscle growth and providing a rich cysteine source critical for glutathione synthesis. Furthermore, the structure of β-LG includes a ligand-binding site that typically binds to hydrophobic ligands such as long-chain fatty acids, triglycerides, retinoids, cholesterol, and more weakly, hydrocarbon molecules. It can also be a source of peptides with diverse functions, like lactokinins, β-lactorphin, and βeta-lactotensin (Mollea et al., 2013). In comparison to other proteins, α-LA's denaturation is highly reversible, making it appear more heat resistant than β-LG (Modler, 2009).

12.1.2 α- Lactalbumin

Alpha-lactalbumin, a primary protein found in human and bovine milk, makes up around 20–25% of whey proteins and contains a broad range of amino acids, including an easily accessible supply of essential and branched-chain amino acids (Marshall, 2004). This relatively small, tightly folded protein functions as a coenzyme in lactose synthesis and is the "B" protein of the lactose synthase enzyme complex, catalyzing the final step in lactose biosynthesis. This protein is a calcium-binding protein with a high affinity for other metal ions, such as Zn + 2, Mn + 2, Cd + 2, Cu + 2, and Al + 3. Calcium strongly binds to α-LA, contributing to the stabilization of its molecular conformation (Modler, 2009). α-Lactalbumin makes up 2–5% of the total protein in skim milk and is generally spherical in shape, not associating except at low ionic strength. Purified α-lactalbumin is commercially used in infant formula due to its structural and compositional similarities to the primary protein in human breast milk, making it highly suitable for use in infant formula production (Walzem et al., 2002).

12.1.3 Bovine Serum Albumin (BSA)

Bovine serum albumin makes up around 6.8% of the total whey protein, a concentration similar to that found in human milk. BSA is a diverse protein nearly identical to the one found in bovine blood. It contains 35 cysteine residues and 17 disulfide linkages per molecule, with an α-helix content of 46% (Walstra et al., 2005). Denaturation of BSA occurs at a temperature of 64 °C, comparable to that of α-lactalbumin (62 °C), but it precipitates before α-lactalbumin due to the latter's reversible denaturation properties (Brown, 1988).

Protein BSA has a molecular weight of approximately 66 kDa and is compact, rigid, and globular. A 17-disulfide bond structure stabilizes its structure, making it relatively resistant to denaturation and precipitation during purification (Peters, 1985). Numerous studies have explored BSA's biological, physicochemical, and structural properties, as well as attempted to modify it to create an ideal protein model for food systems (Peters, 1985; Shin et al., 1994). Moreover, BSA's physicochemical properties can be modified for various functional applications.

12.1.4 Immunoglobulins

Three primary categories of Immunoglobulins (Igs)—IgG (IgG1 and IgG2), IgM, and IgA—can be found in both bovine serum and lacteal secretions. IgG1 is the predominant Ig in bovine milk and colostrum (Tavares & Malcata, 2013). These Igs share a similar fundamental structure, consisting of two identical light chains and two identical heavy chains linked by disulfide bonds, with each chain weighing 23 kDa and 53 kDa, respectively. As Korhonen (2000) points out, numerous investigations have demonstrated the therapeutic potential of Igs, whose biological properties are well-established. Immunoglobulins, chemically referred to as gammaglobulins, are antibodies that make up approximately 10–15% of the total whey proteins in milk. They play an essential role in safeguarding the gastrointestinal tract's mucosa from pathogenic microorganisms. Milk immunoglobulins serve as a vital source of passive immunity for newborns, offering protection against infections and diseases. However, the potential of milk immunoglobulins goes beyond neonatal immunity, as they possess powerful properties that could be utilized to neutralize harmful dietary components or toxins. By incorporating milk immunoglobulins into diets, it may be feasible to strengthen the body's natural defense mechanisms and enhance overall health outcomes. This highlights the potential of milk immunoglobulins as a promising approach for promoting human health and well-being (Mollea et al., 2013).

12.2 Bioactive Peptides in Whey

Various factors influence the concentration of whey protein, including the category of whey (acid or sweet), the milk source (bovine, caprine, or ovine), the season, the type of feed, the lactation stage, and the processing quality. Whey contains a high concentration of proteins and biologically active components like whey protein-derived peptides. Bioactive peptides belong to a group of biologically active molecules found in numerous proteins, including whey protein. These peptides remain inactive while encrypted within the original protein's sequence (Korhonen & Pihlanto, 2006). However, they can be liberated through different protein degradation methods.

One primary way to release bioactive peptides is through digestive enzyme hydrolysis. This natural process occurs in the stomach and small intestine, breaking proteins down into smaller peptides and amino acids. These smaller peptides can then be absorbed into the bloodstream, exerting their bioactive effects. Another method of releasing bioactive peptides involves the action of proteolytic microorganisms. Microorganisms such as lactic acid bacteria can break proteins down into smaller peptides and amino acids during fermentation. This process can occur during the production of fermented dairy products like yogurt and cheese.

Lastly, bioactive peptides can also be released through plant or microbial proteases' action. These enzymes can be added to food products during processing to break down proteins and release bioactive peptides. This approach has been utilized to enhance the bioactivity of whey protein and other food ingredients. This process on general level is presented in Fig. 12.1.

12.3 Biological Activities of Bioactive Peptides

Bioactive peptides exhibit a variety of biological activities that are dependent on their sequence and amino acid composition. These activities include but are not limited to antihypertensive, antioxidative, immunomodulatory, opioid, antimicrobial, and mineral-carrying effects (Kareb & Aïder, 2019, Korhonen & Pihlanto, 2006). Besides the common functionalities, they also have anti-obesity properties (Jakubowicz & Froy, 2013). In addition, some bioactive peptide sequences are capable of carrying out multiple biological functions. As shown in Table 12.2, whey protein and peptides have bioactive properties in the human body.

Hypertension is a significant issue in society due to its high prevalence and association with cardiovascular diseases like coronary heart disease, peripheral arterial disease, and stroke. Some bioactive peptides in whey proteins may protect against hypertension through angiotensin-converting-enzyme (ACE), which facilitates the transformation of angiotensin I, an inactive decapeptide, into angiotensin II, an octapeptide with strong vasoconstrictor properties (Skeggs et al., 1956). Various

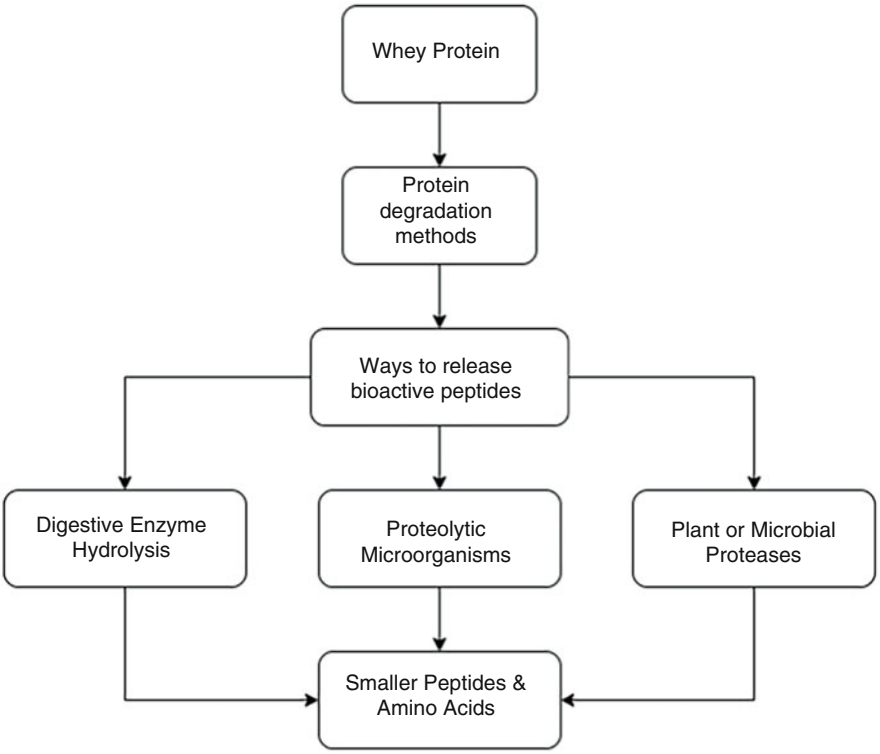

Fig. 12.1 Bioactive peptide release methods

studies have demonstrated that whey's bioactive peptides can aid in lowering blood pressure by inhibiting ACE.

Research by Rizzello et al. (2005) and Silva et al. (2006) identified the YQEPVLGP sequence as having antimicrobial, ACE-inhibitory, and ABTS radical scavenging properties. Hernández-Ledesma et al. (2005) and Otte et al. (2011) reported that peptides TTMPLW and VMFPPQSVL, respectively, possess ACE-inhibitory properties. These peptides also exhibit other health benefits, including immunomodulatory effects for TTMPLW (Gobbetti et al., 2004) and antimicrobial properties for VMFPPQSVL (Rizzello et al., 2005). In animal hypertension models, several peptides derived from milk fermentation by *L. helveticus* LBK-16H have shown ACE inhibitory properties, including Ile-Pro-Pro, Val-Pro-Pro, Tyr-Pro, and Lys-Val-Leu-Pro-Ile-Val-Pro-Gln. These findings suggest that these peptides may contribute to reducing high blood pressure and enhancing cardiovascular health (Jäkälä & Vapaatalo, 2010).

Besides peptides derived from milk fermentation, antihypertensive peptides have also been found in the whey fraction of milk protein. These peptides, called α-lactorphin (Tyr-Gly-Leu-Phe) and β-lactorphin (Tyr-Leu-Leu-Phe), are obtained through enzymatic proteolysis of whey proteins α-lactalbumin and β-lactoglobulin,

Table 12.2 Bioactive properties of whey components in the human body

Physiological functionality	Whey components	References
Antimicrobial	β-lactoglobulin and its derivatives, α-lactalbumin and its derivatives, lactoferrin and lactoferricin, immunoglobulins, lactoperoxidase, lysozyme	Rizzello et al. (2005), González-Chávez et al. (2009), Sachdeva et al. (2014) and Chatterton et al. (2006)
Antihypertensive and hypocholesterolemic	β-lactoglobulin and its derivatives, α-lactalbumin and its derivatives Immunoglobulins	Pihlanto-Leppälä et al. (1999), Jäkälä and Vapaatalo (2010) and Hernández-Ledesma et al. (2005)
Immunomodulatory	Whey protein concentrates (WPC), whey protein isolates (WPI), and their derivatives, immunoglobulins, lactoferrin an lactoferricin, lactoperoxidase	Park and Nam (2015) and Gill et al. (2000)
Antioxidant	Glutathione precursor peptides, lactoferrin	Hernández-Ledesma et al. (2005), Walzem et al. (2002) and Kareb and Aïder (2019)
Opioid	β-lactoglobulin-derived peptides	Antila et al. (1991) and Chiba and Yoshikawa (1986)
Mineral-binding	β-lactoglobulin, α-lactalbumin and lactoferrin, lactoperoxidase	Vegarud et al. (2000)

respectively. Studies have shown that these peptides can effectively lower blood pressure in hypertensive rats, indicating their potential use in hypertension management (Sipola et al., 2002). Recio et al. (2009) discovered that sheep and goat whey protein is a significant source of ACE-inhibitory peptides.

As Ahn et al. (2009) noted, whey protein-derived peptides fermented with Lactobacillus brevis inhibited ACE more efficiently than those fermented with *Lactobacillus casei*, *Lactobacillus plantarum,* and *Lactobacillus acidophilus*. This research emphasizes *Lactobacillus brevis* potential to effectively produce ACE-inhibitory peptides from whey proteins, which could have important implications for developing functional foods and supplements aimed at reducing high blood pressure. Discovering and characterizing new peptides with ACE inhibitory activity is crucial for developing new treatments for hypertension and cardiovascular disease.

The majority of research on the antimicrobial activity of whey proteins has concentrated on lactoferrin and lysozyme, along with their proteolytic fragments (Chatterton et al., 2006). Lactoferrin and lysozyme are two whey proteins known for their antimicrobial properties. LF is an iron-binding glycoprotein that constitutes only a small fraction of skim milk protein but a higher proportion of whey protein. As a major component of the mammalian innate immune system, lactoferrin is an 80 kDa iron-binding glycoprotein from the transferrin family. Its antimicrobial properties include direct effects against bacteria, viruses, fungi, and parasites, as well as anti-inflammatory and anticancer effects (González-Chávez et al., 2009). LF has demonstrated various biological properties, including antioxidant, antibacterial,

antiviral, and antifungal activities. It promotes the growth of beneficial bacteria and has long been considered a crucial component of the host defense against microbial infection (Walzem et al., 2002; Kareb & Aïder, 2019).

LF's antimicrobial effects are credited to its iron-binding activity, which deprives bacteria of essential nutrients required for growth. Additionally, human lactoferrin can damage the outer membrane of Gram-negative bacteria, and the lactoferricin peptide fragment has direct bactericidal activity. LF presents a new source for innovative antimicrobial agents with potential pharmaceutical applications. Clinical trials have shown that bovine lactoferrin is helpful for eradicating Helicobacter pylori, but f derived from LF is not as effective (Sachdeva et al., 2014).

Matijašić et al. (2020) research characterized lactoferrin extracted from acid whey using a unique ion-exchange chromatography technology on CIM® monolithic columns. The lactoferrin samples were analyzed for their properties, including purity, iron-binding capacity, antibacterial activity, and pH- and temperature-stability. The results indicated that monolithic ion-exchange chromatography holds great potential for the industrial processing of acid whey as a source of lactoferrin and other bioactive proteins. There was high antimicrobial activity and purity of the isolated apo-LF. The study also discovered that a protein product's biological activity does not necessarily correlate with its purity (Matijašić et al., 2020).

Another whey protein, lysozyme, causes lysis of microbial cells by hydrolyzing the peptidoglycan of bacterial cell walls. Lysozyme is a protein made up of 129 amino acid building blocks and contains four disulfide bonds. Its molecular mass ranges from 14,300 to 14,600, and its isoelectric point falls between 9.5 and 9.7. However, only two of the four disulfide bonds are necessary for its hydrolytic activity (Modler, 2009). It has been shown to possess antimicrobial activities against S. typhimurium and *E. coli* (Min et al., 2005; Losso et al., 2000). However, lysozyme alone is ineffective against gram-negative bacteria, as a lipopolysaccharide layer in the outer membrane acts as a permeability barrier for lysozyme to penetrate the cell interior.

LF and lysozyme offer potential sources for novel antimicrobial agents with pharmaceutical applications. However, the effectiveness of these proteins against gram-negative bacteria may be limited, and further research is required to optimize their use as antimicrobial agents (Walzem et al., 2002). The antimicrobial effects of β-Lg peptide fragments can also be observed when other enzymes are used for their generation. These peptide fragments demonstrate the ability to combat pathogenic bacteria (Pihlanto-Leppälä et al., 1999).

Immunomodulating properties have been found in bioactive peptides derived from milk caseins and whey proteins, a function that can influence various aspects of immune function. Peptides generated through the fermentation of milk caseins by proteolytic bacteria or via the action of gastric enzymes have been reported to interact with immune functions, including lymphocyte proliferation, antibody synthesis, and cytokine regulation (Park & Nam, 2015; Gill et al., 2000).

Hernández-Ledesma et al. (2005) conducted a study to examine how hydrolysates of the main whey proteins, b-Lg and a-lactalbumin, could function as antioxidants when exposed to various commercial proteases (such as pepsin, trypsin,

chymotrypsin, thermolysin, and corolase PP). Through their research, they discovered that corolase PP was the most effective enzyme for generating b-lg hydrolysates that exhibited strong activity in scavenging oxygen radicals (as measured by ORAC-FL values). Immunoglobulins and other glycoproteins (lactoferrin, transferrin), as well as enzymes (lysozyme, lactoperoxidase), play a crucial role in human immunity (Božanic et al., 2014). A newborn's immune system is protected by immunoglobulins, which combat infections and promote the growth of beneficial gut bacteria (Kareb & Aïder, 2019).

Bovine whey protein fractions, namely α-LA (f 50–53) and β-LG (f 102–105), contain peptides that exhibit opioid activity. These peptides, known as α- and β-lactorphins, have the ability to bind with opiate receptors and can be effectively blocked by naloxone (Chiba & Yoshikawa, 1986). The presence of these bioactive peptides in bovine whey protein has been extensively studied, with research suggesting their potential to influence various physiological functions. When injected into the bloodstream, opioid peptides have been found to produce analgesic and sedative effects by impacting the nervous system. Among these peptides, α-lactorphin has been shown to exhibit mild opioid activity towards smooth muscles. On the other hand, β-lactorphin has been observed to have a contractile effect on smooth muscles, as evidenced by studies such as Antila et al. (1991).

12.4 Enzymes

Enzymes are proteins that function as biological catalysts, speeding up chemical reactions. In whey, enzymes are usually proteases, which break down proteins into smaller peptides and amino acids, and lactase, which breaks down lactose into glucose and galactose. Whey includes enzymes such as lactoperoxidase, hydrolases, transferases, lyases, proteases, and lipases.

One of the most important enzymes in milk, and one that is heat-stable, is lactoperoxidase. It constitutes 0.25–0.5% of whey protein. The molecular mass of lactoperoxidase is 78 kDa, and it is a single polypeptide chain made up of 612 amino acid residues. It contains 15 cysteines, one heme group, and approximately 10% w/w of carbohydrate moieties. The enzyme's activity is influenced by temperature and duration; for example, enzymatic activity is lost at 62.5 °C after 30 min, 70 °C after 15 min, or 85 °C after 15 s (Modler, 2009; Mollea et al., 2013). A catalytic reaction can reduce hydrogen peroxide, and the natural antimicrobial lactoperoxidase system eliminates psychrotrophic bacteria in dairy products (Champagne et al., 1994). By catalyzing the peroxidation of thiocyanates and certain halides (such as iodine and bromine), this enzyme system inhibits or kills bacteria. Lactoperoxidase is heat-tolerant and is not inactivated during pasteurization (Gupta & Prakash, 2017). This enzyme plays a critical role in providing natural protection against microbial contamination and shielding animal cells from various types of damage and peroxidative effects (de Wit & van Hooydonk, 1996). According to Gupta and Prakash (2017), lactoperoxidase serves as a protective factor against infectious microorganisms. The

lactoperoxidase system is recovered during lactoferrin purification and is commercially used in various cosmetics and as a preservative in pastry cream and soft-serve ice cream (Walzem et al., 2002). An inoculum of 104 cells/mL of lactoperoxidase in heat-treated whey has been shown to effectively inhibit the growth of *E. coli*, *Lactococcus lactis* spp. *lactis, Lactococcus* lactis spp. *cremoris*, *Pseudomonas fluorescens*, and *Bacillus cereus* (Modler, 2009).

Shin et al. (2005) conducted a study that examined the effects of orally administered LF and LP in a mouse model of influenza virus infection. The study demonstrated that LF and LP help reduce pneumonia by suppressing inflammatory cell infiltration in the lungs. Additionally, LP was shown to reduce body weight loss and the production of a proinflammatory cytokine. This study suggests that LF and LP have potential as functional milk components for the treatment of infections where the host's exaggerated inflammatory responses are responsible for pathogenesis.

12.5 Organic Acids

Whey's lesser-known but valuable attribute is its organic acid content. Organic acids, also referred to as short-chain fatty acids, are vital compounds that participate in various biological processes such as energy metabolism, gut health, and immune function. Whey is abundant in these organic acids, with the specific composition being dependent on the production method and type of cheese from which the whey is obtained. In addition to their presence in whey, these organic acids are also produced in the human gut through the fermentation of dietary fibers and offer numerous health benefits.

Lactic acid is a natural compound with a long history of use in various industries, including pharmaceuticals, chemicals, and food. It is produced by different types of microorganisms, with the main LAB genera being *Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Enterococcus,* and *Streptococcus* (de Motta & Gomes, 2015). The Lactobacillaceae family, especially *Lactobacillus helveticus*, is the most significant lactic acid producer. This microorganism has several advantages over other lactic acid-producing bacteria, such as reduced phage contamination risk in dairy production, a higher lactic acid production rate in milk, and the ability to ferment lactose and its byproducts. Furthermore, *L. helveticus* generates racemic lactic acid and is well-suited for creating culture starters from milk-based media. Other common lactic acid-producing microorganisms include *Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus delbrueckii, Streptococcus thermophilus, Lactococcus lactis, and Leuconostoc*, among others (Tyagi and Kluepfel, 1998). Industrial-scale production of lactic acid from cheese whey or cheese whey permeate presents challenges due to the low productivity of lactic acid, even though mixed cultures have the ability to produce it. This is because these substrates often lack the necessary nutrients required for optimal lactic acid production. (Plessas et al., 2008, Prazeres et al., 2012).

In terms of lactic acid production, low-cost raw materials are typically preferred, and whey (ultrafiltration permeate) containing a relatively high lactose content (5–6 g/100 g) has proven to be an affordable and suitable raw material source.

Plessas et al. (2008) conducted research to assess lactic acid production using *Kluyveromyces marxianus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Lactobacillus helveticus*, either individually or as mixed cultures, under stirred or static fermentation conditions, with cheese whey as the substrate. The study examined lactic acid production, residual sugar, and cell biomass. The findings revealed that mixed cultures generated more lactic acid than individual cultures. The highest lactic acid concentrations were obtained when *K. marxianus* was combined with *L. delbrueckii* ssp. *bulgaricus* and when all strains were used together, suggesting potential synergistic effects between the yeast and the two lactic acid bacteria.

During lactic acid production, temperature and pH are critical environmental factors (Panesar et al., 2007). Büyükkileci and Harsa (2004) explored the effects of temperature, pH, and medium composition on lactic acid production from whey using *Lactobacillus casei.* The highest lactic acid productivity was achieved by *L. casei* at 37 °C and pH 5.5. The batch productivity reached 1.87 g/L/h at the flask level, while at the fermenter level, it reached 3.97 g/L/h.

12.6 Probiotics

Lactic acid bacteria (LAB) are microorganisms often employed in food applications due to their probiotic qualities. They are utilized in the production of beverages and fermented foods, contributing to both the sensory attributes of the food and the prevention of spoilage (Srinivash et al., 2023). LAB are widely used probiotic bacteria with the potential to offer beneficial effects, making them suitable candidates for industrial and medical applications. Nevertheless, certain yeasts and bacilli are also gaining prominence in probiotic food production.

Probiotics are beneficial bacteria that aid in restoring gut microbiota, promoting human health and well-being. These probiotic bacteria consume non-digestible prebiotic food ingredients and play a vital role in the growth and establishment of intestinal biota. LAB have demonstrated potential for treating gastrointestinal tract's related diseases in numerous studies (Caggia et al., 2015; Gareau et al., 2010).

Cheese whey, with its high organic load, can be an environmental hazard if not disposed of appropriately. However, due to its nutrient content, this material could be used as a culture medium for LAB growth, serving as an alternative to commercial media. This approach could potentially lower costs and reduce environmental impact (Sansonetti et al., 2009).

Probiotics offer multifaceted benefits to human health and can be found in traditional fermented foods or beverages (Kumar & Salminen, 2016). The current definition of probiotics, adopted by experts from the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), describes them as "live micro-organisms which, when administered in adequate

amounts, confer a health benefit on the host" (FAO/WHO, 2001). However, there has been debate over the inclusion of the term "live" in the definition, as positive effects have been observed with dead cells as well. Recent advancements have broadened the concept of probiotics to encompass "probiotic-derived bioactives," which are novel bioactive compounds resulting from bacterial metabolism and/or the hydrolysis of the food matrix. Probiotic foods are reported to offer various health benefits, as they help maintain a healthy balance and composition of intestinal flora and enhance resistance to pathogen invasion (Tripathi & Giri, 2014).

A major scientific challenge faced by the dairy industry is improving the probiotic response capacity by developing effective compounds that can support optimal growth and survival of probiotics under various conditions, especially during their passage through the harsh environment of the gastrointestinal tract. Addressing this issue is crucial, given the challenges associated with maintaining the viability of probiotics in this environment (Kareb & Aïder, 2019).

Whey contains probiotics, which are beneficial bacteria with a range of health benefits. These advantages include enhancing gut health, strengthening the immune system, and preventing certain diseases. Numerous probiotic strains have been identified, and they can be found in various foods and supplements. Proper selection of probiotic strains is essential to ensure the desired health benefits. Cheese whey contains vitamins and nitrogen that are crucial for LAB growth (Tripathi & Giri, 2014; Iosca et al., 2023).

Various strains of rod-shaped LAB, such as *Lactobacillus helveticus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus, and Lactobacillus casei*, have been employed in lactic acid production from whey. *Lb. helveticus* is the preferred strain due to its high conversion rate, as it can generate nearly double the amount of lactic acid compared to other common LAB (Parente & Cogan, 2004. Supplementing whey permeates with yeast extracts or yeast protein autolysates (peptones) can enhance the efficiency of lactobacilli fermentations. This is because these supplements provide the additional nutrients that are required for optimal lactic acid production.

In a study by Wróblewska et al. (2019), the therapeutic potential and immunoreactivity of whey fermented with potentially probiotic bacteria were assessed. The whey was fermented with *S. thermophilus* 2 K and *L. bulgaricus* BK, as well as *L. plantarum* W42 and *B. lactis* Bi30, and administered to mice through gavage for several weeks. Mice fed with fermented whey exhibited a change in the Th1 and Th2 cell ratio, indicating an immune response. In a follow-up experiment, mice immunized with α-CN + β-LG were treated with the fermented whey products, which resulted in a decrease in allergy markers such as IL-4, IgE, and specific IgG1. The Th1/Th2 balance also shifted towards a Th1 response, increasing the secretion of main regulatory cytokines IL-10 and TGF-β. Moreover, the presence of *L. plantarum* W42 and *B. lactis* Bi30 significantly augmented the observed therapeutic effect of the fermented whey. Based on these findings, Wróblewska et al. (2019) suggested that both fermented whey and fermented whey products could serve as a foundation for future research and the development of probiotic products targeting allergy sufferers.

Rzepkowska et al. (2017) examined 25 bacterial strains isolated from raw, whey samples that are not pasteurized and are organic. The strains were phenotypically and genotypically identified, and their enzyme profiles, antibiotic resistance, and antimicrobial properties were investigated. Sixteen strains were identified as belonging to the Lactobacillus genus, with Lb. plantarum and Lb. fermentum being the most common species. The strains exhibited low or average lipolytic, esterolytic, and proteolytic activity, making them suitable as starter cultures for foods with low protein content. The majority of the tested strains were susceptible to several antibiotics and demonstrated high or moderate antagonistic activity against indicator strains, making them potential candidates for food applications. As a result, the fermentums strains of *Lb. fermentum* (S4, S7, S8, S10) were identified as the most suitable.

Whey can be used to produce a variety of probiotic beverages. It can be used directly or supplemented with dairy powders (such as buttermilk powder, skim milk powder, etc.) or incorporated into beverage formulations in different proportions (Turkmen et al., 2019).

Skryplonek and Jasińska (2015) carried out a research to investigate the quality properties of beverages prepared using fresh acid whey and milk, along with either buttermilk powder or sweet whey powder as additives. Two strains of commercial probiotic cultures were introduced into the samples, which were then evaluated for sensory attributes, acetaldehyde content, hardness, titratable acidity, pH acidity, and bacterial count. The samples were stored under refrigerated conditions for up to 21 days, and their quality was monitored throughout the storage period. The results indicated that all samples had a bacterial count higher than 8 log cfu/mL throughout the storage period. Beverages with the La-5 strain exhibited increased hardness and acidity, while those containing Bb-12 had higher acetaldehyde levels. The study concluded that beverages made from acid whey and milk, fortified with buttermilk powder or sweet whey powder, provided a suitable environment for the growth and survival of the tested probiotic bacteria strains. Furthermore, the bacterial count was sufficient to deliver health benefits to consumers.

12.7 Aromatic Compounds

Aroma compounds have widespread applications in food, cosmetic, chemical, and pharmaceutical industries. These compounds are typically produced through chemical synthesis or extracted from plant and animal sources (Izawa et al., 2015). The taste of whey can be affected by various factors, such as milk quality, cheese type, and whey treatment post-curd drainage, leading to diverse chemical compositions. During cheese-making, lactic acid bacteria are introduced to milk, generating volatile aroma compounds like aldehydes, ketones, lactones, sulfur compounds, and others in the whey. Liquid whey's aroma can be influenced by different short-chain volatile acids, contributing to the overall taste of whey. A product's aroma is the most significant contributor to its flavor, making flavor quality a vital factor in

consumer acceptance of dairy and other food items. Whey's primary proteins, β-lg and α-LA, contain aromatic amino acids, but glycomacropeptides lack aromatic amino acids such as phenylalanine, tryptophan, and tyrosine. The sour and salty taste is the initial challenge when incorporating acid whey into food products. Fulfilling consumer demands for flavor and health is essential when using acid whey to create fermented milk drinks (Laye et al., 1995; Lievore et al., 2015; Rocha-Mendoza et al., 2021).

Sweet fluid whey is generally characterized by milky and sweet aromatic tastes and has low levels of cardboard and metallic flavors (Carunchia Whetstine et al., 2003). In contrast, acid whey typically contains higher concentrations of organic acids, calcium, potassium, and iron than other fluid whey types (Gallardo-Escamilla et al., 2005). The increased levels of organic acids and minerals in acid whey result in increased lipid oxidation and sour aromatic, cardboard flavors, and a more distinct sour taste compared to sweet whey (Gallardo-Escamilla et al., 2005; Smith et al., 2016).

Smith et al. (2016) conducted research comparing the flavor and flavor stability of cheese, rennet, and acid wheys. Fresh wheys had sweet aromatic and cooked milk flavors, cheddar wheys had diacetyl/buttery flavors, and acid wheys had a sour aromatic taste. Acid casein whey had a soapy taste, while acid and Greek yogurt wheys had a potato flavor. Cultured acid wheys contained an acetaldehyde taste.

The main reason for the development of off-flavors in fluid whey, dried milk, and whey ingredients is lipid oxidation. During this process, volatile compounds such as aldehydes, ketones, and short-chain fatty acids are produced, which can remain in the whey even after processing and contribute to the undesirable flavors. (Cadwallader & Singh, 2009). These compounds are naturally present in fresh fluid milk and whey, but their concentrations can increase due to factors such as heat, storage, and other processing steps that promote oxidation.

Off-flavors in fluid whey related to proteolysis are mainly caused by chymosin and other proteases (Carunchia Whetstine et al., 2003). Moreover, lipolytic enzymes from starter cultures can release fatty acids in milk, oxidize, and contribute to off-flavors (Cadwallader & Singh, 2009). While free fatty acid degradation occurs naturally, starter cultures can accelerate the release and degradation rate in milk and whey.

Different manufacturing facilities may produce unique volatile compound differences in liquid Cheddar whey due to variations in milk source, processing methods, and starter culture rotation (Carunchia Whetstine et al., 2003). Aroma-active compounds, such as 2,3-butanedione, 2-butanol, hexanal, 2-acetyl-1-pyrroline, methional, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal, along with other short-chain volatile acids, have been identified in liquid Cheddar wheys (Karagül-Yüceer et al., 2003).

Whey has been utilized in the production of various chemicals, such as ethanol fermentation, due to its rich nutrient content. The presence of lactose and other essential nutrients make whey a promising raw material for bio-product manufacturing. Several technologies have been developed to use whey as a raw material for creating valuable food or chemical products, including single-cell proteins, lactic

acids, citric acid, enzymes, glucose, methane, oligosaccharides, ethanol, and others (Panesar et al., 2007).

Izawa et al. 2015 study explored the production of aroma compounds through microbial fermentation of whey using seven strains of Wickerhamomyces pijperi yeast. The fermentation process was carried out on glucose-added whey (whey-g), and 12 aroma compounds were identified in the fermented broth using headspace gas chromatography-mass spectrometry analysis. The primary components were ethyl acetate, acetaldehyde, and isoamyl alcohol. This research offers valuable insights into the potential use of whey as a substrate for aroma compound production.

12.8 Conclusion

In conclusion, whey, once regarded as a waste byproduct, has proven to possess significant biological, nutritional, and technological merits. As a rich source of proteins and biologically active components, whey plays a vital role in maintaining a robust immune system. Extensive research is currently being conducted on bioactive peptides derived from whey protein due to their diverse physiological effects. The development of probiotic whey beverages has also garnered interest because of their health-promoting benefits. The flavor of whey is influenced by several factors, such as the quality of the milk used, the type of cheese produced, and the handling of whey following curd drainage. In summary, the research on the characteristics of sweet and acid whey, their bioactive properties, and the factors impacting their viability and flavors underscores the potential of whey as a versatile raw material with applications across various industries.

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Chapter 13
Bacteriocins Production Using Whey

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Abstract The diverse class of antimicrobial proteins/peptides known as bacteriocins from lactic acid bacteria (LAB) has the potential to be employed as bio-preservatives. They exhibit a variety of antibacterial actions across the antibacterial spectrum at low doses, in addition to heat and pH constancy in food. Eminent bacteriocin synthesis is expected in a complicated medium. Yet, the such broth is too costly for a profitable manufacturing technique. It is necessary to employ food-grade media to create stable temperature, broad-division bacteriocins with utmost particular action before using them as food bio-preservatives. Bacteriocins' under cede in the food-grade environment and labor-intensive, overpriced detoxification techniques, which are appropriate at the laboratory size but not at the

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industrial level, are the main obstacles to their use as food bio-preservatives. The current review focuses on the manufacture of bacteriocins utilizing complicated and food-grade medium and principally examines the bacteriocin manufacture variants, broth utilized, various manufacture techniques used, and the impact of varied generation methods on bacteriocin production. The purifying techniques developed for efficient bacteriocin recovery at both small and large scales are also a focus of this investigation.

Keywords Bacteriocin · Whey · Bio-preservatives · Extraction techniques · Protein

13.1 Introduction

Currently, most food producers forbid the discharge of untreated whey into waterways (this is predominately regulated at the government level); as a result, adequate handling of this by-product is necessary before discharge (Garg et al., 2020). The safest way to dispose of whey is through biological wastewater treatment. However, this type of waste removal is often expensive (Pires et al., 2021). Due to the high cost of this procedure, whey, the primary by-product of the dairy industry, is regularly disposed of in the environment without any treatment (Sabo et al., 2019). Alternatively, lactic acid bacteria (LAB) can be cultured in whey to create value-added compounds such as bacteriocins.

Consumer desire for foods with a fresh appearance, minor processing, and no preservatives has grown over the past few decades (Singh et al., 2021). The food industry has historically utilized thermal and chemical treatments to extend the lengthen shelf life, prevent degradation, and enhance the safety of the food produced (Armghan Khalid et al., 2022). Yet, these processes cause the organoleptic and nutritional characteristics to change, potential loss of vitamins, the structure of proteins to change, food to brown, and harmful substances to be produced (Farooq et al., 2023).

Most milk is consumed in its processed form, which includes cheese, and in its fresh (liquid) form (Pires et al., 2021). A substantial portion of whey is released during cheese production due to product processing waste (Pires et al., 2021). Generally, the amount of the byproduct is higher than that of the primary product. Eight to nine liters of whey can be produced using 10 L milk (Manzoor et al., 2019). Bovine whey proteins and whey are byproducts of the cheese industry, and these byproducts are rich in immunoglobulins, protease peptone, serum albumin, lactoglobulin, and lactalbumin (Mehany et al., 2023). Whey protein contains all nine essential amino acids and has high biological value as compared to other sources of dietary proteins. It can be used in the production of industrialized foods, different beverages and protein supplement (Poonia & Pandey, 2023). While viewed as a byproduct, they contain unique nutritional elements that may be beneficial in the production of infants food or in the applications that assist the growth of microorganisms (Manzoor et al., 2021).

Table 13.1 Categorization of bacteriocins

Bacteria	Categorization	Magnitude (kDa)	Representation	References
Gram-negative bacteria	Colicins	Between 30% and 38%	A, B, E2, E3 Colicins	Rebuffat (2016), Wencewicz and Miller (2018) and Ge et al. (2019)
	Colicin-like bacteriocins	Between 30% and 38%	Klebicins, S-piocins	Rebuffat (2016), Wencewicz and Miller (2018) and Garcia-Gutierrez et al. (2020)
	Bacteriocins, phage-tail like	Between 20% and 100%	R- and F-piocins	Rebuffat (2016), Egan et al. (2017) and Wencewicz and Miller (2018)
	Microcins	Greater than 10	Colicin V, Microcin B17, Microcin C7	Rebuffat (2016), Acedo et al.(2018) and Wencewicz and Miller (2018)
Gram-positive bacteria	Class I	Greater than 5	Mersadicin, lacticin 3147, Nisin	Ongey et al.(2017) and Wencewicz and Miller (2018)
	Class II	Greater than 10	Carnobacteriocin B2, Pediocin RA1	Wencewicz and Miller (2018) and Wiebach et al. (2018)
	Class II	Less than 10	Enterocin AS-48, Helvecin	Bennallack and Griffitts (2017), Wencewicz and Miller (2018) and Garvey and Rowan (2019)
Archaea	Halocins	Less than 5	A4, C8, H1, H4 Halocin	Wencewicz and Miller (2018) and Zou et al. (2018)
	Sulfolobaceae	Approximately 20	Sulfolobaceae	Lv et al. (2017) and Wencewicz and Miller (2018)

Food industries, including the processing facilities in this domain, have implemented new processing techniques to preserve food quality and safety and meet consumer needs (Giannakourou & Tsironi, 2021). Ohmic heating, high-pressure, pulsed electric fields, intense light, and bio-preservation are some new cutting-edge technologies implemented to reduce on-site food waste (Juliano & Reyes-De-Corcuera, 2022). Bio-preservation is one of the most dependable new methods for food preservation since it may replace chemical additives without sacrificing the final product's nutritional value or organoleptic quality. The extension of food safety and shelf life using natural or managed microbiota or their antimicrobial substances is known as bio-preservation (Agriopoulou et al., 2020) (Table 13.1).

The LAB has been utilized in fermented foods as a biopreservation method since the dawn (Pujato et al., 2022). These microbes create not only large amounts of lactic and other organic acids, which have antimicrobial properties, but also diacetyl, acetoin, hydrogen peroxide, and bacteriocins, among other inhibitory substances (Monika et al., 2021). Bacteriocins are ribosomally produced antimicrobial peptides

or proteins with a bactericidal or bacteriostatic mode of action (Darbandi et al., 2022). Due to their potential use in the food industry as natural preservatives, LAB-produced bacteriocins have been the subject of extensive studies in past years (Ibrahim et al., 2021). Only pediocin PA-1, generated by *Pedi coccus acidilactici*, and nisin, derived by *Lactococcus lactis*, are now approved as food additives (Manoharan & Balasubramaniam, 2022).

Nevertheless, bacteriocin-producing LAB can be added to a polymeric matrix or utilized as starter or supplement cultures in food fermentation (Settier-Ramírez et al., 2020). De Man, Rogosa, and Sharpe (MRS) are used as a standard medium for LAB growth and bacteriocin synthesis research (Strafella et al., 2020). Due to the expensive cost, MRS is used less as a food additive and also for bacteriocin synthesis on an industrial scale. Therefore, it's essential to find a low-cost and profitable method of producing bacteriocins in high quantities (Strafella et al., 2020). Organics from food production industries have been investigated for this in recent years.

13.2 Production of Bacteriocin

Traditional food preserves include bacteriocins and ribosomally generated antimicrobial peptides that are either introduced or created by starting cultures during fermentation (de Freire Bastos et al., 2020). Relatively recent study areas were opened by in-depth examinations of a few bacteriocins, which also expanded the uses of these antimicrobial peptides (Ma et al., 2022). With the fast advancements in genetics and nanotechnology, the potential for bacteriocins to be transformed into next-generation antibiotics open-up the door to even extra exciting uses, including the potential treatment of cancer and development of new carrier molecules (delivery systems) (Ali et al., 2022b; Šišková et al., 2023). Moreover, it has been proposed that several bacteriocins control quorum signaling, which raises the possibility of new uses for this class of drugs (Soltani et al., 2021). While there are few fascinating genetics studies on bacteriocins produced by Gram-negative bacteria, most application-oriented studies concentrate on bacteriocins created by Gram-positive bacteria, primarily lactic acid bacteria (Jaumaux et al., 2020). Bacteriocins are now also used in areas other than food, such as human health. Bacteriocins are short polypeptides or proteins produced by ribosomal RNA that have antibacterial action and are intimately tied to the strain that produced them. Bacteriocins engage with and kill cells with particular surface sensors (Motta et al., 2021).

Entities in a microbial community can create bacteriocins and be susceptible to or resistant to them. Since three cell categories collectively coexist and are absolute for limited means, only a small portion of bacteriocinogenic cells will be driven to produce and excrete bacteriocin (Vidovic et al., 2022). Some delicate cells are promptly ruined, while in others, mutations decide how resistant they are resistance-producing cells are swiftly replaced by producing cells due to the "cost" of producing bacteriocin (Flor Duro, 2021).

The operons, including the genes responsible for bacteriocins, a group of immunological proteins, and other auxiliary proteins, are found on the circular chromosome, plasmids, and other mobile genetic components (Aulitto et al., 2022). These genes, which are frequently induced, must be secreted and accumulate extracellularly to be activated (Tintor et al., 2020). Antimicrobial peptides called bacteriocins, made by bacterial ribosomes, stop the culture of similar or comparable bacterial variants. The frameworks of bacteriocins present in various bacteria have also been previously reported (Flynn et al., 2021). According to the accumulated and growing information, bacteriocins appear to have distinctive compositions, ways to function, biosynthesis, self-immunity, and gene regulation mechanisms. In the food and pharmaceutical industries, bacteriocins are seen as promising to stop food from spoiling and developing pathogenic germs. Also, the discovery of their manufacture allowed for the development novel peptides using bacteriocin-controlled gene expression platforms and lantibiotic enzymes, a subclass of bacteriocins (Nazari & Smith, 2020).

It is well-established that different microbes may have a variety of strategies for establishing engagement and defense (Haahtela, 2019). The creation of bacteriocins, peptides with antibacterial activity, is linked to one of these pathways, as Gram-positive and Gram-negative bacteria contain bacteriocins (Soltani et al., 2021). Yet, due to the commercial use of some strains of these bacteria, notably LAB, which have the Generally Regarded As Safe (GRAS) classification, bacteriocins generated by Gram-positive bacteria are of specific involvement (Zimina et al., 2020). The generation of bacteriocin by Gram-positive microbes is discussed with some of the most recent innovations. To the best of our knowledge, there have been 108 patents issued since 1965 related to companies that produce Gram-positive bacteriocins, and 57% of those patents are for related bacteriocins made from strains of *Lactococcus, Lactobacillus, Streptococcus, and Pediococcus* (Ramesh, 2019). Interestingly, the last 10 years have seen the majority of patents for creating heterologous bacteriocins (Muras et al., 2021). Although bacteriocins are primarily used in the food business to prevent spoiling and the spread of pathogenic germs, their applications have expanded recently to include cancer diagnosis and treatment, opposition to plant illness, and culture promotion (Lieke et al., 2020; Ali et al., 2021b; Iqra et al., 2023; Rasool et al., 2023).

The bacteriocins are ribosome-synthesized (poly) peptides generated by practically all bacterial lines (Zimina et al., 2020). Because of their wide range of action, prolonged usage in food fermentation, and belief that these microbes are advantageous to humans, bacteriocins from lactic acid bacteria and probiotic bacteriocin-producing pathogens have been extensively explored (da Costa et al., 2019). Although nisin A, the first and most researched LAB bacteriocin, is frequently used for food preservation, numerous novel bacteriocins are needed to suppress undesirable bacteria (Verma et al., 2022). The majority of study on the biotechnological applications of different bacteriocins has been on their usage as food preservation, with nisin serving as the prototype and being utilized successfully in food (Soltani et al., 2021; Ali et al., 2022a). The therapeutic potential of bacteriocins from LAB, alone or in combination with traditional antimicrobial drugs, is for use in

medicine or veterinary medicine (Todorov et al., 2019). Since antimicrobial medicines used in therapy are becoming less effective, there is even more interest in their potential use (Monteiro et al., 2019).

The bacteriocins that lactic acid bacteria create are anticipated to be certain antibacterial substances (Pang et al., 2022). Their structure, modes of activity, and biosynthetic mechanisms have all been described. Implications for the novel LAB bacteriocins and their biosynthesis pathways include peptide engineering and food preservation (Luo et al., 2022).

Bacteriocins, bacterial proteins or peptides, are also proposed to be potential novel antibacterial agents (Naskar & Kim, 2021). From the molecular basis perspective of their generation and action, examining the characteristics of naturally occurring and genetically engineered bacteriocins is presented (Zimina et al., 2020). The majority of bacteriocins have a constrained range of restrictive action. An ability to form lasting is seen in some wide-ranging bacteriocins (the amino acid chain's C- and N-ends are connected by a peptide bond) (Pang et al., 2022). The protein's performance depends on various factors found on the exterior of marked cells caused by the specific spot of the protein's ends. It is also possible for several different cells, including eukaryotic cells, to express the genes responsible for producing bacteriocins and related proteins (Wang et al., 2021). Moreover, data on site-specific mutagenesis' effects on bacteriocin's characteristics are available (Scholz et al., 2014).

Small peptide bacteriocins have antibacterial effects, and Gram-positive or Gram-negative bacteria can manufacture them (Soltani et al., 2022). A large number of bacteriocins have been discovered and well-described recently. With new information on bacteriocins' composition, amino acid sequencing, and mode of action emerging, the categorization of these substances is constantly changing (Pirtskhalava et al., 2021). Typical amino acids such as lanthionine, methylanthionine, dehydroalanine, dehydrobutyrin, and D-alanine are present in some bacteriocins (lantibiotics) (Shafique et al., 2022). Nisin, which is formed by varieties of *Lactococcus lactis*, is one of the most well-known bacteriocins formed by LAB. These bacteriocins are known to be very safe for human use (both oral and external). Nisin is commonly used as a preservative in several food products (Verma et al., 2022).

13.3 Effect of Fermentation Conditions

It is well established that the impact of food production and consumption on public health is significant. In addition to providing nourishment, the food available on the market can also contain toxins that could be developed during production or shelf storage (Lustig, 2020). Carcinogens and other hazardous substances can also be present in the foods due to food manufacture and consequently be ingested during regular dietary practices (Adwas et al., 2019). High-temperature beef cooking creates heterocyclic aromatic amines (HAA), which are suspected to be carcinogenic

and mutagenic. Furthermore, high HAA levels were also found in 24 samples of various commercial ultra-high heat products (Sumon et al., 2021). Spray-dried whey probably contains HAA since exposure to high temperatures can lead to its development.

Nevertheless, there is no evidence of HAA development in most published studies on spray-dried dairy products (Ali et al., 2021a; Zhao et al., 2022). Other potentially dangerous substances present in food are nitrosamines, which alter DNA transition through methylation. Biogenic amines (BA) can have negative health consequences such as nausea, headaches, and even organ failure and are found in various foods and beverages (Dasa et al., 2022). In conclusion, the total BA should not exceed 1000 mg/kgL. The value of some meals is pointed by a BA index (BAI), which orders samples as ample, adequate, or degraded.

Multifunctional foods and beverages made with LAB might lessen some of the negative impacts of what is regularly consumed (Zapaśnik et al., 2022). The LAB is widely utilized as a fermentation process for food products that provide customers with nutritional support and potential health value exceeding the nutrition contribution. *Pediococcus pentosaceus* ENM104 and *Lactiplantibacillus plantarum* SPS109 were the starter for fermented milk (Jitpakdee et al., 2022). After fermentation utilizing these starter cultures, the resulting milk had less cholesterol but more angiotensin-converting enzyme (ACE) restrictive and higher antioxidant action (Aksakalli-Magden et al., 2023). Whey is a by-product produced during the cheese-making process when milk is used. Whey is a protein that creates nutritional drinks and formulas (Arab et al., 2023). It contains 94% water (6% total solids), 4.5% lactose, 0.8% protein, and 0.7% minerals.

Once whey was fermented with natural and LAB beginning, it was discovered that ACE minimum inhibitory concentration was higher (Jitpakdee et al., 2022). Nevertheless, whey and regular FW can include foodborne microbes such as *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*. As a result, an operational whey drink fermentable using LAB beginning that can hold in these microbes might offer a commercial refreshment and raise the value of this by-product (Rocha & Guerra, 2020).

A functional whey drink may protect against *Salmonella Typhi*, which often contaminates improperly cooked food, since a mouse model demonstrated the gastrointestinal persistence of a probiotic in LAB culture (Jitpakdee et al., 2022). As LAB-generated whey has demonstrated action in opposition to an active dental cavity, an operational LAB-generated whey drink may aid in eradicating pathogens like *Streptococcus mutans*, which is frequently initiated in the oral cavity produces dental caries by producing biofilm (Garner, 2022). In addition, a key strategy to increase production capacity and adequate cost examination is suggested that LAB-fermented whey may be commercialized with appropriate animal/human intervention trials (Cichońska & Ziarno, 2022).

Users of LAB-fermented whey items may also experience several health advantages, including lowered cholesterol, GABA-filled neurotransmitters, and reduced free radicals (Castellone et al., 2021). The *P. pentosaceus* ENM104 and *L. plantarum* SPS109 LAB strains were used in a study to examine the possible

advantages of whey fermentation (Jitpakdee et al., 2022). A study was conducted to know how these isolates grew aerobically and anaerobically, how susceptible they were to antibiotics, how they performed against bacteria in MRS medium, and how well they could decrease HAA, nitrosamines, and BA in phosphate-buffered saline (PBS) (Jitpakdee et al., 2022).

Using agro-industrial effluents as an inexpensive, acceptable fermentation substrate has attracted much attention in recent generations (Raina et al., 2022). In the process of making cheese, whey is a greenish-yellow fluid left over after the generation and isolation of the curd, while permeate is the liquid that emerges after ultrafiltered milk or whey (Goulding et al., 2020). These dairy by-products have significant chemical and biological oxygen spending levels. Because whey and permeate require considerable pre-treatment before discharge, the dairy sector has an issue with their removal because doing so raises operating expenses in the dairy facility (Ahmad et al., 2019). Whey and saturate typically have few uses but are frequently utilized in creating protein, lactose, whey, diffuse powders, and animal feed. These by-products make the ideal culture medium for fermentative bacteria because they have an abundant supply of lactose as a fuel source and the right amount of nutrients (Rodríguez et al., 2021).

13.4 Bacteriocin Production Strain

Either Gram-positive (*Lactobacillus, Lactococcus, Streptococcus, Enterococcus, Leuconostoc, Pediococcus,* and *Propionibacterium*) or Gram-negative (other bacteria) bacteria can create bacteriocins (Escherichia coli, Shigella, Serratia, Klebsiella, as well as Pseudomonas) (Soltani et al., 2022). The LAB that produces bacteriocin has traditionally been regarded as desirable since it increases the strain's suitability for many industrial uses (Mora-Villalobos et al., 2020). Bacteriocin-producing LAB variants are thought to have superior bacterial health because they have better growth in the beginning for food generation and/or probiotic isolates since they can quickly establish themselves in the desired microbial niche (Chen et al., 2022). Rapid progress in genome sequencing over the past decade has revealed certain innovative LAB strains' genuine capacity to produce bacteriocins, which increases their usefulness in various application systems. These unique strains share specific astonishing components in the biosynthetic pathway, enabling them to create many bacteriocins without using their energy (Walsh et al., 2021).

Contrary to popular belief, biosynthetic enzymes are not unique in their relationship with bacteriocins; instead, different bacteriocin-producing variants use joint biosynthetic components for each of their different bacteriocins (Nisa et al., 2023). Many bacteriocins in these variants share the quorum-sensing three-element regulation method, maturation, and transport processes (Yin et al., 2019). However, as this novel variant has a comprehensive range of possible applications, thorough genotypic evaluation is still required to validate their safety regarding potential virulence and pathogenicity.

Bacteriocins differ from antibiotics in three ways: they originate in ribosomes, are active at small doses (in the nanomolar ambit), and have a specific range of activity (Simons et al., 2020). Furthermore, multi-enzymatic synthetase combinations produce the latter, often refrain at more excellent distribution (in the micromolar ambit), and have broad antimicrobial activity. Nearly 100% of bacterial variants are thought to produce leastwise in single bacteriocin. A helpful database named BACTIBASE (www.http://bactibase.hammamilab.org) has been combined with these molecules' sequencing and physicochemical properties and details regarding their shape, taxonomy, literature, and biological activity (Zhang et al., 2023). More than 200 entries from Gram-negative and Gram-positive bacteria can be found in BACTIBASE (Telhig et al., 2020). These entries describe a variety of peptides and proteins with different sizes, shapes, modes of action, and gene order. Furthermore, this diversity makes categorizing bacteriocins, which is constantly contested, much more difficult (Table 13.2).

13.5 Production System

The primary by-product created by the dairy industry, whey, is collected during the manufacture of cheese after casein is removed and clotted (Franceschi et al., 2023). Several food industries have looked for inexpensive disposal techniques for a long time, including the disposition of waste into rivers, lakes, sewage systems, oceans, or fields (Mohamed et al., 2022). Given the high organic content of whey and its widespread production, these practices have resulted in numerous environmental issues (Zandona et al., 2021). It is well established that these by-products and wastes can severely pollute the environment when disposed of on land, changing the physicochemical properties of the soil and lowering crop yields. Waste substances, especially from industries, threaten aquatic life by hindering biodegradability and reducing dissolved oxygen levels in the marine environment (Zandona et al., 2021).

Using whey as a substrate to create additional-valued elements naturally is an affordable and advantageous alternative because it preserves roughly 55% of the total milk contents (Castro-Muñoz et al., 2022). Its most numerous elements include lactose (which accounts for 75% of the dry matter), soluble proteins (between 12% and 14%), lipids, and mineral salts (between 1% and 10%), all of which are required for the development of microorganisms, notably LAB (Peydayesh et al., 2022). Antimicrobial compound synthesis by LAB is typically regarded as being relatively expensive. According to estimates, the growth medium and additives needed to support the growth of the microbe producing these compounds account for 30% of the process' overall expenditures (Lim et al., 2021). By taking into account the whey above ingredients, this by-product can primarily be seen as an affordable carbon origin that can lower the monetary value related to LAB growth media. In addition to being cost-effective, using whey as a component of the LAB medium can be seen as an environmentally benign technique that can lessen the effects of its removal (Sakr et al., 2021; Ali et al., 2022c).

Table 13.2 Bacteriocins yield employing free/immobilized producer strains in different manufacturing modes

Microbial agent	Bacteriocin	Manufacturing modes	Bacteriocin output	Broth	Immobilized or free cell	References
Leuconostoc mesenteroides subsp. mesenteroides UL5	Mesenterocin	Assembled growth	4096 AU/mL(MRS), 2048 AU/mL(whey), 2048 AU/mL (diffuse whey)	In addition, MRS, whey and whey diffuse with YE (2%), tween 80 (0.1%), $MnSO_4$ (0.005%), $MgSO_4$ (0.01%)	Free cell	Daba et al. (1993)
Pediococcus acidilactici PO_2	Pediocin PO_2	Retain manufacturing in a packed-bed bioreactor	6400 AU/mL	MRS broth	Immobilized cell	Cho et al. (1996)
L. lactis UL 719	Nisin Z	Retain fermentation	During continuous free cell and immobilized cell with aeration, maximum Nisin production is 2560 IU/mL and 2430 IU/mL, respectively	Whey diffuse powder (6%) addition with 0.2 M KCL	Free cell and immobilized cell	Desjardins et al. (2001)
Lactococcus lactis subsp. lactis and P. acidilactici UL5	Nisin Z and pediocin	Mixed-strain assembled growth	Nisin and pediocin 3000 AU/mL and 730 AU/mL or 1060 AU/mL and 1360 AU/mL, after 18 or 16 h incubation, respectively	Whey diffuse (6%) with YE (2%) and tween 80 (0.1%) [glucose (0.5%) supplement to SWPM]	Free cell	Goulhen et al. (1999)
L. lactis subsp. lactis CECT 539 and P. acidilactici NRRL B-5627	Nisin and Pediocin	Assembled growth	Increase pediocin titer from 55 BU/mL to 195 BU/mL and 185 BU/mL of YE and casitone, respectively, and 21–74 BU/mL and 59 BU/mL for nisin, respectively	Whey addition with lactate four nitrogen sources (YE, casitone, NH_4Cl and glycine)	Free cell	Guerra and Pastrana (2003)
P. acidilactici UL 5	Pediocin PA1	Repeated cycle assembled growth (RCB)	187 AU/mL/h and 342 AU/mL/h in SPM and MRS resp. in the free cell. 5461 AU/mL/hand 2048 AU/mL/h, respectively in immobilized cell	MRS broth add-on. (1% glucose) and whey diffuse (SWP) medium	Free and immobilized	Daba et al. (1993)

L. lactis UL 719	Nisin Z	Assembled fermentation	Without aeration, 8200 AU/mL; with aeration, 41,000 AU/mL	Whey diffuse powder (6%) with aeration addition with YE (1%) and tween 80 (0.1–0.4%)	Free cell	Amiali et al. (1998)
L. lactis subsp. lactis CECT 539 and P. acidilactici NRRL B-5627	Nisin and pediocin	Assembled growth	DW two strains were slightly higher than in CW, and production is lower as a comparison to MRS medium in Bacteriocin production	Thinned whey (DW) and saturated whey (CW)	Free cell	Guerra and Pastrana (2003)
L. lactis subsp. lactis CECT 539 and P. acidilactici NRRL B-5627	Nisin and pediocin	Assembled growth	Nisin and pediocin titers are 6.2 and 9.7 times lower in whey than in MRS broth, respectively	Determine pH drop on bacteriocin manufacture in non-cushion whey and cushion whey	Free cell	
Bacillus licheniformis P40	Bacteriocin	Assembled growth	3200 AU/mL	Cheese whey powder addition with YE (1%)	Free cell	Cladera-Olivera et al. (2004)
L. lactis subsp. lactis ATCC11454	Nisin	By using a packed-bed bioreactor assembled fermentation	5.1 9104 AU/mL a maximum of nisin titre	Whey diffuse addition with YE or casein hydrolysate	Immobilized cells	Liu et al. (2005)
L. lactis UL 719	Nisin Z	Repeated cycle assembled growth	8200 IU/mL	Whey diffuse (6%) addition with 0.2 M KCL	Immobilized cell	Bertrand et al. (2001)
P. acidilactici C20	Pediocin C20	Assembled growth	150 9103 AU/mL	Whey diffuse addition with 2% YE	Free cell	Halami and Chandrashekar (2005)

While whey may support the growth of most LAB, it is deficient in nitrogen and other essential elements, necessitating frequent replenishment to continue bacteriocin synthesis satisfactorily (Yuvaraj et al., 2021). Furthermore, specific LAB, like *lactococcus,* heavily relies on exogenous supplies to synthesize peptides and proteins because of their ineffective proteolytic mechanism (Harwood & Kikuchi, 2022). In recent work, it was shown that the isolate *Lb. plantarum* ST16 could create a bacteriocin with potent antibacterial activity against various microbes when rising in de Man, Rogosa, and Sharpe (MRS) broth and various food ingredients (Sabo et al., 2019). Although this strain produced a reasonable amount of biomass cultivated in cheese whey, it failed to generate bacteriocin (Parlindungan et al., 2021).

13.6 Recovery and Strategies for Purification of Bacteriocins

To create bacteriocins for foodstuff biopreservation, it is vital to extract bacteriocins in a purified form on a big scale (Strack et al., 2020). When bacteriocins are utilized for biopreservation, inappropriate media components may be present in their basic form (Hussein, 2022). Even though bacteriocins are locked away into the nutrient broth, most techniques begin with a step to focus on bacteriocins from the growth filtrate, and these techniques typically need a better purity index, regardless of their primary goal of reducing working volume. Converse liquid chromatography, gel filtration, cation exchange, and other chromatographic isolation, such as cation exchange, hydrophobic interaction, and isoelectric focusing, are all necessary for the bacteriocins' considerable purification (Quintela-Baluja et al., 2022).

Three main approaches or techniques may be recognized for the homogeneous cleaning of bacteriocins: the traditional multi-step approach (Yap et al., 2022), the straightforward three-step method (Maviza et al., 2022), and single-step bed adsorption (Riabinin et al., 2020). In cases where conventional procedures are used, protein yields are typically relatively low (Drummond et al., 2020). It is possible because the technique has more steps than necessary, which results in low yield, one of the issues with the industrial-level cleaning of bacteriocins (Verma et al., 2022).

Several protocols have been developed for the wider-scale detoxification of bacteriocins from different conditions using (1) ammonium sulfate precipitation, (2) chloroform/methanol removal, and (3) cation-exchange/hydrophobic engagement high-pressure liquid chromatography (Goyal et al., 2021). Furthermore, several large-scale methods for isolating bacteriocin from complex growth mediums have been developed, employing bacteriocins' cationic and hydrophobic characteristics (Yap et al., 2022).

Bacteriocins can also be separated by a specific single technique called enlarged adsorb using a hydrophobic connection gel after raising the available bacteriocin titer by altering the pH of the stark generation broth (Kountoupis, 2019). Unclarified

L. lactis A164 culture broth was run through an enlarged ion-exchange chromatography, and the split was purified; nisin Z was produced by eluting the phase with 0.15 M NaCl (Peng et al., 2021). This relatively simple one-step detoxification technique yielded 31-fold filtration with a 90% yield. Because of its benefits, such as fewer filtration processes, a shorter computation overall, higher productivity, eminent processing volume, and high flow rates while operating, expanded ion-exchange chromatography can be used in large-scale processes. As a result, this approach may offer a more inexpensive alternative to standard multi-step methods for the scale-up filtration of nisin Z (Yap et al., 2022). Bacteriocin was directly extracted from *Lactobacillus sakei* CCUG 42687's non-clarified fermentation channel employing macroporous octyl- and phenyl-monolith sections, and its testing showed that at pH 6.2, approximately 80% of the bacteriocin action could be regained with a detoxification component in between 150 and 160 in the cell-free elution (Escobar-Sánchez et al., 2023). It offers a potential technique for swiftly and accurately isolating bacteriocin from several samples. The latter two ways, speedier than the first conventional but still effective, have been used to purify many bacteriocins with fascinating industrial potential. They contain nisin, macedocin, enterocins, and amylovorin L (Padhi et al., 2022) (Table 13.3).

13.7 Conclusion

Gram-positive and gram-negative bacteria may be used for the production of bacteriocins, which are proteinaceous antimicrobial compounds with antagonistic activity against other, typically similar bacterial species. These chemicals are divided into four groups based on their morphological characteristics, molecular magnitude, characteristics of their modes of activities, and the existence of adapted amino acids. Bacteriocinogenic The class IIa bacteriocin pediocin PA-1/AcH, which is often acquired by Pediococcus variants, is expressed via an operon composed of functional genes in supplemented to PedB (which codes the resistance peptide that safeguards the generator variants), PedC (which codes the ABC conveyer) and PedD (codification of the additive peptide to extracellular genetics).

Several studies have concentrated on the quorum sensing system, which depends on genetic aspect synchronization to create beneficial metabolites like bacteriocins, which induce bacteriocin production by comporting another variant in the growth broth. As a stress signal, the extra bacteria in the co-culture often increase bacteriocin synthesis. Lactobacillus spp. can operate as an inducer, enhancing the bacteriocin synthesis of the *Lactococcus lactis* and *Lb. plantarum* variants, according to previous studies. This research has given credence to co-culture, where two or more bacteria are used to produce a desired metabolite under aseptic circumstances. These methods lead to cell-to-cell connections that stimulate metabolite creation and aspects with practical uses in industry, medicine, and the environment. The creation of a firm's production with uses in food preservatives may be made possible by this enhancement in bacteriocin manufacturing.

Table 13.3 Various techniques used for bacteriocin detoxification with their recuperation and detox fold

Bacteriocin	Medium	Steps of refining	Detoxification	Cede/ Refine	References
Carnocin KZ213	MRS medium (101)	Hydrophobic interaction chromatography	911	0.58 mg/ L	Saint-Hubert et al. (2009)
		Cation-exchange chromatography	34,000		
Bacteriocin by *E. faecium* MMT21	MRS medium	Cation-exchange chromatography	ND	ND	Ghrairi et al. (2008)
		Hydrophobic interaction chromatography			
		Reverse-phase HPLC (RP-HPLC)			
Enterocin EJ97	CM medium (101)	Cation-exchange chromatography	8.46	59.56%	López et al. (2007)
		Reverse-phase HPLC (RP-HPLC)	30.8	48.85%	
Mesenterocin Y105	MRS medium	Cation-exchange chromatography	60	120 l g/L	Guyonnet et al. (2000)
		Hydrophobic interaction chromatography			
		HPLC			
Leucocin C	MRS medium	Cation-exchange chromatography	ND	ND	Fimland et al. (2002)
		Reverse-phase HPLC (RP-HPLC)			
Macedocin	Skim milk with yeast extract	Centrifugation	ND	ND	Georgalaki et al. (2002)
		Ammonium sulfate precipitation			
		Reverse-phase HPLC			
Pediocin from *P. acidilactici* MM33	MRS medium	Centrifugation	1	100%	Lozano et al. (1992)
		Cation-exchange chromatography	725	50.7%	
		Rotavapor	5725	40%	
		Freeze drying	36,500	50.7%	

(continued)

Table 13.3 (continued)

Bacteriocin	Medium	Steps of refining	Detoxification	Cede/ Refine	References
Sakacin P	MRS medium	Macroporous monolith column	156	87%	Desjardins et al. (2001)
Pediocin SA-1	MRS medium	Centrifugation	ND	ND	Anastasiadou et al. (2008)
		Tricin SDS-PAGE			
Bacteriocin by *Leuconostoc mesenteroides* E131	MRS medium	Centrifugation	1	ND	Todorov et al. (1999)
		Ammonium sulphate precipitation	5.5		
		Resource S	4.6		
		Ammonium sulphate precipitation	0.6		
		Reverse-phase HPLC (RP-HPLC)-I	24.7		
		Reverse-phase HPLC (RP-HPLC)-II	9.3		
Pediocin PD-1	MRS medium	Centrifugation	1	100%	Bauer et al. (2005)
		Precipitation	8	86%	
		Dialysis			
		Lyophilization	6	55%	
		Methanol–chloroform extraction	11	47%	
		Cation-exchange chromatography	1700	34%	
AMP by *L. sakei*	MRS medium (81)	Acid extraction	2.9	3.33%	de Carvalho et al. (2010)
		Cation-exchange chromatography	55.2	3.2%	
Nisin Z	MRS medium (51)	Expanded-bed ion-exchange chromatography	31	90%	Cheigh et al. (2004)
Bacteriocin from *Carnobacterium divergens*	MRS medium	Centrifugation	1	100%	Etivier et al. (2000)
		Triton X-114 phase partitioning	ND	0.1%	
		Cation-exchange chromatography	13,000	0.04%	

(continued)

Table 13.3 (continued)

Bacteriocin	Medium	Steps of refining	Detoxification	Cede/ Refine	References
Pediocin PA-1	MRS medium	Centrifugation	ND	73%	Beaulieu et al. (2006)
		Cation-exchange chromatography			
		Hydrophobic interaction chromatography			
Enterocin E-760	Brucella medium (6.51)	Cation-exchange chromatography	ND	ND	Line et al. (2008)
		Hydrophobic interaction chromatography			
AMP from *L. helveticus*	Whey	Centrifugation	1	120 AU/ mL	Owusu-Kwarteng et al. (2013)
		Ultrafiltration	1.3		
		Precipitation	2.3		
		Gel-filtration chromatography	10.3		
		Ion-exchange chromatography	27.3		
Acidocin CH5	MRS medium	Centrifugation	1	100	Chumchalova et al. (2004)
		Solid-phase extraction	0.2	3.9	
		Cation-exchange chromatography	66	4.0	
		Hydrophobic interaction chromatography	49	2.7	
		Reverse-phase HPLC (RP-HPLC)	ND	ND	
Sakacin A	MRS medium	Centrifugation	ND	100%	Holck et al. (1992)
		Ammonium sulphate precipitation		96%	
		Cation-exchange chromatography		14%	
		Hydrophobic interaction chromatography		51%	
		FPLC		83	

(continued)

Table 13.3 (continued)

Bacteriocin	Medium	Steps of refining	Detoxification	Cede/ Refine	References
Pediocin PA-1	MRS medium	Cation-exchange chromatography	ND	85%	Uteng et al. (2002)
		Reverse-phase HPLC (RP-HPLC)		110%	
Enterocin AS-48	CM medium (251)	Cation-exchange chromatography	11.87	95.99%	Abriouel et al. (2003)
		Reverse-phase HPLC (RP-HPLC)	24.3	74.95%	
Plantaricin ST31	MRS medium	Centrifugation	1	100%	Ray and Hoover (1993)
		Cation-exchange chromatography	110	5.94	

On the contrary, it is well known that LAB growth conditions and agar medium composition, which must include carbohydrates, organic nitrogen sources, amino acids, proteins, minerals, and vitamins, significantly impact bacteriocin synthesis by LAB. Because of the cost-expensive MRS broth and the desire to lower the cost of bacteriocin output, various studies have concentrated on constructing an alternative medium. The development of more accessible (and less expensive) growth medium derived from various natural origins, such as molasses, corn syrup, and cheese whey, has been the center of attention of bacteriocin studies over the past decade to produce high levels of bacteriocins at low cost and with the least amount of use of industrial waste products.

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Chapter 14
Whey: As a Low-Cost Substrate for the Production of Biosurfactants

Vandana Chaudhary, Priyanka Kajla, Ankur Luthra, and Ruby Siwach

Abstract Biosurfactants have become attractive microbial products in the emerging biotechnology industry due to their advantages over synthetic surfactants in terms of environmental sustainability, global public health, and the concerns of industries to produce environmentally friendly goods. Surfactants are chemical compounds composed of amphipathic molecules containing hydrophilic and hydrophobic moieties that partition at physical interfaces. Owing to their amphipathic nature biosurfactants have wide applications in the food industry as emulsifiers, foaming agents, dispersion, and antimicrobial agents as well as in other industries including the petroleum industry, pharmaceuticals, cosmetics, detergents, etc. Most commercial surfactants are synthetic or petrochemical based that have varied limitations of less cost-economic, toxicity, and hazardous to the environment. Therefore, an alternative to this problem is using biosurfactants prepared from cheap renewable sources that are safe, environmentally compatible, and have good biodegradability. One such source is whey, which is generated as a waste product from the dairy processing industry and can be utilized to synthesize biosurfactants by employing different microbes/enzymes in fermentation systems. Dairy whey is an excellent source of different nutrients which serves as the best alternative to synthesize biosurfactants with varied properties and applications. In this chapter different biosurfactants using cheese or paneer whey are discussed along with applications. Production strategies of whey-based biosurfactants for enhancing and improving yield are briefly discussed.

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Emphasis is given on food applications of different whey-based biosurfactants as well as application in other industries is also discussed.

Keywords Biosurfactants · Whey · Production strategies · Applications · Bioremediation

14.1 Introduction

Surfactants are also known as surface-active agents and when added to a liquid they reduce its surface tension, thus increasing the spreading and wetting properties of the liquid. Surfactants adsorb themselves at the interface between a liquid and a different phase i.e., gases or solids and therefore, make self-assembled molecular clusters known as micelles in a solution. To show this adsorption at interface between two different phases, structurally a surfactant must have an amphiphilic structure with two different functional groups (hydrophobic as well as hydrophilic group) with different affinity (Fig. 14.1). Usually, the surfactant has a long carbon chain with 8–22 carbons which is termed as hydrophobic (water hating), if the surfactant is being used in water systems and lipophilic (lipid loving), if the surfactant is being used in lipid systems. The surfactant molecules also possess a functional group which has water loving nature and these are called the hydrophilic groups if the surfactant is being used in water systems and lipophobic groups (lipid hating), if the surfactant is being used in lipid systems. This type of structure of a surfactant with two parts with opposite affinities is known as an amphiphilic structure, which imparts remarkable functional properties to the surfactants. Few examples of the commonly used surfactants are alkylbenzene sulfonates (detergents), (fatty acids, sodium stearate) soaps, lauryl sulphate (foaming agent), di-alkyl sulfosuccinate (wetting agent), lignosulfonates (dispersants) etc. (Nakama, 2017).

The majority of synthetic surfactants come from the petrochemical sector, making it possible to produce them at more economically and with high yield. Unfortunately, this kind of manufacturing process employed for production of synthetic surfactants is regarded as being non environment friendly, unsuatainable. and at contrasts with many government initiatives to create a sustainable green economy. Furthermore,

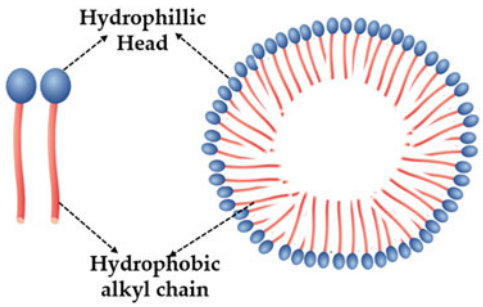

Fig. 14.1 Structure of bio/surfactants

synthetic surfactants frequently have biocompatibility and toxicity contentions and affect ecosystems, which further restricts their use (Johnson et al., 2021).

Biosurfactants are biological surfactants that are microbial in origin i.e., either bio-synthesized as a component of cell membrane or produced by some yeasts, fungi or certain bacteria extracellularly. Biosurfactants action is similar to synthetic surfactants as both reduce surface and interfacial tension but the biosurfactants are either produced at the cell surface or excreted out by microbial cells (Banat et al., 2010). These usually exist in variable chemical structures such as glycolipids, protein-polysaccharide complexe, lipoproteins, phospholipids etc. As a result, it is logical to anticipate that distinct groups of biosurfactants will have a variety of characteristics and physiological activities (Nikolova & Gutierrez, 2021). In addition to it, the biosurfactants can be designed to fit a wide array of applications by altering its production processes. Although, majority of biosurfactants fall under the category of secondary metabolites but many of them play very crucial role for the producing microorganisms such as some are required for the survival of the producing microbes as these are associated with improving food transport, enhancing host-microbe interactions and many more (Bhadra et al., 2022). Its other functions include heavy metal binding, bacterial pathogenicity, quorum sensing, and biofilm development. Microbial surfactants offer many advantages over their synthetic counterparts e.g., low toxicity, low critical micellar concentration (CMC) and high biodegradability, and remain active at extreme levels of temperature, pH and salinity.

Due to these benefits, the biosurfactants are considered as a superior alternative to their synthetically counterparts and their dominance in the global surfactant market has grown over the past 15 years. According to a test study, the global Microbial Biosurfactants market size was US$30 million in 2022 and is likely to grow to US$41 million by 2029 with a CAGR of 4.6% during this period (Anonymous, 2023). Despite showing immense potential, the widescale use of biosurfactants commercially is a challenge and is limited due to several factors such as high production and extraction costs, low yields in production processes and lack of toxicity studies in human systems. Often, the amount and type of a raw material imparts the significant contribution towards the production cost; and can account for 10–30% of the total production costs in most biotechnological processes used for production of biosurfactants. Therefore, in order to decrease the production cost, it is essential to use low-cost raw materials as substrates for production of biosurfactants. A wide array of low cost raw materials such as, plant-derived oils, oil wastes, starchy substances, distillery wastes and cheese whey have been used in biosurfactant production. Cheese whey—a by-product obtained during cheese manufacturing, is a rich source of nutrients that can be used to cultivate microorganisms that produce biosurfactants. There are several microorganisms that can produce biosurfactants from cheese whey, including bacteria such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Lactobacillus acidophilus*. The biosurfactants extracted from cheese or paneer whey have various applications in industries such as food, pharmaceutical, and cosmetic industry. They can be used as emulsifiers, foaming agents, and detergents. Overall, the extraction of biosurfactants from cheese/paneer whey is a promising approach for utilizing a by-product of the dairy industry and producing

valuable compounds with various industrial applications (Kachrimanidou et al., 2022). For instance, a strain of *Pseudomonas aeruginosa* known as SR17 was sequestered from hydrocarbon-contaminated soil which could effectively use paneer whey for the manufacturing of rhamnolipids (a type of biosutfatant). These biosurfactants significantly reduced the surface tension of the medium from 52 mN/m to 26.5 mN/m. It was also deduced that the yield of biosurfactant was 2.7 g/L which further augmented to 4.8 g/L with the administration of glucose @ 2% as well as mineral salts. The biosurfactant was reported to have 83%, 88%, 81%, 92%, 86%, and 100% emulsification activity against n-hexadecane, olive oil, kerosene, diesel, and engine oil, respectively (Patowary et al., 2016).

The current chapter firstly provides insight regarding the various types of biosurfactants and their producer microorganisms. Further, it summarizes the production strategies of whey based biosurfactants and its applications in various industries. It is contemplated that this chapter will empower the industries as well as other stakeholders to exploit the full potential of biosurfactants by providing deep insights into various clean and eco-friendly production strategies.

14.2 Classification of Biosurfactants

The biosurfactants produced by the microorganisms have different molecular compositions and can be glycolipids, lipopeptides, lipoproteins, phospholipids and polymeric surfactants. Besides that, neutral lipids, fatty acids and particulate compounds can also be considered as biosurfactants (Table 14.1).

Biosurfactants can be classified into different categories on basis of their molecular weight, source microorganism and chemical composition.

1. On basis of molecular weight, the biosurfactants are classified into:

   (a) High molecular weight biosurfactants
   (b) Low molecular weight biosurfactants

   High molecular weight biosurfactants: Lipoproteins, lipopolysaccharides, proteins, polysaccharides and biopolymer complexes have been categorized as high molecular weight biosurfactants.

   Low molecular weight biosurfactants: Lipopeptides, glycolipids and phospholipid-based compounds are grouped as low molecular weight biosurfactants (Drakontis & Amin, 2020).

2. On basis of producer microorganism, the biosurfactants are classified as:

   (a) Biosurfactants produced by bacteria: The microbial biosurfactants may be produced by bacteria, yeast or fungi, however those produced by various bacterial species are the dominant ones. The major bacterial species producing biosurfactants are *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp. *and Arthrobacter* sp. However, the pathogenic nature of these producing

Table 14.1 Major groups of biosurfactants and producer microorganisms

Biosurfactant		
Group	Biosurfactant type	Producer microorganism
Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa*
	Trehalolipids	*Mycobacterium tuberculosis* *Rhodococcus erythropolis* *Arthrobacter* sp. *Nocardia* sp. *Corynebacterium* sp
	Sophorolipids	*Torulopsis bombicola Torulopsis petrophilum Torulopsis apicola*
Fatty acids, phospho-lipids and neutral fats	Corinomycolic acid	*Corynebacterium lepus*
	Spiculisporic acid	*Penicillium spiculisporum*
	Phosphatidylethanolamine	*Acinetobacter* sp. *Rhodococcus erythropolis*
Lipopeptides	Surfactin	*Bacillus subtilis*
	Lichenysins	*Bacillus licheniformis*
	Viscosin	*Pseudomonas fluorescens*
	Serrawettin	*Serratia marcenscens*
	Gramicidin	*Bacillus brevis*
	Subtilisin	*Bacillus subtilis*
	Molecules composed of proteins and lipids	*Yarrowia lipolytica MTCC9520* *Penicillium chrysogenum SNP5* *Aspergillus mulundensis* *Fusarium* sp.
Polymeric biosurfactants	Emulsan	*Acinetobacter calcoaceticus*
	Alasan	*Acinetobacter radioresistens*
	Biodispersan	*Acinetobacter calcoaceticus*
	Liposan	*Candida lipolytica*
	Yasan	*Yarrowia lipolytica*
	Manoproteins	*Saccharomyces cerevisiae* *Kluyveromyces marxianus*
Particulate surfactant	Vesicles	*Acinetobacter calcoaceticus*

Source: Amaral et al. (2010), da Silva et al. (2021), de Cássia et al. (2014)

mico-organisms, limit the application of the biosurfactants produced by them for use in the pharmaceutical and food industries.

(b) Biosurfactants produced by fungi and yeast: The major biosurfactant producing yeasts are *Candida* sp., *Pseudozyma* sp. *and Yarrowia* sp. Majority of these species have GRAS (generally regarded as safe) status which gives them extra advantage over bacterial species which may be pathogenic in nature. Yeasts species having GRAS status are *Saccharomyces cerevisiae, Yarrowia lipolytica and Kluyveromyces lactis*. These yeast species having GRAS status are non-toxic and non-pathogenic in nature which allows the application of the biosurfactants produced by them in the pharmaceutical and food industries (Amaral et al., 2010).

3. On basis of chemical composition, the biosurfactants are classified as:

   (a) Glycolipids: The biosurfactants having carbohydrates linked to long-chain aliphatic acids or hydroxyaliphatic acids by an ester group are known as glycolipids. Rhamnolipids, trehalolipids and sophorolipids are major sub-categories of glycolipids. Rhamnolipids are those glycolipids, where, one or two molecules of rhamnose are linked to one or two molecules of hydroxydecanoic acid and is produced by *Pseudomonas aeruginosa*. Trehalolipids are produced by most species of *Mycobacterium, Nocardia* and *Corynebacterium*. Trehalolipids from *Rhodococcus erythropolis* and *Arthrobacter* sp. reduced the surface tension and the interfacial tension in culture broth to 25–40 mNm and 1–5 mNm, respectively (Asselineau & Asselineau, 1978). Glycolipids having sophorose are known as sophorolipids. Sophorose is a dimer of glucose having an unusual β-1,2 linkage between two glucose moieties and constitutes the hydrophilic portion of sophorolipids. In sophorolipids the sophorose is linked to a long fatty acid chain containing 16 or 18 carbons which constitutes the hydrophobic portion of sophorolipids. Sophorolipids may be synthesized either in lactonic or acidic form. Acidic sophorolipids are alicyclic and carry a carboxylic acid group at the terminal of the hydrophobic portion, while lactonic sophorolipids are cyclic with ester functionality (da Silva et al., 2021).

   (b) Lipopeptides and lipoproteins: Lipopeptides (LPP) contain a portion of fatty acids with varying degrees of branching and oxidation, linked to linear or cyclic oligopeptides having varying number and type of amino acids. Many bacterial and yeasts species produce lipoproteins mainly surfactin and lichenysin. Surfactin is a very effective biosurfactant which is a cyclic lipopeptide and contains a seven amino-acid ring structure linked to a fatty-acid chain via lactone linkage. Lichenysins produced by *Bacillus licheniformis* show remarkable stability under extreme temperature, pH and salt conditions.

   (c) Neutral lipids, phospholipids fatty acids: Many bacterial and yeast species synthesize large quantities of fatty acids and phospholipid biosurfactants when grown on n-alkanes. In *Acinetobacter* sp. 1-N, phosphatidyl ethanolamine-rich vesicles are produced which form optically clear micro-emulsions of alkanes in water.

   (d) Polymeric biosurfactants: The major biosurfactants of this type are emulsan, liposan, alasan, lipomannan and other polysaccharide-protein complexes. Emulsan is a powerful emisifying agent for hydrocarbons in water, even at a concentration as low as 0.001–0.01%. Liposan is an extracellular water-soluble emulsifier produced by *Candida lipolytica* and contains 83% carbo-hydrate and 17% protein.

   (e) Particulate biosurfactants: Some bacteria produce the extracellular membrane vesicles partition to form a microemulsion which plays a crucial role in uptake of alkane by microbial cells. Vesicles of *Acinetobacter* spp. strain

HO1-N with a diameter of 20–50 nm are made up of protein, phospholipids and lipo-polysaccharide (Chakrabarti, 2012).

14.3 Production Strategies for Whey-Based Biosurfactants

The main issues with the production of biosurfactants are the reasonably high cost of raw materials and the low final yield. It is customary for the quantity and quality of raw materials to have a significant impact on the cost of manufacturing and in the majority of the biotechnological processes, it is thought that the expense of raw materials makes up 10–30% of the total cost of production. So, it becomes necessary to employ agro-industrial wastes, such as whey obtained from dairy businesses, as inexpensive raw materials in order to reduce costs (Kapadia Sanket & Yagnik, 2013). By using whey in food and other uses it will boost global competitiveness, promote sustainable economic growth and generate employment (Poonia, 2020). Due to a lack of cost-efficient production methods, only a few biosurfactants are currently being used on an industrial basis. Due to the incompatibility of these compounds, some applications that called for large amounts of inexpensive biosurfactants were impeded (Helmy et al., 2011). High-yielding strains, the use of enzymes, and improved fermentation and downstream conditions appeared to be successful techniques for large-scale biosurfactant production to outcompete their synthetic counterparts.

14.3.1 Factors Affecting the Production of Biosurfactants

The final yield and production of biosurfactants are governed by a variety of parameters, such as the sources of carbon, nitrogen, and phosphate as well as other sources like additives and metal ions (Sekhon Randhawa & Rahman, 2014).

14.3.2 Nutrient Formulations

Lipids and carbohydrates are the primary carbon sources used in the manufacture of biosurfactants. The substrates used by microorganisms are carbohydrates and lipids, which are subsequently directed to biosynthetic processes involved in cell formation and the production of biosurfactants (Marcelino et al., 2020). When carbon sources with various polarities were combined, other experiments revealed that the yields in the manufacturing of biosurfactants were satisfactory (Fontes et al., 2012). The carbon source utilized in the synthesis of biosurfactants is crucial since it affects the molecule's structure and, as a result, its physicochemical properties (Santos et al., 2016). In addition to carbon sources, nitrogen supplies are necessary for the

formation of biosurfactants, and the synthesis of nucleic acids, amino acids, protein, and enzymes is required for cellular metabolism. The production of biosurfactants can use a variety of materials as nitrogen sources, such as yeast extract, rice bran extract, soybean meal extract, corn steep liquor, beef extract, urea, peptone, ammonium sulfate, and ammonium nitrate (Franco Marcelino et al., 2017). As their ingestion does not significantly alter pH, it is advised to choose organic compounds with a complex composition while choosing a nitrogen source for the synthesis of biosurfactants. The presence of inorganic salts hydrolyzes cations or anions which might hinder the fermentation process by altering the pH of the culture medium. The C: N ratio is generally kept high during biosurfactant synthesis. In order to control biosynthesis nitrogen act as a limiting element in this bioprocess, thus, its source is strategically exploited (Marcelino et al., 2020).

Micronutrients are necessary in culture media in addition to carbon and nitrogen sources to produce biosurfactants. Multivalent cations typically serve as cofactors in a variety of enzymatic pathways and may facilitate the creation of biosurfactants (Fontes et al., 2008).

14.3.3 Operational Conditions for Fermentation

Production of biosurfactants can also be hampered by physicochemical characteristics and operational elements including temperature, pH, agitation, and aeration. The enzymatic activities of microorganisms employed to make biosurfactants may be hampered by temperature and pH. These microorganisms typically create biosurfactants between the temperatures of 25 °C and 40 °C (Marcelino et al., 2020). *Cryptococcus*, *Rhodotorula, Candida*, and *Tremella,* are some of the yeasts that are recently been shown to synthesize biosurfactants at 25 °C (Chaves, 2017). (Van Bogaert et al., 2011) found that pH values between 7 and 7.5 can cause a distorted structure of sophorolipids produced using yeast.

In addition to ensuring that the culture medium is homogenized, agitation and aeration are crucial factors in the formation of biosurfactants since these have a direct impact on the rate of oxygen transfer (estimated as kLa -volumetric coefficient of O_2 transfer) in the culture medium. The kLa defines the respiratory chain, as well as the catabolic and anabolic pathways important for the synthesis of biosurfactants and other metabolic pathways that are utilized by the cell to promote cell growth (Marcelino et al., 2020). According to (Yeh et al., 2006), high agitation and aeration rates caused yeasts to yield more biosurfactants. However, vigorous stirring and aeration during fermentation may lead to foaming problems (Joshi-Navare & Prabhune, 2013).

The optimization of biosurfactant production depends on how the bioprocess and bioreactor designs are carried out, in addition to studies of the nutritional, physicochemical, and operational aspects. Whilst stirred tank reactors are often used reactors in the production of biosurfactants, this type of reactor has the potential to create foam, which is a major concern (Marcelino et al., 2020). The ability of the

submerged fermentation process to produce biosurfactants is constrained by an excess of foam during fermentation because, along with the foam, nutrients, products, and biomass are lost, which either lowers productivity or, in the worst situations, renders fermentation impossible (Winterburn & Martin, 2012). To reduce foaming issues in submerged fermentation in stirred tank bioreactors, different approaches are employed viz., the use of mechanical and acoustic, techniques, "switchable foam control" (using the pH sensitivity of specific biosurfactants), membranes, and foam fractionation (Pereira et al., 2013) (Winterburn & Martin, 2012). Due to its affordability and simplicity, solid-state fermentation is being employed to produce biosurfactants without foaming.

14.3.4 Production Enhancement Through High-Yielding Strains (Genetic Engineering Strategies)

The employment of hyperproducing microbial strains is frequently required for large-scale production of biosurfactants in order to produce commercially viable products, despite the application of better and optimized bioprocess engineering. For the production process to be more economical and to generate goods with superior commercial features, the availability of twitchy strains and recombinants is crucial (Helmy et al., 2011). The utilization of mutant, recombinant, and genetically altered strains of microorganisms is one technique that is frequently employed to increase productivity. Using biotechnological methods, genetic engineering involves modifying naked genes, plasmids with BioS genes, or genetically modified microbes. This strategy makes it easier to change genes or operons of microbes including both heritable and non-heritable DNA, generate alternative metabolic pathways, and change the sequence of previously existing BioS synthetic genes (Jimoh et al., 2021). The producer strains received genetic editing to boost rhamnolipid production. The primary enzyme in charge of producing rhamnolipids is the rhamnosyltransferase 1 complex (RhlAB). In order to make the rhamnolipid-producing *E. coli lac* ZY genes were introduced into the chromosome of *P. aeruginosa* to enable growth and the use of whey for improved biosurfactant synthesis (Koch et al., 1988).

Recombinant DNA technology has been successfully applied by researchers to boost BioS production yield. The activation of the polypeptides, fatty acids, and non-ribosomal peptide synthetase enzymes depends on phosphopantetheinyl transferases. According to (Jimoh & Lin, 2019) theory, the biosynthesis of the lipopeptide BioS depends on the sfp phosphopantetheinyl transferase, which is a critical requirement for peptide synthesis systems. In a similar study, (Porob et al., 2013) discussed the role of surfactin genes in biosurfactant production from 6 distinct *Bacillus* species and the 224 amino acid coding capacity of an sfp gene cloned from *Bacillus tequilensis*. Also, the recombinant microbial strains BioSa, BioSb, and BioS were cloned with the genes sfp, sfp0, and srfA, which enhanced BioS activity. Another

way to alter the gene sequence of a microbial strain in order to activate it is through mutagenesis (Bouassida et al., 2018). Mutant variants could also be produced by other techniques including ultraviolet radiation, physical and/or chemical mutagenesis utilising methyl-N′-nitro-N-nitrosoguanidine, or selection based on resistance to ionic detergents like CTAB (Bouassida et al., 2018; Xu & Zhang, 2016)). After being subjected to ion induction, *B. subtilis* E8 mutant produced more surfactants. Similar to this, gamma irradiation allowed the *Pseudomonas aeruginosa* MR01 mutant to produce BioS at a rate that was more than 1.5 times higher than normal (Lotfabad et al., 2010).

14.4 Downstream Processing/Extraction/Purification of Biosurfactants

The overall yield of any commodity is determined by the upstream supply chain and the downstream recovery processes. A biosurfactant's ionic charge, solubility in water, cell-boundness or extracellularly, and, of course, the cost of recovery, all influence the method of extraction that is used. Common methods for recovering biosurfactants include solvent extraction, adsorption followed by solvent extraction, precipitation, crystallization, centrifugation, and foam fractionation. Biosurfactant recovery usually entails precipitation using ammonium sulfate, acetone, ultrafiltration, ion exchange, dialysis, lyophilization, isoelectric focusing, and thin-layer chromatography (Satpute et al., 2010). *Pichia fermentans*, isolated from fermented dairy whey waste, were employed to synthesize biosurfactants that were recovered using three different methods by (Johny & Saravanakumari, 2014). Acid precipitation, ethyl acetate precipitation, and acetone precipitation followed by gravity separation are some of the techniques. According to their findings, acetone recovery provided the maximum oil clearance (45 mm) and dried product yield per liter (6.24 g).

Similar to this, the potential for efficient extraction and recovery of biosurfactants using a novel electrokinetic approach was assessed. The applied voltage varied from 30 V to 10 V to assess its impact on the extraction and recovery process. The largest extraction and recovery occurred at the highest voltage of 30 V, with a result of 69.33%. This was followed by 20 V with 9.63% and 10 V with 4.98%. Compared to the extract recovered by acid precipitation, the biosurfactants extracted utilizing the electrokinetic technique were of high purity with minimum contamination (Gidudu & Chirwa, 2021). Table 14.2 depicts the bioprocessing technologies for enhanced biosurfactant production using whey as the base material.

Table 14.2 Use of bioprocessing technologies for enhanced biosurfactant production using whey as substrate

Raw material	Type of microorganism/ strain	Fermentation conditions	Product/type of biosurfactant produced	Yield obtained	Properties of biosurfactant	Downstream processing/ purification conditions	References
Paneer whey	*Pseudomonas aeruginosa,* SR 17	Mineral salt medium with 2% glucose (w/v) with incubation at 35 °C for 4 days	Rhamnolipid	2.7 g/L and increased to 4.8 g/L with added 2% glucose and mineral salts	Casein micelle conc. (CMC) of 110 mg/L resulted in enhanced emulsification activity (100%) in crude oil	Solvent extraction using ethyl acetate followed by chromatography using chloroform/methanol mobile phase	Patowary et al. (2016)
Curd whey	*Pseudomonas Aeruginosa strain*-PP2 and *Kocuria turfanesis* strain-J	Sterilized whey was incubated at 150 rpm at 30 °C for 96 h	----	0.568 g/L in case of *Kocuria turfanesis* strain-J and 1.233 g/L in case of *Pseudomonas aeruginosa strain*-PP2	Surface tension reduction was stable at pH 2–11 and biosurfactant of strain-J exhibited high emulsification of imidacloprid at 60 °C	Centrifugation at 10,000 rpm for 10 min	Dubey and Juwarkar (2001) and Dubey et al. (2012)
Cheese whey permeate	Lactobacillus strains	Initial lactose concentration was set at 20–25 g/L. fermentation was carried out in 2 L duran bioreactor for 24 h and 72 h with 10% inoculum (w/v) at 30 °C and 37 °C with pH of 6.7–6.9 & agitation at 600–800 rpm	Proteinaceous type biosurfactant	----	*L. fermentum* ACA-DC 0183 resulted in maximum surface tension reduction (34.9 mN/m) after 32 h of fermentation	Centrifuges and washed with distilled water followed by agitation overnight in demineralized water at 4 °C and further dialysis using a membrane of 6–8 kDa molecular weight cut off	Kachrimanidou et al. (2022)

(continued)

Table 14.2 (continued)

Raw material	Type of microorganism/ strain	Fermentation conditions	Product/type of biosurfactant produced	Yield obtained	Properties of biosurfactant	Downstream processing/ purification conditions	References
Permeate from whey ultrafiltration	*Bacillus methylotrophicus and Bacillus pumilus*	50 mL of medium was inoculated with 2 mL inoculum and maintained in a shaker at 100 rpm at 30 °C for 2 days and 5 days	Surfactin	---–	Reduction of surface tension, obtaining a minimum value of 35.07 mN/m for *B. methylotrophicus* and 26.02 mN/m for *B. pumilus*	Sterilized medium was centrifuged at 5000 rpm for 20 min followed by microfiltration using 0.4 μm membrane	Decesaro et al. (2020)
Distillery and whey waste	*Pseudomonas aeruginosa* strain BS2	Glucose was used as carbon source and incubated on an orbital shaker at 30 °C and 150 rev/ min for 120 h	---–	0.97 g/L	Significant reduction of surface tension from 72 mN/m to 27 mN/m and formed 100% stable emulsions with low CMC of 0.028 mg/ mL	Diethyl ether extraction and crystallization method	Dubey and Juwarkar (2001)
Whey and cassava waste water	*Bacillus subtilis*	Media inoculated with strain was incubated at 30 ° C, 150 rpm for 12 h	Surfactin	27.07 mg/L	Whey (27.7–34 g/L), activated carbon (25 g/L) and cassava wastewater (74 g/L) was the optimum substrate concentration	Centrifuged (104 g for 10 min at 5 ° C), acidified with acidified solution, again centrifuged and neutralized followed by drying at 50 °C	de Andrade et al. (2016)
Cheese whey	*Bacillus licheniformis* K51, *B. subtilis*	Whey was inoculated with 2% (v/v) seed culture	---–	---–	Stable biosurfactant (for 09 days at 80 ° C), in the pH range	Centrifuged at 11,292 g for 20 min,	Joshi et al. (2008)

	20B, *B. subtilis* R1 and *bacillus* strain HS3	and then incubated at 160 rpm with controlled shaking and also without shaking conditions at 45 °C for 72 h			of 6.0–12.0, and salt concentration up to 7% (w/v)	precipitated using 6 N HCl, and dried.	
Whey	*Streptococcus thermophilus, Lactobacillus acidophilus* and *Lactobacillus rhamnosus*	600 mL of whey was inoculated with 15 mL lactic acid bacteria and incubated for 48 h at 37 °C with 150 rpm agitation	---–	9.20–11.80 g/L	Exhibited emulsification index 19.5–58%, biosurfactant conc. (10 mg/L.) showed antiadhesive effect against E. *coli* and S. *aureus*	Centrifuged at 6500 × g at 4 °C for 20 min, precipitated using 6 M HCl and dried at 40 °C	Alkan et al. (2019)

14.5 Applications of Biosurfactants in Various Industries

The Environmental Protection Agency considers biosurfactants like rhamnolipids and sophrolipids to be safe and nontoxic for use in cosmetic, pharmaceutical, and food applications. (Nitschke & Costa, 2007). The cosmetic and food industries value these compounds for their antibacterial, antifungal, and antiviral characteristics as well as their solubilizing abilities. For this reason, biosurfactants are used in sophisticated food formulations as thickeners, stabilisers, and emulsifiers (Campos et al., 2013); (de Cortés-Sánchez, 2020). In the food industry, biosurfactants are typically utilised as bio-emulsifiers that contributed to emulsion stability in order to improve food shelf-life and texture. (Nitschke & Silva, 2018). Owing to versatile attributes viz., solubilization, softening, penetration, dispersion, emulsification, wetting, and detergency biosurfactants have broad spectrum applications as cleansing agents in textile industry (de Oliveira et al., 2015) in cosmetic formulations as antiaging agent, in pharmaceutical industries due to antimicrobial, antiviral and antioxidative properties and many more as illustrated in Fig. 14.2. In this section, authors have focused on the wide applications of whey based biosurfactants in particular.

14.5.1 Food Industry

14.5.1.1 As Emulsifiers

Emulsions are present in a wide range of culinary products, whether they are processed or natural, including milk, butter, cream, kinds of margarine, mayonnaise, cream liquors, whippable toppings, ice creams, tea, and coffee whiteners (Kralova & Sjöblom, 2009). In order to prevent phase separation, emulsifiers are surface-active chemicals that enable ordinarily immiscible liquids to form stable emulsions. These are therefore essential ingredients in food compositions. Phospholipids, amphiphilic proteins, and synthetic surfactants are the most often used emulsifiers in food (Freire et al., 2010). The use of microbial-derived surfactants as emulsifiers in food compositions can be investigated due to their "natural or green" origin owing to environmentally friendly traits (Mohan et al., 2006). As per the investigation conducted by (Suryanti et al., 2017), rhamnolipids produced using *Pseudomonas fluorescens* cultured on whey tofu reduced surface tension for the emulsion of water with benzene, n-hexane, pentane, and kerosene or other lubricants by more than 40%. The findings were comparable to those of surfactants that are easily accessible on the market, such as Triton X-100 and Tween 80, and stable emulsions could persist up to 30 days when employing toluene, paraffin, palm oil, or other lubricants as an immiscible chemical. In the food business, rhamnolipids are generally used as emulsifiers and wetting or foaming agents/stabilizers, which helps food products last longer on the shelves. Muffins and croissants were given a shelf-life boost by adding 0.10% rhamnolipid, which enhanced moisture retention, texture, and freshness.

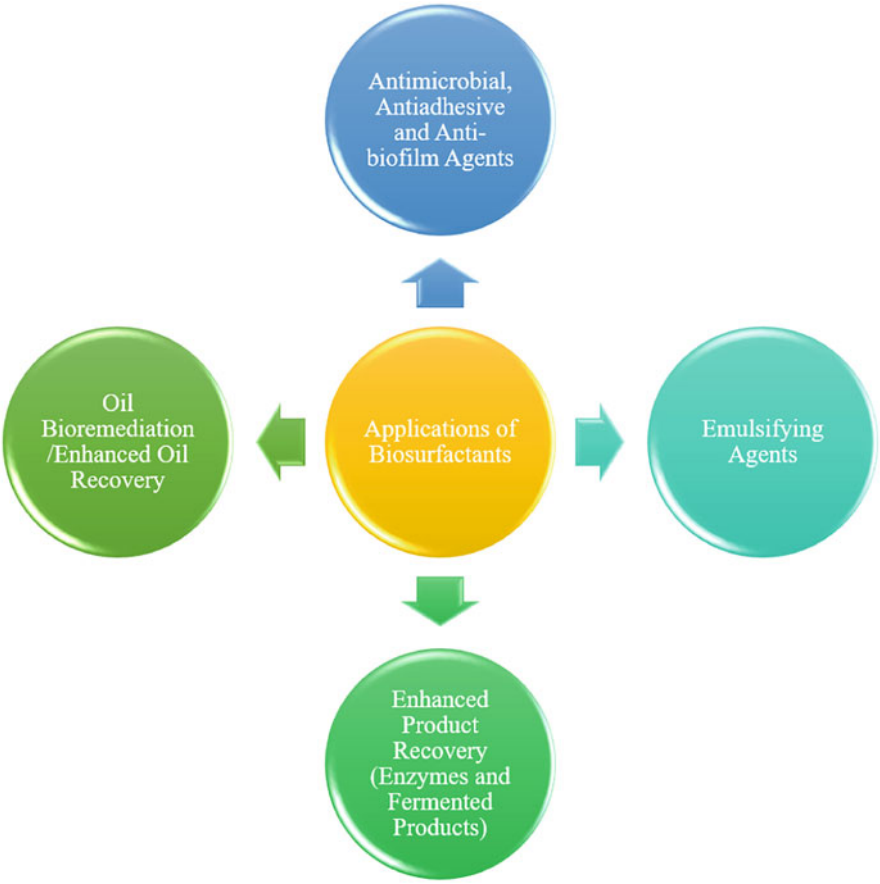

Fig. 14.2 Applications of whey-based biosurfactants

Candida glabrata UCP 1556 produced a bio-emulsifier with whey and corn-steep spirits that reduced surface tension to 28.8 mN/m, had a CMC of 2%, and exhibited an anionic profile. The biosurfactant also decreased the viscosity of canola oil, cottonseed oil, and soybean oil. It also had an oil dispersion capability of 81.54% at temperatures ranging from 0 °C to 120 °C, a pH range of 2 to 12, and a NaCl range of 2–12% (Lima et al., 2017). Another study was planned to compare the emulsification index (EI) by using *Yarrowia lipolytica* of synthetic medium encompassing yeast extract (YE), glycerol, ammonium sulphate (AS), glucose to numerous low cost media. It was revealed that EIs of media containing leftover liquid obtained during the processing of butter or cheese, and supplements of YE and AS were comparable to the EI of the synthetic medium. It was also demonstrated that when butter whey was tested with corn steep liquor (CSL) without incorporation of AS, identical EI (66.8%) was displayed to that of the same medium with AS (66.3%).

This concoction could be successfully employed to get to obtain vegetable oil-in-water emulsions confirming its possible usage in food items (Santos et al., 2021).

14.5.1.2 Anti-biofilm Activity of Biosurfactants

Biofilms are a layer of aggregated communities of microflora adhering to either each other and/or to the surface. These are contemplated to be a matter of crucial concern for industries, our surrounding environment and medical field, as they are found practically everywhere and have a high level of resilience to environmental stressors and antibiotics. They are crucial in the biological field because they can subvert the host immune system (Usui et al., 2023). In the industrial setting, they are capable of contaminating food, detrimental to machinery, and also interfere with the regular working cycle of industries (Galié et al., 2018). In a food industry, biofilms build very swiftly. The initial steps of biofilm production are: prepping of the material's surface and the reversible attachment of the cells. After that, the binding becomes irreversible, and microcolonies start to grow. Ultimately, the biofilm develops a tri-dimensional structure, creating a sophisticated ecosystem that is prepared for dispersal (Carrascosa et al., 2021). The development of disease and the deterioration of food are commonly linked, and bacterial biofilms on surfaces are substantial contributors to contamination in the food industry. Hence, reducing microbial adherence to contact surfaces with food is essential for providing consumers with high-quality, secure products (Campos et al., 2013). Also, the development of biofilms results in a number of problems, such as impaired heat transfer mechanisms, increased surface corrosion, and reduced production effectiveness (Maddela & Abiodun, 2022). The approach based on biosurfactants that have shown the most promising for eliminating microbial biofilms (Sharma et al., 2015) (Kiran et al., 2010). (Meylheuc et al., 2006) proposed a method to reduce the amount of *Listeria* cells attaching to stainless-steel surfaces by prepping the surfaces with a *Pseudomonas fluorescens* anionic biosurfactant, which favoured the disinfectant's bactericidal effects. The goal of the study by (Khiralla et al., 2015) was to examine the antibiofilm properties *of Lactobacillus pentosus* and *L. plantarum* HG against *P. aeruginosa* and *B. cereus*, two prevalent pathogens (plant pathogen). In the presence of 20 μL of cell-free *Lactobacillus* supernatant, both pathogens demonstrated a considerable decrease in biofilm formation. When employed as polystyrene surface conditioners, surfactin and rhamnolipids were likewise effective at reducing adhesion from *S. aureus, L. monocytogenes*, and *Micrococcus luteus* at different temperature profiles (Zeraik & Nitschke, 2010). Mechanism of anti-biofilm activity of biosurfactant is depicted in Fig. 14.3.

14.5.1.3 Antiadhesive and Antimicrobial Activity of Biosurfactants

Antimicrobial action is relevant in many applications due to biosurfactants' general tendency to break membranes, which causes increased membrane permeability,

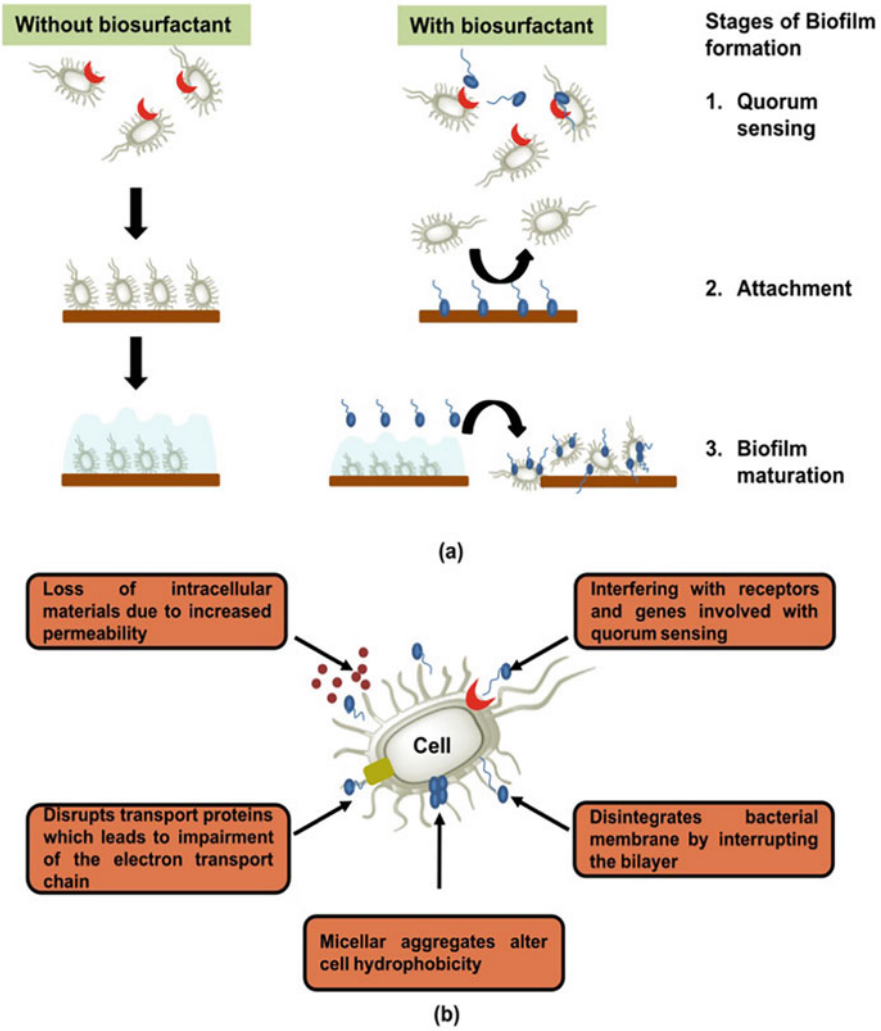

Fig. 14.3 Anti biofilm activity of biosurfactants (**a**) Action of biosurfactants during different phases of biofilm formation. (**b**) Mechanism of Anti biofilm activity of biosurfactant (Bhadra et al., 2022)

metabolite leakage, and cell lysis (Campos et al., 2013). Many biosurfactants exhibit antibacterial and antiadhesive properties. Biosurfactants may be used as coating agents for food-related surfaces and utensils or to lower the rate or occurrence of antifouling due to their anti-adhesive effect against bacteria. Using whey as the substrate, researchers discovered that the biosurfactant produced by *Lactobacillus agilis* CCUG31450 has significant anti-adhesive action against *Staphylococcus aureus* as well as antibacterial activity against *agalactia* and *Pseudomonas*

aeruginosa (Gudiña et al., 2015). In a subsequent investigation by (Gudiña et al., 2010) biosurfactant (25 mg/mL and 50 mg/mL) was isolated from *Lactobacillus paracasei* ssp. *paracasei* A20 demonstrated potent antimicrobial and antiadhesive activity against a variety of microorganisms, including pathogenic *Candida albicans, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus.* It is possible to commend the biosurfactant specifically generated from *L. lactis* as a broad-spectrum antibacterial. As a result of its probiotic origin, *L. lactis* biosurfactant is safe to use as a medicinal agent or food additive (Sharma et al., 2016).

14.5.2 Other Industries

14.5.2.1 Bioremediation and Enhanced Oil Recovery

Despite being helpful to society, petrochemical factories and oil refineries also generate a lot of dangerous waste. Moreover, oil spills during discovery, transportation, and refining have had significant negative effects on the environment (Sobrinho et al., 2013) (Souza et al., 2014). The normal outcome of conventional purification techniques is the straight forward transfer of toxins from one medium to another, which result in contamination thus, causing serious repercussions. Nevertheless, physicochemical techniques cannot totally remove crude oil from the environment. Hence, biological alternatives are receiving more attention (Malik & Ahmed, 2012) (Lin et al., 2014) (Table 14.3). Biosurfactants play an important role in remediation operations because of effective dispersion and cleaning action along with its environmentally friendly characteristics viz., low toxicity & high biodegradability (Silva et al., 2014) (Fig. 14.4). The biosurfactants help in the emulsification of hydrocarbons to create a blend of water-soluble compounds. Surfactants such as rhamnolipids, lichenysins, and surfactin which are usually extracted from whey-based substrates play a very efficient role in cleaning up the oil spillage (Ng et al., 2022; Patowary et al., 2016). A comparison of oil spreading, surface tension reduction properties and emulsification index of biosurfactants produced from whey waste and from MRS broth was done. Surface activity of lactic acid bacteria strains' created a zone with a diameter between 1.87 cm and 5.92 cm. A decrement in surface activity was recorded in both biosurfactants prepared by MRS broth and whey medium. The range of the emulsification index values after 1 h, 24 h, and 1 week was substantial and ranged from 19.50% to 58.00%. *L. acidophilus* from whey medium demonstrated the maximum emulsification activity in the first few hours. (Alkan et al., 2019). By decreasing surface and interfacial tensions, altering wettability, and creating oil/water or water/oil emulsions, biosurfactants are highly successful at boosting or improving oil recovery from diminishing oil reservoirs (Sen, 2008). Studies are limited pertaining to utilization of whey based biosurfactants for bioremediation purposes. However, utilization of biosurfactants prepared from agrowastes are abundant. Biosurfactants are utilized to remove heavy

Table 14.3 Biosurfactants producing microorganisms and uses in the bioremediation of oil-contaminated environments (de Cássia et al., 2014)

Microorganisms	Type of Biosurfactant	Applications
Rhodococcus erythropolis 3C-9	Glucolipid and trehalose lipid	Oil spill clean-up operations
Pseudomonas aeruginosa S2	Rhamnolipid	Bioremediation of oil-contaminated sites
Rhodococcus sp. TW53	Lipopeptide	Bioremediation of marine oil pollution
Micrococcus luteus BN56	Trehalose tetraester	Bioremediation of oil-contaminated environments
Nocardiopsis alba MSA10	Lipopeptide	Bioremediation
Pseudozyma hubeiensis	Glycolipid	Bioremediation of marine oil pollution
C. glabrata UCP1002	Protein-carboydrate-lipid complex	Oil recovery from sand
C. lipolytica UCP0988	Sophorolipids	Oil recovery
C. lipolytica UCP0988	Sophorolipids	Oil removal
C. sphaerica UCP0995	Protein-carboydrate-lipid complex	Removal of oil from sand
C. lipolytica UCP0988	Sophorolipids	Control of environmental oil pollution
C. glabrata UCP1002	Protein-carboydrate-lipid complex	Oil removal
C. guilliermondii UCP0992	Glycolipid complex	Removal of petroleum derivate motor oil from sand
C. tropicalis UCP0996	Protein-carboydrate-lipid complex	Removal of petroleum and motor oil adsorbed to sand
C. lipolytica UCP0988	Sophorolipids	Removal of petroleum and motor oil adsorbed to sand
C. sphaerica UCP0995	Protein-carboydrate-lipid complex	Oil removal

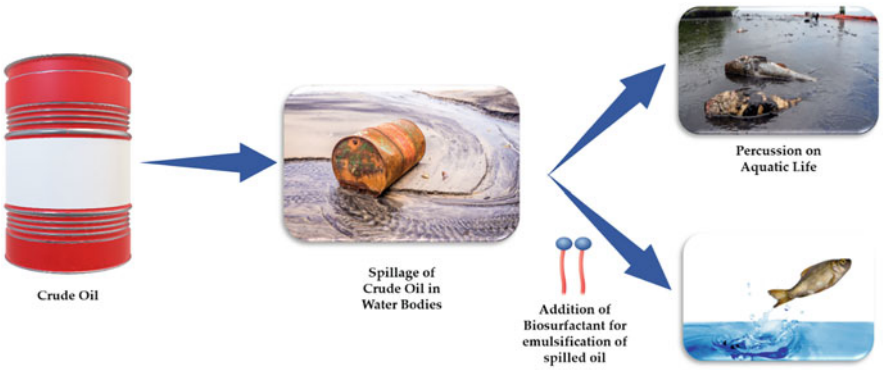

Fig. 14.4 Role of biosurfactant in reducing the harmful effects of oil spillage

Table 14.4 Common applications of biosurfactants in petroleum industry (de Cássia et al., 2014)

Step in Petroleum Production Chain	Applications
Extraction	Reservoir wettability modification
	Oil viscosity reduction
	Drilling mud
	Paraffin/asphalt deposition control
	Enhanced oil displacement
	Oil viscosity reduction
Transportation	Oil viscosity reduction
	Oil emulsion stabilization
	Paraffin/asphalt deposition
Oil tank/container cleaning	Oil viscosity reduction
	Oily sludge emulsification
	Hydrocarbon dispersion

metals from soil (Guan et al., 2017), as ion collectors in waste water treatment (Akbari et al., 2018; Ng et al., 2022). There are two ways that microbial enhanced oil recovery (MEOR) technology can be applied both in situ and ex situ. The in situ method involves injecting either potential bacteria or selective nutrients into the oil well, followed by phases of water flooding and shutting the well down, respectively. Microbial load in the column rises during the shut-in phase to improve oil recovery. Ex situ process produces microbial products outside and injects them into the oilwell, resulting in enhanced oil recovery ((Geetha et al., 2018), (Satpute et al., 2010); (Suthar et al., 2009; Varjani & Upasani, 2016) (Table 14.4).

14.5.2.2 Medicinal Applications

According to reports, biosurfactants could be used in gene therapy, vaccines, substantial adjuvants for vaccine antigens, and antibacterial, anti-malarial, anti-fungal, anti-viral, as well as immunomodulatory activity. They could also operate as sticky and anti-adhesive agents (Rodrigues et al., 2006; Varjani et al., 2021). In the instance of pseudomonads, biosurfactants like rhamnolipids have been used for their noncytotoxic anti-tumorigenic effects against colon cancer cell lines and antimicrobial uses, however, the biosurfactant and its production are associated with pathogenicity. Because they might trigger allergic reactions and skin irritations, biosurfactants derived from pathogens should be used with caution in cosmetic and healthcare goods (Adu et al., 2020), (Haque & Hassan, 2020), (Shaikh et al., 2020). It has been demonstrated that the glycolipid biosurfactant mannosylerythritol lipid (MEL) derived using *Candida* has the potential antibacterial and neurological properties. Surfactin and Iturin A are two important lipopeptides that have been extensively reported for medical use. These two have been noted to have antibacterial, antiviral, and anticancer properties as well as immunomodulatory properties for particular toxins and enzyme inhibition (Rodrigues et al., 2006).

With regard to MRSA *Staphylococci*, *Acinetobacter indicus* M6 developed a glycolipoprotein biosurfactant that shown anti-proliferative action against lung cancer cell (A459) as well as antibacterial and antibiofilm properties (Karlapudi et al., 2020).

14.6 Conclusion

A wide range of prospective applications in the disciplines of oil recovery, environmental bioremediation, food processing, and medicine have been made possible by a number of intriguing properties of biosurfactants. These compounds have a number of exceptional qualities, including a high biodegradability, low toxicity, and efficacy at high and low temperatures, pH levels, and salinities. Even though biosurfactants show a wide range of possible applications, the economics of the bioprocess are a key factor in whether a biotech product is successfully commercialised. Due to their high production costs and low yields, biosurfactants are now more expensive than their chemical counterparts. As a result, there have been reports of significant efforts to cut their production and recovery costs. Biosurfactant manufacturing may be made economically possible by employing techniques including the use of less expensive substrates such as dairy waste whey, optimisation of their synthesis, new and effective multistep downstream processing techniques, and the use of recombinant and mutant hyperproducing microbial strains. Therefore, it has been stated that the manufacture of biosurfactants can be supported by a number of inexpensive raw materials, including plant-derived oils, oil wastes, starchy substances, cheese whey, and distillery wastes.

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Chapter 15
Utilization of Whey for Production of Bioenergy and Biofuels

Vikram Kumar, Ananya Rana, Jayesh J. Ahire, and Neetu Kumra Taneja

Abstract Whey must be recycled, composted, or incinerated in responsible manner because it is produced in large amounts as a byproduct during the production of cheese and casein. Whey's perspective as a "waste item" and an "opportunity" for further processing have to be changed if this resource is to be effectively utilized. The manufacturing of cheese has significantly expanded in our nation, which has increased whey availability. According to estimates, India produces 650,000 tons of paneer per year, along with 3.3 million tons of whey. The environment receives about 47% of the 115 million tons of whey generated annually worldwide. As a result of its versatility and nutritional worth, whey protein is utilized in a variety of food applications. It has been discovered that deproteinized whey or serum presents a potential source of ethanol. Lactose powders were already being produced using sweet wheys, such those produced when cheese is made. Since they include significant levels of sulphate ions and lactose acid, acid whey produced during the synthesis of lactic and sulphuric casein are not optimal for this purpose, but it has been discovered that they can be utilized to ferment lactose into ethanol. By utilizing whey for the manufacturing of ethanol, the expense of processing dairy waste to minimize the biochemical oxygen demand can be decreased. Additionally, the ethanol generated can be used as a biofuel for cars and power plants, reducing further treatment costs.

Keywords Whey · Lactose · Biofuel · Biochemical · Power plants

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15.1 Introduction

Whey disposal is a concern on a global scale. When making cheese and casein, whey is produced in large amounts as a byproduct, and therefore requires special handling before it may be discarded that doesn't harm the environment. For every kilogram of cheese produced, around 9 L of whey are produced. The environment receives about 47% of the 115 million tons of whey generated annually worldwide (Zandona et al., 2021). Because the majority of the constituents have low molecular weights and are soluble, they can rapidly reduce the oxygen content of natural water systems. Raw whey has a COD (Chemical Oxygen Demand) of roughly 60 kg^{-3}, BOD value of 35,000–45,000 mg/L and COD values of 55,000–70,000 mg/L (Poonia, 2020).

The secret to making the most of this resource has been to shift the way whey is thought of from a "waste material" to a "opportunity" for more processing. Whey from making cheese contains about 7% solids, of which 10–12% is protein (Macwan et al., 2016). Apart than water, lactose (74%), minerals (8%), and fat (3%), there are no other components. Heavy and light chain immunoglobulins, alpha-lactalbumin, alpha-lactoglobulin, bovine serum albumin, and other proteins make up the bulk of whey. The small but crucial proteins lactoferrin and lactoperoxidase are also present. Due to its versatility and nutritional worth, whey protein is employed in a variety of food applications (Vincent et al., 2016).

It has been discovered that deproteinized whey or serum offers a potential source of ethanol. Lactose powders were already being made using glucose wheys, which produced during the making of cheese. Since they include significant levels of sulphate ions and lactose acid, acid wheys produced during the synthesis of lactic and sulphuric casein are not optimal for this purpose (Moulin & Galzy, 1984), but it has been discovered that they can be utilized to ferment lactose into ethanol. About 20 years ago, ethyl alcohol production from deproteinized whey was technologically established in Europe. A good illustration of how technology is used to solve a problem is the extraction of ethanol from a stream that was earlier waste (Okamoto et al., 2019). The manufacture of whey-to-fuel ethanol is examined and pertinent information is addressed. It is predicted that 203 million gallons of gasoline ethanol might be made from whey in 2006, of which dairy cooperatives could receive 65 million gallons. Dairy cooperatives presently run two whey-ethanol plants, which together produce 8 million gallons of ethanol annually (Ling, 2008). During the 1980s, the successful functioning of the factories demonstrates that (1) whey-to-fuel ethanol production technologies and processes are mature and suitable for adoption for commercial operations, (2) whey-to-fuel ethanol production is technically feasible, and (3) making ethanol from whey is economically viable. However, in light of the price volatility of whey products today, the calculation of the opportunity cost of employing whey as a feedstock for fermentation should play a major role in establishing the profitability of a new whey-ethanol project (Bušić et al., 2018).

15.2 Potential of Ethanol Production from Whey Sources

The lactose in whey, whey permeate, or permeate mother liquor is fermented to produce ethanol from whey. Hence, the amount of surplus lactose that is accessible and not being used in whey-derived products determines the potential volume of ethanol production from whey feedstock. An estimated 4433 million pounds of lactose were present in the 90.5 billion pounds of whey produced by the cheese industry in 2006 (Ling, 2008). Condensed whey, dry whey, reduced lactose and minerals whey, whey protein concentrates, whey protein isolates, and lactose were the principal whey products, using an estimated 1857 million pounds, or 42% of the available lactose. Due to these whey products, an estimated 2576 million pounds of lactose were unaccounted for in 2006. This unreported volume may have been contained in secondary, tertiary, or additional whey- or lactose-derived products (Minj & Anand, 2020). As a result, it is possible to estimate that there was an excess of lactose of roughly 2.5 billion pounds in 2006. This is the quantity of lactose that might be used to make ethanol.

Theoretically, 0.538 pounds of ethanol might be produced from 1 pound of lactose. Consequently, it is possible to estimate that 203 million gallons of ethanol might be produced from excess lactose in 2006 (Kemaa & Guedri, 2016). Similar estimates were made of the possible amounts of ethanol that may be produced from excess lactose for earlier years: 182 million gallons in 2003, 195 million gallons in 2004, and 199 million gallons in 2005. The two ethanol manufacture units run by dairy cooperatives combined to make 8 million gallons of ethanol in 2006. However, 195 million gallons of potential remained unrealized (Hertel et al., 2007). The idea that using lactose for food, feed, industrial, and other purposes should take precedence and that ethanol production is the last resort usage for whey and lactose is implicit in the assessment of potential ethanol volume. Whey and lactose would only be used as a last resort in the manufacturing of ethanol to keep the feedstock cost as low as feasible (Ling, 2008).

15.3 Process of Whey to Ethanol Conversion

Although the methods used to produce ethanol differ from plant to plant, but they consistently follow a similar set of fundamental guidelines. Reverse osmosis is used to concentrate the leftover permeate to increase the lactose content necessary for effective fermentation after whey protein has been extracted from it using ultra filtration. With some specific, highly effective strains of the yeast *Kluyveromyces marxianus*, the lactose in whey permeate is fermented (Zandona et al., 2021). The yeast is inoculated into the fermentation vessels after being added to the substrate that is fermenting. When fermentation is finished, yeast is removed from the substrate that has been fermented, and ethanol is extracted from the residual liquid (beer) using the distillation process. The rectifier is then used to dehydrate this

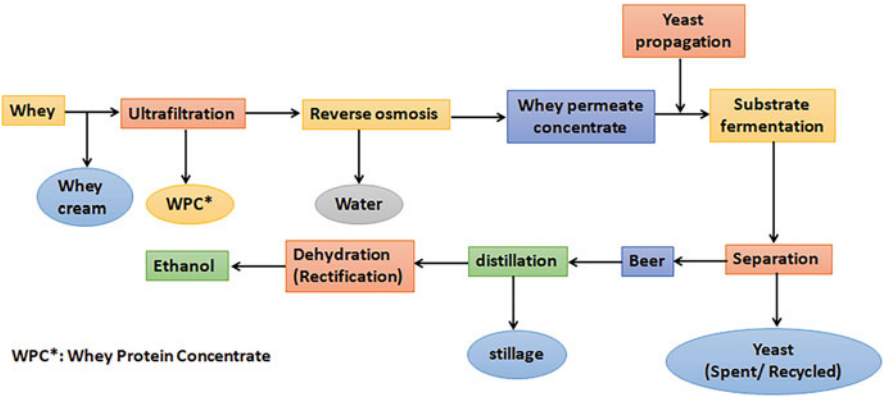

Fig. 15.1 The foundational processes involved in the manufacture of ethanol from whey

ethanol (Maicas, 2020). In order to prevent abuse, the resulting anhydrous ethanol is denatured by adding gasoline if it is intended for use as fuel. After the ethanol in the beer has been removed (stillage), the biomass (spent yeast) and the effluent left in the liquid may be disposed of, digested for methane gas, sold as feed, or processed further into food, feed, or other products (Ling, 2008). The procedure is shown in Fig. 15.1.

Distilleries receive serum or deproteinized whey as a byproduct of the processes used to create lactic casein, mineral acid, or total milk protein (TMP). Due to the fact that during fermentation, 20% of the lactose in milk is transformed to lactic, the lactose concentration of whey made from lactic casein manufacturing is only about 4% (Figueroa Pires et al., 2021). Whey with a modest increase in lactose content comes from the formation of TMP or sulphuric acid casein. Depending on the intended use, yeast ferments lactose, and the resulting ethanol is then distilled and purified into one of eight classes (Lievore et al., 2015).

15.3.1 Cooling of the Serum or Deproteinated Whey and Fermentation

Whey typically arrives at the distillery at a temperature of at least 60 °C. The first step of the process is to use a plate heat exchanger to chill the serum to the fermentation temperature since the serum is sterile and free of microorganisms at this temperature (Stanbury et al., 2017). This serum is cooled, and yeast is added before it is poured into the first of three fermentation tanks. The fermenting serum moves from one tank to the next over the course of around 24 h (Magazoni et al.,

2009). As fermentation continues, the serum specific gravity declines from roughly 1.022 kg L^{-1} to 1.008 kg L^{-1} as the fermentation progresses, as well as the processing flow rate (m^3 h^{-1}) are also monitored during in-process control. The decrease in specific gravity is a result of lactose converting to ethanol during the evolution of carbon dioxide (Environmental Sustainability, 2016).

15.3.2 Chemical Aspects of Conversion Process

Yeast is grown (or multiplied) in different vessels. Yeast biomass is enhanced on the serum by the presence of air, allowing for faster growth. After the serum is chilled, the yeast from the container is added to it. The yeast *Kluveromyces fragilis* employed in fermentation produced -galactosidase, an enzyme necessary for the breakdown of lactose (a disaccharide) into glucose and galactose (Zhou et al., 2021). (Eq. 15.1; Fig. 15.2).

$$\text{Lactose} + \text{water}\ \text{Galactose} + \text{Glucose}\ 4\ \text{ethanol} + 4\ CO_2 \qquad (15.1)$$

The required processing speed determines the fermentation temperature, which is kept as low as possible to reduce bacterial contamination of the process.

The calculation of fermentation efficiency is based on the highest percentage of reactants to products, which is 51%. There is nothing more that needs to be added to the fermentation because it has been found that milk serum contains everything the yeast needs to flourish (Chang et al., 2018). Using separators or decantation, yeast is extracted from the fermented serum once the fermentation is finished. Beer is the term for the fermented serum after the yeast has been removed. Before distillation to extract the ethanol, the beer is stored (Environmental Sustainability, 2016).

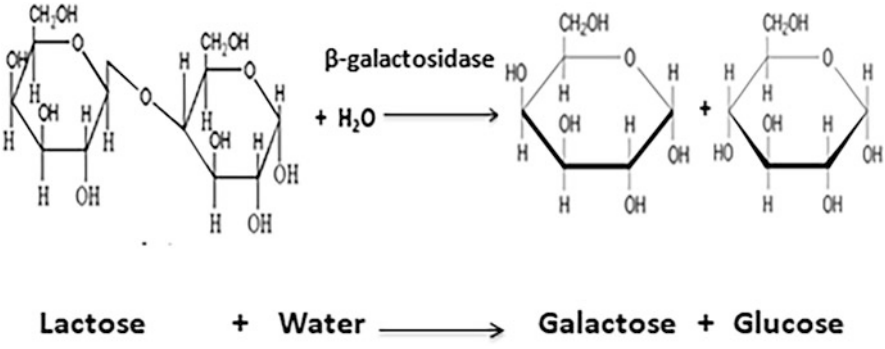

Fig. 15.2 Basic chemical reaction in whey to ethanol conversion

15.3.3 Distillation

The resulting liquid (the beer) is then sent through a distillation process to remove the water and produce ethanol after the yeast is removed from the substrate that has undergone fermentation. After that, the rectifier is used to dehydrate the ethanol. To avoid misuse, the resulting anhydrous ethanol is denatured by adding gasoline if it is meant to be used as fuel (Maicas, 2020). The biomass (spent yeast) and the effluent, the liquid that remains after the ethanol has been extracted from the beer (stillage), The material can be sent to a treatment facility, digested to produce methane gas, used as animal feed, or refined into other consumables (Lievore et al., 2015).

15.4 Estimated Cost of Producing Biofuel from Whey

Only two large-scale whey-to-ethanol plants exist in the United States; hence there are no publicly accessible production cost statistics. Expenses listed by various sources lack sufficient information and likely represent the most accurate "informed" estimations. But, a June 2005 New Zealand publication makes a current, thorough cost estimate of manufacturing fuel ethanol from whey available to the public (Manochio et al., 2017). According to the New Zealand research, with a level of uncertainty of +/− 20%, the anticipated "at gate" cost (operating and capital service expenses) of manufacturing ethanol from whey permeate was N.Z. $0.6–0.7 per liter. The anticipated price was translated to U.S. $1.60–1.85 per gallon using the exchange rate of N.Z. $1 = U.S. $0.7 (Ling, 2008). The projected cost comprised the following scenario and assumptions, and it took into account economy-of-scale impacts, transportation costs, and competing waste uses:

- Native plants undergo fermentation.
- Distillation of 96% ethanol at native fermentation plants.
- Transportation of 96% ethanol to a central dehydration facility.
- Assuming a mix of debt and equity financing and a nominal interest rate of 10%, the annual capital service cost was equal to 20% of the capital cost.
- The dehydration plant required a minimum daily capacity of 60,000 L of ethanol to be commercially feasible (about 15,850 gallons a day or 5 million gallons a year).
- Biogas from effluent treatment was used as fuel for the distillation and dehydration steps in the recovery of alcohol. (Excess steam from the cogeneration or dairy plants would be beneficial.)
- It was possible to produce ethanol that was 9–10% (by volume) of the fermenting beer using wet feedstock that included at least 15% (by weight) fermentable sugar. The resulting cost of recovering the ethanol could be less than NZ $0.02 (about $0.52 USD) per liter.

The price of manufacturing ethanol from whey permeate, which the New Zealand research assessed to be between $1.60 and $1.85 per gallon with a level of uncertainty of +/− 20%, is comparable to the price ranges given by US sources. These American sources' estimates resulted in an operational cost of around $1 per gallon (Ling, 2008). Moreover, there was a capital service cost that ranged from $0.30 to $0.80 per gallon and was computed at an expected rate of 20% of capital cost. If the estimated rate had been different, the capital service cost would have either been greater or lower. Depending on the size of the plant, capital costs (the price of the construction project) could range commercial use between $1.50 and $4.00 per gallon per year (Battelle Memorial Institute, 2016).

15.5 Roles of Dairy Cooperatives in Whey-Biofuel Plant

If a new whey-ethanol factory is found to be economically viable and were to be built, the business might be structured using these forms:

- A dairy cooperative's massive cheese plant and an ethanol plant next to it, both with configurations comparable to the two current plants (June & Thompson, 2000).
- A dairy cooperative's multiple cheese plants are combined into one ethanol plant that ferments whey permeate (Pasotti et al., 2017).
- A unit that produces ethanol from whey permeates collected from various cheese factories. The coordination of whey handling may take place between the cheese plants of a cooperative and those of other cooperatives, or between the cheese plants of a cooperative and those of other cooperative and non-cooperative businesses. The coordination may be done through a joint venture or by contract (Koushki et al., 2012).
- Small cheese plants may be drawn to such an endeavor if they are seeking for ways to increase the value of their whey (Gould, 1963).

15.6 Challenges in Whey-Biofuel Production

There are some challenges unique to the manufacture of whey-ethanol because of the makeup of whey:

- Whey is particularly prone to contamination and deterioration, as is whey permeates concentrate (Buchanan et al., 2023).
- Transporting whey permeate concentrate is expensive (mostly water).
- Lactic contamination is a risk during the fermentation process. In general, the fermentation systems must be constructed and run with extreme care to provide food-grade cleanliness or, in the case of some systems, even aseptic standards (Mahboubi et al., 2018).

- Scaling of the distillation column could be an issue or a concern because the calcium salts in the whey are "reverses soluble," becoming insoluble at higher temperature.
- The discharge contains a lot of chloride. If land-spreading is used, this restricts the application rate on fields. A significant operating expense may just be the land-spreading of the effluent. Two possibilities exist for higher-value products made from used effluent. If whey permeate concentrate is the substrate, then (1) a basis for a sports drink, and (2) a mineral salt block for animals if permeate mother liquor is the substrate (Bank, 2009).

15.7 Application of Ethanol Produced from Whey

The main end product of fermentation process is ethanol and its derivatives, whose names and industrial applications are shown in Table 15.1.

15.8 Blends Diesel-Biodiesel-Ethanol to Use in Automobiles

Diesel-biodiesel ethanol mixtures require multiple methods to attain optimal results. Ethanol has a typical 95% mass, making mixing difficult in diesel, biodiesel, and ethanol blends; yet, 95% ethanol makes the optimum fuel mixture to yield the best physical and chemical properties. However, some adjustments to the engine may be necessary when utilizing this gasoline blend to accommodate the unique characteristics of ethanol (Battelle Memorial Institute, 2016). Nevertheless, modern compression-ignition (CI) diesel engines have trouble running on a blend of diesel, biodiesel, and ethanol. The stability of the diesel-biodiesel-ethanol combination is just as crucial as the volumetric ratio itself if you want your injection-type engine running on biodiesel mix to work at its best. It is believed that the optimal diesel-to-

Table 15.1 Ethanol derivatives and their industrial applications

Industrial name	Origin of the name	Industrial application
EA95	Ethyl alcohol 95%	Used as solvent in industries
95WS	95% white spirit	Used in food flavoring, food coloring, white vinegar, surgical spirit, medicines
NS	Neutral spirit	In perfumes, cosmetics, high quality deodorants; alcoholic beverages, food flavoring, food coloring
XNS	Extra neutral spirit	Top quality deodorants and perfumes; alcoholic beverages
EA99	Ethyl alcohol 99%	Packaging industries, paint, printing ink, industrial solvent
AA	Anhydrous alcohol	Hospitals applications, pharmaceuticals, aerosols products
HGAA	High grade aerosol alcohol	Aerosols-specifically hail care products, cosmetics, pharmaceuticals

biodiesel ratio varies depending on the engine's configuration and the fuel's performance and emissions. We'll go into more depth about the output and the emissions in the following chapters (Mahboubi et al., 2018). The amount of particulate matter (PM) released into the atmosphere during combustion is used as a performance metric. Use of low sulphur diesel in a biodiesel-ethanol/bioethanol blend can cut particulate matter emissions by about 15%. When 10% ethanol was added to 100% diesel, PM emissions dropped by 30%, leading to enhanced engine performance. In addition, giving a mixture of ethanol and diesel fuel improves the cold stream's (air stream's) characteristics over supplying diesel fuel alone. As a result, the engine generates less heat, which improves its efficiency (Mahboubi et al., 2018). When ethanol is added to diesel, the resulting fuel has a higher density and lower viscosity, two qualities that characterize its usability. Fuel properties such as viscosity, lubricity, energy content, flash point, and calorific value are all altered throughout the mixing process. As there are only a few variables to take into account, including the amount of carbon in the mixture, the amount of hydrogen in the mixture, and the biodegradability of the fuel, these factors must be taken into account during the mixing process (Buchanan et al., 2023). Nonetheless, the qualities of the mixture are emphasized because they are the only way to boost the efficiency of a DI engine. While maintaining the stability of the combination is crucial, it is necessary to investigate it at every scale, from the atomic to the structural. Because the fuel mix's density and viscosity affect combustion, it's helpful to know what they should be for a given DI engine type. In some situations, such as when the engine is running at a greater temperature, the combination remains unstable in comparison to pure diesel (Buchanan et al., 2023).

15.9 Conclusion

If all the lactose in surplus whey and whey permeate, which is whey that is not used in value-added whey-derived products, is fermented for the purpose, the nation's fuel ethanol supply may be augmented by an estimated 203 million gallons annually (according to data from 2006). 65 million gallons of this potential might go to dairy cooperatives. In India, the production of paneer has significantly expanded day-to-day, which has improved whey accessibility. According to estimates, India produces 650,000 tons of paneer per year, along with 3.3 million tons of whey. The expense associated with treating dairy waste to lower the BOD can be reduced by efficiently using the whey for the production of ethanol, moreover, the ethanol that is created can be repurposed as a renewable source of fuel for both vehicles and power plants.

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Chapter 16
Recent Trends in Membrane Processing of Whey

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Abstract Whey is a liquid fraction derived by separating the coagulum from milk, skim milk or cream and it is a valuable by-product of cheese manufacturing. Whey exhibits an excellent nutrient source, as it contains many classes of different-sized components like lactose, salt, residual casein, various soluble proteins, vitamins and minerals. Due to their high nutritional value, whey products like whey powder in form of whey protein isolate (less than 25% protein), whey protein concentrate (more than 90% protein), fractionated whey protein isolates, and whey butter are increasing in demand. Thus, for large-scale production of whey and whey products—membrane processing techniques are used. In comparison with traditional extraction methods like acidification and coagulation, these membrane processing techniques were observed not to destroy the micellular structure but one of the key issues is membrane fouling caused by the absorption of ions and proteins on membranes. To minimise the limitation of conventional membrane processes and traditional extraction processes, recent approaches integrate different membrane process techniques for the recovery of whey. Advanced membrane processing techniques like two-stage nanofiltration and ultrafiltration process, hydrophilic modified UF membranes, hydrophobic membrane distillation, shear enhanced membrane filtration and transverse vibrating membrane filtration with modified membrane tubules developed. The decline in the fouling rate can be observed in shear-enhanced membrane filtration by applying a high shear rate ($>10^5$/s) in two stages—the first adsorption fouling stage and the cake fouling stage. Single NF process with shear-enhanced membrane filtration is preferable for recycling and leads to lower energy

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consumption. The better anti-fouling performance of hydrophilic modified UF membrane made it preferable for recovering whey protein efficiently from dairy effluent. Fractionation of whey protein from a complex mixture using innovative membrane process techniques dual-grading pH-responsive membranes-sequential stepwise pH gradient (3.0–8.0) and membrane adsorption chromatography (macroporous membrane) by coupling anion and cation membranes, both techniques recovered an individual fraction of protein with a purity of 90% or more. A transverse vibrational hollow fibre membrane can increase the transmission rate of whey protein, maintain the structure of the protein at low operating temperatures and improve the performance in fouling reduction. Membrane technology for defatting of whey uses a tubular membrane with a static mixer (vibration) which increases the efficiency of defatting without affecting the whey proteins and flux decline. High mineral content gives raw whey a salty flavour during the preparation of whey powders, while electrodialysis is one of the membrane processes that help in the desalination of whey and it further enables the use of desalted whey powders in various products. Electrodialysis may lead to protein denaturation when it is operated at extremely high temperatures, thus its operating temperature should be around 5–60 °C. Cleaning membrane is one of the effective alternatives to reduce fouling, application of ultrasound at low frequency is reported to have higher cleaning efficiency than the normal application of chemicals like sodium hydro-oxide solution as it weakens the binds between membrane and foulants. This paper provides a detailed overview of advanced membrane processing techniques of whey, whey protein fractionation using membranes, integrated membrane tubules, membrane fouling and polarization reduction techniques, and various challenges and limitation due to membrane processing in whey.

Keywords Membrane processing · Whey · Whey proteins · Fouling · Ultrasounds · Electrodialysis · Microfiltration

16.1 Introduction

Whey is one of the important by-products in the dairy industry with a high nutritional profile. In certain cases, under the absence of sustainable practices, whey having high chemical oxygen demand is considered to cause serious environmental issues due to excess disposal during the manufacturing of cheese (Zandona et al., 2021). Significant loss of nutrients is observed due to the disposal of whey, thus to utilize the nutritional benefits of whey and to minimize the environmental issue caused by whey, the disposed of whey should be reused/processed and converted into valuable dairy products according to the 17 Sustainable Development Goals (SDGs) agenda (Tegarwati, 2019). By using different types of whey in food and other uses, it will boost global competitiveness, promote sustainable economic growth and generate employment (Poonia, 2020). Whey gained more acceptance among food processors, as it imparts beneficial functional properties due to its gelatinization property, water absorption capacity, high solubility and emulsifying capacity (Wen-qiong et al.,

2019). Whey can be processed by biological (aerobic/anaerobic digestion and fermentation to hydrogen) or physical–chemical methods (chitosan and trichloroacetic acid treatment). Both the techniques showed limitations like process instability and low recovery efficiency due to the presence of high organic content in whey (Addai et al., 2020).

Whey recovery and processing were effectively done with the use of membrane techniques like microfiltration, ultrafiltration, nanofiltration and electrodialysis. Thus, whey components in membrane processing are separated based on the size and trace amount of protein present in whey like ά-Lactalbumin (ά-LA), β-Lactoglobulin (β-LG), Bovine Serum Albumin (BSA), Lactoferrin (LF) and Lactoperoxidase (LP) can be recovered at a high rate by fractionation using different membrane modulus (Steinhauer et al., 2015a). Membrane techniques have a reported recovery rate of 50–55% of whey protein (WP) with better functional quality and this method is relatively simple, cost-effective and consumes less energy compared to other methods (Das et al., 2016). Further, to increase the recovery rate, integrated membrane combined with non-thermal techniques like HPP, membrane assisted with enzyme and two-stage assisted method of membrane processing reported to produce a protein permeate yield of around 70–89% and reduced the fouling rate up to 10–15% (Wen-qiong et al., 2017; Rastogi et al., 2022). Figure 16.1 depicts the membrane processing of whey.

One of the major drawbacks of membrane processing is membrane surface fouling and boundary layer (cake) formation. This fouling during whey processing is due to the deposit of lactose, ions, and proteins (BSA and β-LG aggregates) on the membrane (Steinhauer et al., 2015b). To mitigate fouling, membrane cleaning is an important measure, chemicals used for cleaning can decrease the efficiency of the membrane and also generates effluents like organochlorine compounds (Luján-Facundo et al., 2016). To limit the use of chemical cleaning of membranes, non-conventional physical methods are used that include ultrasound application. Ultrasound-integrated membrane processing increases the cleaning efficiency by up to 90% and reduces the accumulation of proteins on the membrane surface at the lowest ultrasound frequencies (20–50 kHz) (Yu et al., 2017).

The chapter aims to investigate advances in different membrane techniques used in the processing of whey and its products. This review also includes the composition and properties of whey, and different techniques (Ion-exchange; Counter diffusion) on modulus like flat sheet modules and tubular modules for feasible recovery of whey components. Whey protein fractionation with the use of membrane techniques, operability parameters of WP recovery and the impact on permeability flow rate and whey desalination with the help of membrane electrodialysis are discussed. Furthermore, membrane fouling reduction strategies and several integrated techniques to tackle boundary layer formation are discussed briefly.

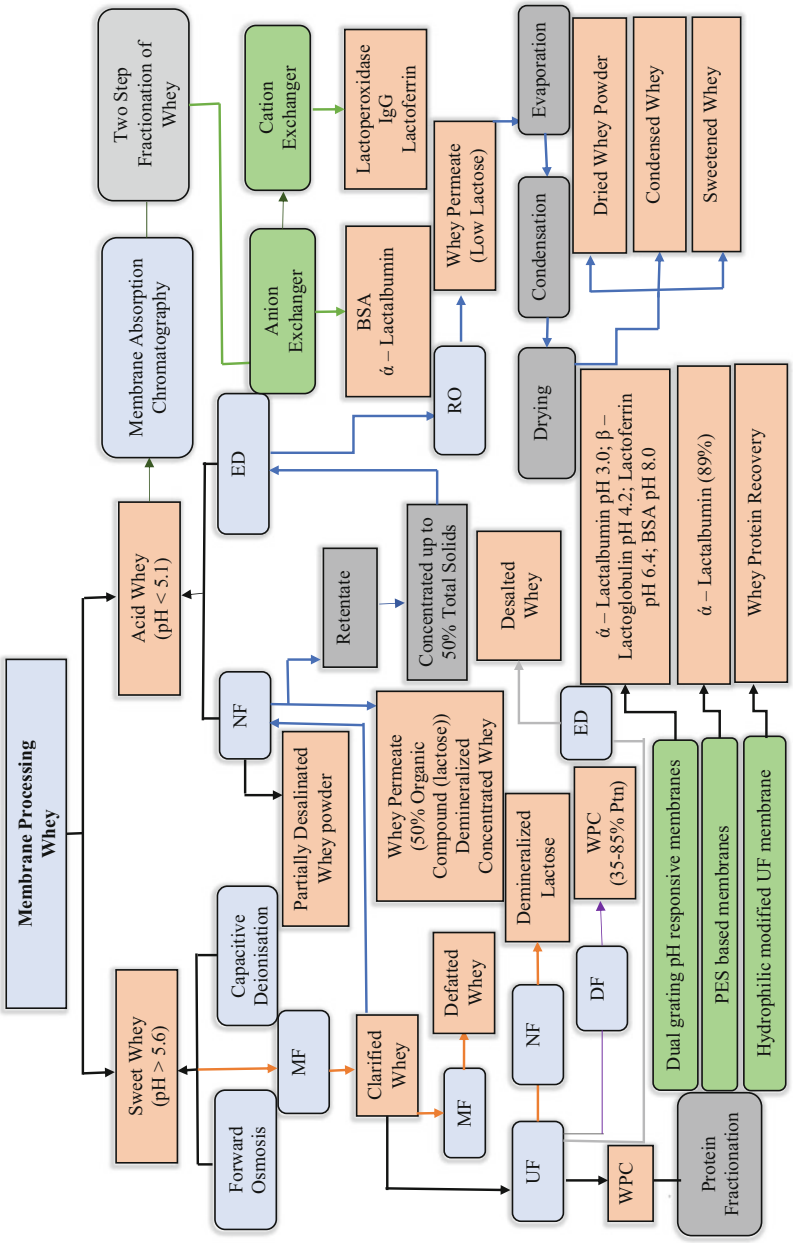

Fig. 16.1 Membrane processing of whey. (Voswinkel & Kulozik, 2011; Hausmann et al., 2013). *BSA* bovine serum albumin, *DF* diafiltration, *ED* electrodialysis, *IgG* immunoglobulin G, *MF* microfiltration, *NF* nano filtration, *PES* polyethersulfone, *RO* reverse osmosis, *UF* ultrafiltration, *WPC* whey protein concentrate

16.2 Whey: Properties & Composition

Whey (yellowish-green liquid fraction) comprised about 50% of total nutrients (lactose, Ca, Na, K, P, soluble proteins (ά-La; β-LG; BSA; LF) and minerals in milk (Westerik et al., 2015). Usually two types of whey: Sweet whey—produced when milk is treated with rennet, which usually ranges in the pH 5.9–6.6 (Sithole et al., 2006) and acid whey—produced by coagulation of casein by the addition of lactic acid, sulphuric acid, hydrochloric acid or with the addition of lactobacilli, it usually ranges in the pH 4.3–4.6 (Chandrapala et al., 2015). Both acid whey and sweet whey comprise 0.3–0.7% proteins including ά-LA (25%), β-LG (50%), BSA (6.5%), IG (15%); 3.3–6.0% lactose; 0.15–1.0% fat; 0.5% ash and few traces of salt content (Aider et al., 2007). WP relatively have low molecular weight; thus, it is soluble in its isoelectric point at a pH 5 especially ά-LA soluble at pH 4.2; β-LG soluble at pH 5.1 and BSA soluble at pH 5.1.

Minerals with cation properties like calcium, magnesium, and potassium and minerals with anion properties like chloride, citrate, and phosphates are present in the whey. These ionic (cation & anion) conditions existing in the streams of liquid whey facilitate changes in the protein properties of whey (Nishanthi et al., 2017b). Whey protein functionality gets changed due to protein conformation (formation of a viscoelastic film, protein network formation) changes due to the interaction of minerals (cations) with WP, interaction occurs by direct attachment to WP binding sites or creating intermolecular salt bridges with WP or by creating electrostatic shielding (Barral et al., 2008).

Calcium (divalent cation) present in whey is reported to have efficient properties on the gelation of the WP system (whey protein polymers). The addition of sodium chloride and calcium chloride at a temperature around 20–37 °C produced WP polymers due to heat polymerization, in certain studies it is reported at a pH of 6.8 (basic condition) polymerization decreased the gelation time (sixfold to tenfold) of whey protein isolate and gel stiffness was more in addition of sodium chloride than the addition of calcium chloride (Caussin et al., 2003).

WP has a significant impact on properties like forming foam and acts as an emulsifier. Polymerization leads to the formation of viscoelastic and cohesive films, due to the presence of disulphide bonds and hydrophobic interactions, this, in turn, helps to obtain optimum and efficient foaming and emulsifying properties and the protein undergoing polymerization must have lower interfacial tension to diffuse to interface that is formed newly and should be easily soluble (Bouaouina et al., 2006).

The acidic condition created in whey streams by the presence of organic acids like citric acid and lactic acids alters the properties of the proteins. Wherein lactic acid leads to the aggregation of WP by hindering hydrophobic interactions that are caused by the occurrence of covalent, electrostatic and Vander Waal forces (Nishanthi et al., 2017a). As WP is soluble at their isoelectric point, below isoelectric point polymerization mediated by disulphide and thiol group that is responsible thiol oxidation is

limited in the WP and above isoelectric point, more reactive sites for polymerization occurs due to denaturation of proteins (Bazaria & Kumar, 2016).

Whey proteins are excellent foaming and emulsifying agents. To obtain optimum foaming and emulsifying characteristics, the protein must be substantially soluble, diffuse to the newly formed interface, unfold and reorient in ways that lower interfacial tension, and form cohesive and viscoelastic films by polymerization mainly via disulfide bonds and hydrophobic interactions (Bazaria & Kumar, 2016; Nishanthi et al., 2017b).

16.3 Different Membrane Techniques and Membrane Tubules

Membrane techniques like counter diffusion, ion exchange and osmotic distillation are considered to be the basic principles behind the functioning of membrane processing techniques Microfiltration, Ultrafiltration, Nanofiltration and Reverse osmosis. Commonly four modules: hollow fibre, ceramic multi-tube, tubular and spiral wound cartridges are used for membrane processing, which, in combination with membrane surface made of Poly Sulfone (PS), Poly Ether Sulfone (PES), Poly Vinyl Alcohol (PVA) are effective in reducing the fouling content and also facilitate permeability rate of whey proteins/products (Rektor & Vatai, 2004).

Counter diffusion helps in demineralization up to 50% and also facilitates a higher removal rate of monovalent up to 70%. Counter diffusion increases the efficiency of ceramic membranes and silica membranes by improving their hydrophobicity drastically (Alsyouri et al., 2006). Seshadri et al. (2010) examined the effect of the counter diffusion self-assembly method for the preparation of silica membrane, as this method provides both sides of the membranes to react/contact with the synthesis solution. The study showed that the CDSA method produced thin film silica membranes with desired pore size and these membranes are of good quality with high hydrophobicity.

Electrodialysis is a method that works on the principle of ion exchange and has a positive impact on the desalination and protein recovery from whey. The ion exchange membrane helps in maintaining the overall electroneutrality, basically, it is a reversible reaction between the target ion/protein and functional group of the membrane i.e., the ion present in solution exchanged with similarly charged ions that are attached to the solid material (immobilized) (Ghosh et al., 2019). The attraction of ions occurred through the process of electrostatic adsorption. These exchange membrane porous matrices are generally made of silica, dextran, polyacrylamides or polystyrenes. Two categories of ion exchange membranes: Cation exchange (Negative charge due to the presence of carboxylic and sulfonic acid) membrane attract positively charged proteins present in the whey like lactoferrin and lactoperoxidase; wherein anion exchange (Positive charge due to the presence of ammonium or

diethylamine) membrane attract negatively charged proteins present in the whey like ά-Lactalbumin, β-Lactoglobulin and BSA (Goodall et al., 2008).

Higher packing density present in tubular-type membrane modules is not commonly used due to excessive boundary layer formation and membrane integrity problems. Popović et al. (2010) examined the effect of ceramic tubular membrane fouling in forward osmosis to recover the whey proteins. The study showed tubule membranes are highly fouled with whey proteins, thus the application of ultrasound at 35 kHz reduced the cake formation and increased the flow rate. Flat sheet membrane has lesser packing density compared to tubular membrane, but are effective in reducing the fouling rate. Flat sheet membrane module comprises plate and frame type and spiral wound membranes (Aydiner et al., 2013). Arunkumar et al. (2016) in their study compared the effect of flat type and spiral wound charged membrane (A-7 m^2) in ultrafiltration, the study revealed charged membrane of spiral wound successfully in increasing the membrane area, reduced the ionic strength and further increased the permeability flow rate in comparison with flat sheet membranes.

16.4 Advances in Membrane Processing of Whey

Apart from the removal of micro-organisms, in recent years membrane processing techniques have been extensively used for whey processing in the dairy industry to develop a broad array of whey protein concentrates/isolates, whey protein fractionation and to particularly enhance the functional properties like removal of lipids, aggregation of lipoproteins, hydrophobicity of whey proteins (ά-lactalbumin and β-lactoglobulin) (Carter et al., 2021). *Different membrane processing techniques used for whey processing with various key points are listed in* Table 16.1. Microfiltration, Ultrafiltration, Nanofiltration and Electrodialysis most commonly used membrane processing techniques with membranes made of polymeric or ceramic and processing parameters of temperature, pressure, pH, and pore size ranging from 4 °C to 50 °C, 5 kPa to 170 kPa, 5 pH to 8 pH and 0.1 μm to 0.5 μm (Coşkun et al., 2023; Salunke et al., 2021). Several studies aimed to develop advances in membrane processing techniques by introducing two-stage NF & UF, attaching non-thermal techniques (HPP, Ultrasound), shear enhanced/vibratory membrane to reduce the fouling rate and thereby increase the efficiency of the whey (Chai et al., 2017; Chai, 2019). *Various trends in membrane processing of whey are shown in* Fig. 16.2.

16.4.1 Microfiltration

Whey protein concentrate/isolate is manufactured mainly with the use of microfiltration. Generally, the pore size of the microfiltration membrane ranges

Table 16.1 Different membrane processing techniques used for whey processing

Membrane processing techniques	Type of membrane	Source	Pore size/ MWCO	Factors influencing selectivity & permeability of membrane			Permeability rate	Total protein obtained %		Dry solid weight ratio %	References
				Duration/ vibration amplitude	Pressure/ EFS/ velocities	Temp		Permeate	Retentate		
Microfiltration	Polymer membrane	Curd whey	0.1 μm and 0.2 μm	100–150 min	0.15–0.2 MPa	13–15 °C	17–19 kg/m^2 h	0.402%	0.499%	6.0–6.1	Babenyshev et al. (2019)
	PVDF spiral wound membrane	Cheddar cheese whey	0.3 μm	60 min	115 kPa	50 °C	56.20 kg/m^2 h	0.324%	1.317%	25.80	Carter et al. (2021b)
	Ceramic membrane	Sweet whey	0.3 μm	6–7 h	25 kPa	50 °C	54.0 kg/m^2 h	0.507%	0.735%	2.01	Carter et al. (2021c)
Ultrafiltration	Dead end filtration	Coconut whey	5 kDa, 50 kDa, 100 kDa, 300 kDa	300 min	2–4 bar	25 °C	14–18 L/m^2 h	0.5–10%—5 kDa, 50 kDa, 100 kDa	86–90%—5 kDa, 50 kDa, 100 kDa	21.43	Vijayasanthi et al. (2019)
Ultrasound assisted ultrafiltration	PES membrane	Paneer whey	5 kDa, 10 kDa	10 h	0.4 bar	60 °C	12–2.4 L/m^2 h	–	20.02% at 10 kDa; 15.09% at 5 kDa	0.39%	Prabhuzantye et al. (2019)
	Hydrosart membrane	Paneer whey	10 kDa	40 min	3 bar	30 °C	4.8–3.4 L/m^2 h	88.6% at 120 W US	–	–	Khaire et al. (2019)
Ultrafiltration followed by nanofiltration	UF: Regenerated cellulose acetate and composite fluoropolymer membrane	Goat cheese whey	10 kDa and 1 kDa		0.2 MPa	16–22 °C	$1.73 \times 10^{-10} \pm 2.83 \times 10^{-11}$ ms^{-1} pa^{-1}	0.53%	–	–	Macedo et al. (2021)
	NF: NFT50 (poli-piperazine) membrane		0.13 kDa		2 MPa		$1.48 \times 10^{-11} \pm 4.56 \times 10^{-13}$ ms^{-1} pa^{-1}	0.50%			

Nanofiltration	NF—99 and DL membrane	Acid whey	<0.8 μm/20 Da	20–30 min	1–55 bar	50 °C	60 L/m^2 h	NF-99: 0.60% DL: 0.80%	–	38.2%	Simonič and Pintarič (2021)
	Poly-pyrazinamide membranes	Whey concentrates	200 Da	–	2.5 MPa	20 °C	–	9.0%	1.13%	20%	Bobrova and Ostretsova (2021)
Electrodialysis with filtration membrane (EDFM)	PES membrane	Whey protein isolate	5 kDa, 10 kDa, 20 kDa, 50 kDa, 100 kDa, 300 kDa/ 23 mm	120 min	2 V/cm	20 °C	–	32.6 at 5 kDa 33.2 at 10 kDa 30.2 at 20 kDa	–	–	Kadel et al. (2019)
Direct contact membrane distillation (DCMD)	Hydrophobic PTFE membranes	Whey	0.5 μm	20 h	10 kPa	54 °C	0.047 m/s	–	20%	2.1%	Hausmann et al. (2013)
	Hydrophobic ceramic membranes	UF whey permeate	5 μm	6 h	0.6 MPa	20 °C	4.23 × 10^{-7} kg/m^2 s pa	50%	–	0.66%	Zmievskii (2015)
Membrane distillation and thermopervaporation	Polyelectrolyte coated PVDF membrane	Whey	0.16 μm	4 days	1 bar	60 °C	15 L/m^2 h—MD 6.5 L/m^2 h—thermopervaporation	15%	–	–	Bell (2016)
Forward osmosis and reverse osmosis hybrid system	Thin film—Porous matrix membrane	Cheese whey	–	90 h	83 bar	–	12.62 L/m^2 h	68%	–	17.9%	Arjmandi et al. (2020)
Shear enhanced membrane filtration (UF and NF)	UF: PES membrane	Diary waste water whey	3 kDa, 7 kDa, 10 kDa, 30 kDa	0.0250, 0.0125	0.6 MPa, 0.8 MPa, 1.0 MPa	–	110 L/m^2 h MPa	27.2%	–	–	Szerencsés et al. (2021)
	NF: Thin film composite		240, 200 Da	0.0250, 0.0125	2.5 MPa, 3.0 MPa, 3.5 MPa		40 L/m^2 h MPa	18.4%			
Submerged membrane vibrational system	PVDF hollow fibre membranes	Skim milk	0.04 μm	10.3 Hz—5 h	50 kPa	–	10 L/m^2 h	71.2%	14%	–	Chai et al. (2017)
				21.8 Hz—10 h	6 kPa		17 L/m^2 h	73.8%			

(continued)

Table 16.1 (continued)

Membrane processing techniques	Type of membrane	Source	Pore size/ MWCO	Factors influencing selectivity & permeability of membrane			Permeability rate	Total protein obtained %		Dry solid weight ratio %	References
				Duration/ vibration amplitude	Pressure/ EFS/ velocities	Temp		Permeate	Retentate		
Rotational vibration hollow fibre membrane system	Ceramic membrane	Bovine milk	0.05–0.2 μm	10.3 Hz, 21.8 Hz	13 kPa/ 4–8 m/s	50–55 °C	15 L/m^2 h	84% (44% ά-La and 40% β-Lg)	–	–	Chai (2019)

MF Microfiltration, *NF* Micro filtration, *PES* Polyether-sulfone, *PTFE* Poly-tetra-fluoroethylene, *PVDF* Polyvinylidene difluoride, *UF* Ultra filtration, *US* Ultrasound

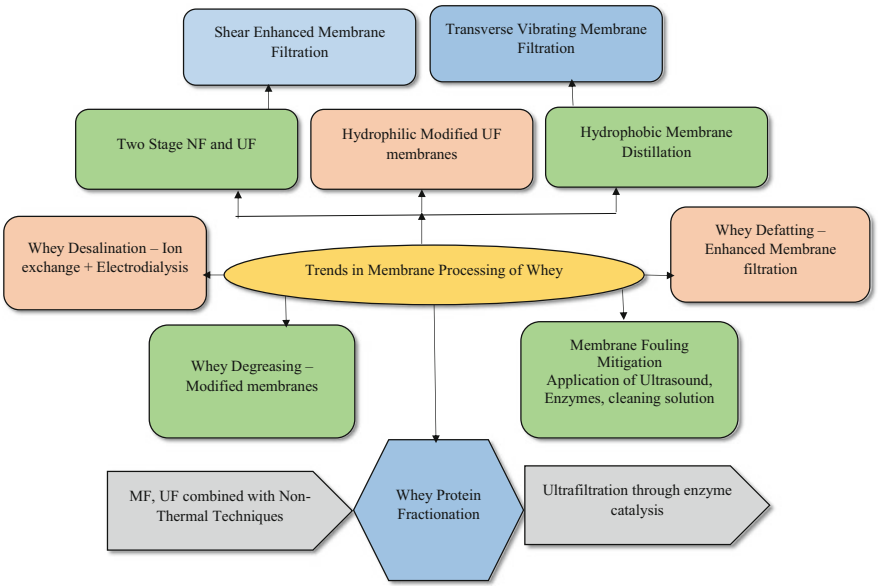

Fig. 16.2 Trends in membrane processing of whey

from 0.2 μm to 2 μm. Transmembrane pressure (TMP) is very low compared to other membrane filtration for high solvent flux and contaminant rejection as its hydrodynamic resistance is low (Anis et al., 2019). A study was conducted by Babenyshev et al. (2019) between cheese whey and curd whey to determine the efficiency of operating parameters of microfiltration. Polymer membrane (0.1 μm and 0.2 μm) used in the study showed a 17–19 kg/m^2 h permeability flux rate and yield of 0.402% protein permeate at particular temperature 13–15 °C and TMP 0.15–0.2 MPa. Carter et al. (2021b) compared polymeric and ceramic spiral wound membranes for the determination of protein removal efficiency at 50 °C from cheddar cheese whey and observed that comparatively 20% whey protein isolate was extracted higher with the use of polymeric spiral wound membrane (0.3 μm) and a permeability rate of 56.20 kg/m^2 h showed a yield of 0.324% total protein permeate and 1.317% protein retentate comprising ά-lactoalbumin and β-lactoglobulin. Ceramic membrane (0.3 μm) was used for the removal of whey protein from the sweet whey using uniform transmembrane pressure of 25 kPa and constant temperature of 50 °C, this membrane reported a yield of 0.507% total protein to permeate at a permeability flux rate of 54.0 kg/m^2 h, the study concluded higher passage rate of protein ά-lactoalbumin and there was no trace for the passage of lactoferrin through the ceramic membrane (Carter et al., 2021c).

16.4.2 Ultrafiltration

Whey protein fractionation is mainly done with the help of ultrafiltration. The pore size of membranes used polyamides, Poly Sulfones (PS), polystyrene, Poly Vinyl Chloride (PVC), polyesters and polycarbonate for ultrafiltration ranges 10^3–10^6 Da (Hampu et al., 2020). Ultrafiltration operates at a low transmembrane pressure that ranges from 0.05 bar to 10 bar (Pinto et al., 2017). A study conducted on coconut whey by Vijayasanthi et al. (2019) to concentrate coconut whey protein using ultrafiltration—dead-end filtration, the study was conducted in four different membrane pore sizes 5 kDa, 50 kDa, 100 kDa, 300 kDa and at constant temperatures of 25 °C. A total of 0.5–10% coconut whey protein permeate was obtained at a pore size of 5 kDa, 50 kDa, and 100 kDa at a permeability flow rate of 14–18 L/m^2 h, and the obtained protein was converted into powder by spray drying (Inlet Air temperature 150 ± 2 °C and Outlet Air temperature 110 ± 2 °C), this spray dried powder was reported to have good flow and sensory properties. To increase the efficiency and stability of whey protein, ultrafiltration was combined with ultrasound, ultrasound-assisted method of ultrafiltration was used to enhance the permeability flux and to reduce the fouling, a study conducted to recover whey protein from paneer was carried out by Prabhuzantye et al. (2019) using ultrasound-assisted ultrafiltration. This study observed that ultrasound increased the permeability flux up to 12–2.4 L/m^2 h for the PES membrane and reduced the fouling rate by 10%. Ultrafiltration using hydrostat membrane at a pressure of 3 Bar and temperature 30 °C along with ultrasound (20 kHz, 120 W) was reported to have a permeability flux of 4.8–3.4 L/m^2 h, a yield of 88.6% total protein permeate and application of ultrasound reported 32% less fouling of the hydrostat membrane (Khaire et al., 2019).

16.4.3 Nanofiltration

The intermediate pressure-driven membrane technology between reverse osmosis and ultrafiltration is Nanotechnology (Román et al., 2009). This membrane processing is highly efficient that mainly helps to retard organic compounds with a molecular weight that ranges from 300 Da to 1000 Da and effectively removes/rejects certain salts (calcium, magnesium, potassium, sodium) (Richards et al., 2011). Nanofiltration is used especially for the demineralization of concentrated whey protein (Pan et al., 2011). A study conducted by Simonič and Pintarič (2021) for the determination of treatment of acid whey using two membranes NF—99 and DL membrane after fractionation of proteins, concluded that at 60 L/m^2 h, permeability flow rate 0.60% and 0.80% total protein permeate consisting fractionation of ά-lactalbumin and β-lactoglobulin increased the fouling rate of NF-99 up to 1.4 times compared to that of DL membrane. Membrane Poly-pyrazinamide with a molecular weight cut-off of 200 Da were used to concentrate whey at a pressure of 2.5 MPa and temperature of 20 °C, and at the mentioned operating parameters, the

whey concentrates 25% was obtained from the milk base and it was reported 9.0% total protein permeate and 1.13% retentate with final dry weight solid ratio 20% (Bobrova & Ostretsova, 2021).

16.4.4 Electrodialysis and Membrane Distillation

Electrodialysis is based on the principle of ion-exchange membranes, and the driving force used is electrochemical potential. Based on ion-exchange membranes the processes like continuous electro-deionization, energy generation and capacitive deionization are carried out in the electrodialysis process (Strathmann, 2010). In electrodialysis, two types of ion-exchange membranes are used—cation-exchange membranes (polymer matrix fixed with negatively charged groups) and anion-exchange membranes (polymer matrix fixed with positively charged groups) (Uliana et al., 2021). Kadel et al. (2019) conducted a study for the determination of whey protein isolate peptide migration selectivity during electrodialysis using a PES membrane with six different Molecular Weight Cut-offs (MWCO) ranging from 5 kDa, 10 kDa, 20 kDa, 50 kDa, 100 kDa, and 300 kDa, the study concluded that at 20 °C temperature and 2 V/cm velocity, the range of total protein permeate was 32.6%, 33.2% and 30.2% particularly at 5 kDa, 10 kDa, 50 kDa MWCO, and all the six PES membrane had a significant impact on the selective separation of whey protein isolate hydrolysate cationic and anionic peptides.

One of the thermally driven separation processes where a microporous hydrophobic membrane is used for the transfer of vapour molecules is known as Membrane Distillation (MD). The driving force used in MD is the vapour pressure difference that is induced across the hydrophobic membrane due to temperature difference (Alkhudhiri et al., 2012). Membrane distillation was beneficially used for the concentration of whey protein above 30%, which is not economically attractive due to the low water activity of the whey and concentration polarization of the membrane used at high temperatures (Christensen et al., 2006). Recent studies aimed to increase the resistance for concentration polarization and to increase the water flux passing through the membrane to make membrane distillation economically attractive. Hausmann et al. (2013) conducted a study using Direct Contact Membrane Distillation (DCMD), Hydrophobic PTFE membranes used for the characterization of milk constituents and their impact on interaction during membrane fouling/concentration polarization. The study reported that to generate high-quality water retention of about 20% and the membrane used didn't wet up to 20 h runtime, so the deposit (fouling on PTFE membrane) due to whey protein has reduced to a lesser extent.

16.4.5 Multi-Stage Membrane Filtration

Multistage membrane processing with ultrafiltration, reverse osmosis, nanofiltration or microfiltration helps to enhance the recovery and purity of whey protein (Noronha, 2002). Multistage membrane processing comprises either two-stage or three-stage membrane filtrations with membranes ranging in different pore sizes selected according to the whey protein (ά-lactalbumin, β-lactoglobulin or lactoferrin) that needs to be filtered. This multistage membrane filtration would be an alternative to reduce the high energy consumption and increase the fouling resistance. Macedo et al. (2021) conducted a study on goat cheese whey to determine the efficiency of the integrated process of nanofiltration and ultrafiltration according to their membrane pore size and permeability flow rate. The study concluded sequential membrane processing i.e., ultrafiltration followed by nanofiltration using regenerated cellulose acetate/composite fluoropolymer membrane (1/10 KDa) and poli-piperazine—NFT50 (0.13 KDa) allowed good separation of protein approximately up to 0.53% and 0.50% at the pressure of 0.2 MPa and 2 MPa. Another study with a hybrid system used forward osmosis and reverse osmosis (F0-FO-RO) to concentrate whey from the diary wastewater, the study also concluded that the utilization of Thin Film Porous Matrix Membrane (TF—PMM) increased the potential concentration of cheese whey and increased the permeability flow rate of 12.62 L/m^2 h that leads to a yield of 68% total protein permeate. According to the study, this multistage membrane filtration posed to achieve profitability by producing higher concentrated whey protein (Arjmandi et al., 2020).

Whey permeates obtained from ultrafiltration, were further passed through Hydrophobic ceramic membranes of direct contact electrodialysis to obtain purified whey protein, the results showed an increased permeability flux 4.23×10^{-7} kg/m^2 s Pa at a constant pressure of 0.6 MPa and reported a yield of 50% of UF whey to permeate (Zmievskii, 2015). To compare the permeability flux of two membranes hydrophobic and polyelectrolyte (negative charge) coated PVDF membrane, membrane distillation followed that thermoper-vaporation was used at transmembrane pressure 1 bar and temperature 60 °C. This comparative study concluded that polyelectrolyte coating can increase high whey permeability and a combination of both membrane techniques solved the problem of fouling by feed components adsorption from whey and then slow pore wetting was observed (Bell, 2016).

16.4.6 Shear/Vibration Enhanced Membrane Processing

Membranes attached with shear-enhanced/dynamic vibration-enhanced filtration tubules have emerged as a promising alternative to reduce membrane fouling, due to shear and vibration developed by developing turbulence in the membrane surface limits the formation of cake and boundary layer (Villafaña-López et al., 2019). On application of pressure (1–10 bar), the pressure vessel attached to the membrane

moves vigorously in rotatory motion, this creates shear waves that are tangent to the direction of membranes (Petala & Zouboulis, 2006). Apart from reducing membrane fouling, shear/vibration-enhanced membranes increase the efficiency of the separation of whey proteins. High shear stress is directly proportional to the reduction of boundary layer formation (removal of solids and foulants) (Zouboulis et al., 2019). Szerencsés et al. (2021) examined shear-enhanced membrane operating parameters (temperature and transmembrane pressure) and vibration parameters in ultrafiltration and nanofiltration, for the determination of permeate flux of whey and its fouling impact on membrane surface; the study concluded vibration due to pressure NF—PES membrane (2.5 MPa, 3.0 MPa, 3.5 MPa) and UF—Thin Film Composite (0.6 MPa, 0.8 MPa, 1.0 MPa) caused higher permeate fluxes at a permeability rate of 110 L/m^2 h MPa and 40 L/m^2 h MPa and the shear force reported to improve casein rejections by allowing acceptable whey protein transmission.

Chai et al. (2017) studied the effect of submerged shear enhanced—PVDF hollow fibre membranes (0.04 μm) at a vibrating amplitude of 10.3 Hz and 21.8 Hz, reported to increase the transmission rate of whey protein at a permeability flux ranging from 10 L/m^2 h to 17 L/m^2 h and the membrane surface also exhibited better performance in terms of fouling/boundary layer formation. Chai (2019) studied the effect of rotational vibration hollow fibre membrane system to increase the permeability flux of the whey from bovine milk, study concluded the increased vibration of the hollow fibre membrane (0.05–0.2 μm; ceramic membrane) reduced the membrane fouling 50% by resisting the casein transmission and it increased the transmission rate of whey at a rate of 15 L/m^2 h at a temperature ranging between 50 °C and 55 °C and pressure ranges 13 kPa, vibration also increased the whey protein fractionation 84% comprising 44% ά-La and 40% β-Lg.

16.5 Whey Protein Fractionation

Various techniques have been adopted to fractionate whey in a more purified form. Salting out, heat/acid separation based on thermal stability/acidic conditions and precipitation in presence of solvents based on selective solubility are some of the conventional techniques used for the fractionation of proteins. The mentioned techniques are considered simple and rapid, but it leads to protein denaturation by affecting the product quality and it leads to a poor yield of protein (El-Sayed & Chase, 2011). Membrane separation and Membrane adsorption chromatographic separation are usually volume-dependent processes, that are very significant to recover a fixed mass of protein in purified form. Membrane separation's adsorbent capacity is directly dependent on the processed volume of the solution not the mass of the protein recovered, whereas chromatographic separation's adsorbent capacity is dependent on the mass of the protein recovered (Saufi & Fee, 2013).

Whey contains a wide array of proteins at low concentrations, these proteins (ά-Lactalbumin, β-Lactoglobulin, Lactoferrin, Lactoperoxidase, Bovine serum albumin) have unique functional, nutritional and various biological properties

(Toro-Sierra et al., 2011). ά-Lactoalbumin rich in high tryptophan content, thus it has nutraceutical properties (Fernández et al., 2011); β-Lactoglobulin reported to act as a better foam stabilizer in the production of confection and BSA was reported to have immunological properties (Arunkumar & Etzel, 2014); Strong antibacterial activity, anti-thrombotic activity and anti-opioid (biologically active peptides) has been reported by protein lactoferrin and lactoperoxidase (Liang et al., 2011).

Kelly (2019) examined the effect of crossflow ultrafiltration for the whey protein fractionation, in this study ceramic polysulfone and PES membrane used for the fractionation reported to facilitate large-scale production of selective whey protein, purity and recovery of the fractionated protein are as follows: ά-Lactalbumin R—89%; P—96%, β-Lactoglobulin R—96%; P—76%, Bovine serum albumin R—60% at pH 9; Lactoferrin R—60% at pH 9. To increase the mass of protein recovered and to avoid fouling the membrane, membranes were combined with ultrasound/High-pressure processing; these integrated techniques helped to increase the efficiency and purity of the recovered proteins. Romo et al. (2023) conducted a study with microfiltration combined with High-Pressure Processing (HPP) in different combinations of pH, transmembrane pressure and temperature to obtain ά-La and β-LG enriched fraction; the study concluded at TMP—1.2 Bar and 600 MPa (HPP); temperature—50 °C and 23 °C (HPP) produced a fractionation of ά-La R—51.43%; P—52.19% and β-LG R—41.25%; P—84.66%.

Whey protein that differs in molecular mass at a factor of 10 can be more efficiently fractionated using ultrafiltration. Mazzei et al. (2021) in their study, used charged cellulose membrane 30 kDa, transmembrane pressure 0.5 bar, cross flow velocities 0.014 m/s and pH 3.4 to increase the yield, efficiency and purity of the fractionated whey protein recovered. The study concluded charged membrane used decreased the boundary layer formation and facilitated the recovery of protein in large mass; further, it reported a fraction of ά-La R—33%; P—95% and β-LG R—27.1%; P—95%.

Wang et al. (2020) examined the operating condition of an electrodialysis filtration membrane, where, the membrane was fabricated using polyvinyl alcohol by using the phase inversion method, particularly to provide size selectivity to fractionate lactoferrin and bovine serum albumin. Filtration membrane washed with glutaraldehyde (crosslinking agent) and catalysed by sulfuric acid decreased the fouling rate and increased the mass of protein recovered. This study reported a fraction of protein recovered at an Electric Field Strength (EFS) 38.5 V/cm and 77 V/cm and Cross Flow Velocities (CFV) 3.2 cm/s are ά-La R—27–34%; β-LG R—34–37; BSA R—50–55%; Lactoferrin R—55–27% and lower ionic strength/partial demineralization prevented the protein loss. The study concluded EDFM is best suited for the separation of whey (eightfold dilution) with lower salt concentration and this separation consumed lower energy; in the case of whey having higher salt concentration can be separated more effectively using electrodialysis ultrafiltration (EDUF) than EDFM.

Recent studies used enzymes (Hydrogen peroxidase, Tyrosinase, Laccase, and Transglutaminase) to increase the whey protein molecular size by catalysing whey protein crosslinking (Saricay et al., 2013); this enzyme catalysis further helps to

reduce boundary layer formation and increase the rate of whey protein recovery and membrane permeability flux during membrane filtration processes. Wen-qiong et al. (2017) investigated the performance of ultrafiltration for the fractionation of protein ά-Lactoalbumin and β-Lactoglobulin from cheese whey under Enzyme (Trans Glutaminase) catalysis conditions. In this study, enzyme TG was added to whey protein to increase the separation efficiency during ultrafiltration, TG catalysis was performed at different operating parameters pH of 5.0, 6.0, 7.0, 8.0; the temperature of 30 °C, 35 °C, 40 °C, 45 °C, 50 °C and catalysis time of 30, 60, 90, 120 min. The study concluded enzyme catalysis at pH 5.0 significantly decreased the cake formation and increased the recovery rate of whey protein by 15–20%. Moreover, enzyme catalysis helped to fractionate recovered whey protein ά-La R − 20% and β-LG R—15%, further TG catalysis reduced the antigenicity of the β-Lactoglobulin.

16.5.1 Membrane Adsorption Chromatography

Membrane adsorption chromatography is directly dependent on the mass of protein recovered, this technique investigated to be the most reliable and fast method for the fractionation of whey protein in highly pure fractions (Voswinkel & Kulozik, 2011) and separation is based on the wide range of isoelectric points (pH) (Voswinkel & Kulozik, 2011). This separation method is more advantageous due to its high selectivity for whey proteins (ά-LA, β-LG, LF, BSA) having similar molecular weight. Two types of membrane adsorption chromatographic techniques were used: Cationic ion exchange membrane and Anionic ion exchange membrane. A study conducted by Fong Shiew et al. (2015) examined the effect of cation exchange membrane chromatography on the fractionation of whey protein (lactoferrin and lactoperoxidase). This study resulted in lactoferrin and lactoperoxidase whey protein fraction in a purity of 88% and 95%. Similarly, Saufi and Fee (2011) investigated the effect of anionic ion exchange membrane on the fractionation and adsorption behaviour of ά-LA, β-LG and BSA, this study resulted in β-Lactoglobulin had a strong affinity towards anion exchange membranes compared to that of ά-Lactalbumin and Bovine Serum Albumin and produced a yield of >99% pure β-LG. Voswinkel and Kulozik (2011) studied the effect of both cationic and anionic exchange membranes for the fractionation and purification of whey proteins under non-denaturing conditions. According to this study, the recovered protein fraction was 90% pure and among ά-LA, β-LG, LF and BSA separation using coupled anion and cation exchange membrane, lactoferrin protein at a pH 4.3 was very less separable due to desorption of higher ionic strength.

16.6 Mitigation to Prevent Membrane Fouling of Whey

Fouling and concentration polarization are considered to be one of the most common inevitable hurdles that limit the permeability flux of the membrane and the performance of the membrane processes to recover the proteins (Lin et al., 2010). Fouling occurs in all types of membrane techniques like microfiltration, nanofiltration, ultrafiltration and reverse osmosis. Fouling of membrane can be alleviated using four common parameters maintaining cross-flow velocities of the boundary layer (Koo et al., 2014); shear inducers/vibration generators in membranes (Li et al., 2014); membranes modified with the addition of tubules (ceramic or polymer) (Issaoui et al., 2017); integrated membrane processes (ultrasound approach) (Thombre et al., 2020). One of the best fouling alleviation techniques is to clean the membrane surface using chemical cleaning agents. When the membranes are applied with cleaning agents (Range 0.5–1.5% solution), it reacts with foulant physically or chemically to weaken the cohesive force between the boundary layer formed and the adhesion between the cake formed and the membrane surface (Zondervan & Roffel, 2007). Apart from the positive reaction of cleaning agents on membranes, it also causes negative impacts like affecting the membrane performance by chelating the membrane surface. Thus, cleaning agents used should be selected according to the membrane type and feed concentration (Rasouli et al., 2019).

Luján-Facundo et al. (2017) investigated the fouling behaviour of Hydrophilic Flat-sheet PES in ultrafiltration using ultrasound-assisted cleaning agents. The study reported application of ultrasound at a frequency of 20 kHz to chemical cleaning agents, increased the separation efficiency of the membrane from 5.47% to 17.23%, and enhanced the protein recovery rate. Also, the application of ultrasound in cleaning agents decreased the fouling rate by loosening the molecules in the membrane and the ultrasound (sonication) generated turbulence in the fluid increasing the movement of the foulants in the membranes. Talebi et al. (2019) studied the effect of electrodialysis using Cation exchange membranes and Anion exchange membranes on fouling behaviour during whey processing. To mitigate the fouling condition, the membrane surface was washed with cleaning solution—HCL and 3% NaCl at particular pH 1.0 ± 0.15 and pH 9.2 ± 0.2, this increased the flowrates, lower energy densities and reduced the mineral deposits on the membrane surface. Compared to acidic and alkaline conditions of the membrane, acidic was more desirable to avoid the precipitation of calcium phosphate and to remove lactate ions. The use of reverse electrodialysis in acidic conditions helps to mitigate protein fouling in the cation and anion exchange membranes.

Azami and Amirinejad (2019) conducted a study by applying ultrasound to a prepared membrane (Comprising Polysulfone (PS), Polyvinylpyrrolidone (PVP), N-methyl pyrrolidone (NMP)) to increase the flow efficiency and to reduce the fouling rate of whey. The study suggested application of ultrasound improved the flux recovery up to 91% at a frequency of 20 kHz, further it was observed that an increase in intensity/frequency simultaneously increases the number of bubbles in

the cavitation area, thus it causes more collapses leading to turbulence in the fluid and this reduces the cake/boundary layer formed on the membrane surface. *Mitigation to Prevent Membrane fouling in whey with operating parameters, methods and key findings has been listed in* Table 16.2.

Rastogi et al. (2022) focused to recover protein by crosslinking membranes with an enzyme (transglutaminase or Enzymes—β-galactosidase or Lignin peroxidase), this enzyme catalysis further reduced the membrane fouling to an extent of 9%. In this study, the mentioned enzymes were encapsulated using calcium alginate beats, thus after treatment with whey, these enzymes can be reused and the enzyme catalysis reaction increased permeability flux at a transmembrane pressure of 1.5 Bar. Madaeni et al. (2020) devoted a study to developing low fouling membrane using self-cleaning nasturtium leaf added to the PES membrane. The study reported the addition of self-cleaning nasturtium leaf used to roughen the smooth surface membrane (Roughness 81.1 nm and 152.4 nm), as a smooth surface is more prone to fouling whereas a rough surface is much more porous, thus it leads to less fouling. This natural self-cleaning without any addition of chemical agents makes the membrane surface more porous and this further increases the permeability flux rate and the lower fouling tendency of the membranes.

16.7 Whey Defatting Using Enhanced Membrane Filtration

Whey defatting is generally done to remove residual lipids, as these lipids that are present in the whey might lead to fouling of the membrane surface (Faucher et al., 2020). Lipids are of two types occurring in whey: non-polar lipids that consist of triglycerides and polar lipids that mainly consist of phospholipids (Damodaran, 2011). A common method for the removal of lipids is centrifugation, but some residual whey is not completely centrifuged due to the presence of a negative charge that usually stabilizes by causing electrostatic repulsion in the whey (Dufton et al., 2019). Thus, to remove residual lipids from the whey, methods like thermocalcic precipitation, zinc-induced precipitation and chitosan precipitation have been commonly used, but these methods are used only on a lab scale not on a large industrial scale due to difficulty in temperature and pH parameters setting up (Damodaran, 2010). Defatting whey (removal of residual lipids) using membrane technology (Electrodialysis, Nanofiltration, Reverse osmosis & Microfiltration) is considered eco-friendly (without the addition of any chemicals). In the case of whey electrodialysis, it creates the formation of complex lipoproteins through a reduction in ionic strength and pH (Kareb et al., 2017). Moreover, these complexes can be easily centrifuged, which are found in the precipitates. *Whey protein fractionation using membrane technology with various operating parameters has been discussed in* Table 16.3.

Faucher et al. (2020) examined the defatting rate of whey using electrodialysis with bipolar membranes to determine the composition of precipitates. The study concluded, the application of electrolysis with bipolar membrane to the sweet whey

Table 16.2 Whey protein fractionation using membrane technology

Membrane techniques	Type of membrane	Operating parameters	Whey protein fractionation (recovery & purity %)				References
			ά—lactalbumin	β—lactoglobulin	BSA	lactoferrin	
Ultra-filtration	Ceramic PS and PES membrane	MWCO—20 kDa Pore size—0.5 µm Temp—20 °C	R—89% P—96%	R—96% P—76%	P—60% pH—9	P—60% pH—9	Kelly (2019)
Microfiltration combined with HPP	Ceramic membrane	Pore size—1.4 µm Temp.—50 °C TMP—1.2 Bar	R—51.43% P—52.19%	R—41.25% P—84.66%	–	–	Romo et al. (2023)
		HPP Pr.—600 MPa Temp.—23 °C					
Charged ultrafiltration	Cellulose membrane	Charge—30 kDa TMP—0.5 bar CFV—0.014 m/s pH -3.4	R—33% P—95%	R—27.1% P—95%	–	–	Mazzei et al. (2021)
Electrodialysis	PVA membrane	EFS—38.5 and 77 V/cm Temp.—25 °C CFV—3.2 cm/s	R—27–34%	R—34–37%	R—50–55%	R—55–27%	Wang et al. (2020)
Ultrafiltration through enzyme catalysis	PES membrane	MWCO—10 kDa TMP—0.15 MPa	R—20%	R—15%	–	–	Wen-qiong et al. (2017)
	Enzyme—TG	TG pH—5.0, 6.0, 7.0, 8.0 Temp.—30 °C, 35 °C, 40 °C, 45 °C, 50 °C T—30 min, 60 min, 90 min, 120 min					

CFV Cross-flow velocities, *ESF* Electric field strength, *HPP* High pressure processing, *MWCO* Molecular weight Cut Off, *P* Purity, *PES* Polyether sulfone, *Pr.* Pressure, *PS* Polysulfone, *PVA* Poly vinyl alcohol, *R* Recovery, *T* Time, *TG* Trans glutaminase, *TMP* Transmembrane pressure

Table 16.3 Mitigation to prevent membrane fouling in whey

Membrane technique	Type of membrane	Product	Operating parameters of Membranes	Methods used to mitigate fouling	Fouling mitigation parameters	Findings	References
Ultrafiltration	Hydrophilic flat-sheet PES	Cheese whey	TMP—2 Bar Temp.—25 °C Velocity—2 m/s Time—2 h	UF membrane equipped with ultrasound. US applied to chemical cleaning solution	Power—4000 W Frequency—20 kHz EC—4 kW.h	Application of US, improved the cleaning efficiency of membrane—5.47% to 17.23%	Luján-Facundo et al. (2017)
Electrodialysis	Cation exchange membranes and anion exchange membranes	Acid whey and skimmed sweet whey	Voltage—7 V Current—166 A/m^2, 305 A/m^2, 444 A/m^2 Temp.—45 °C	Cleaning solution—HCL and 3% NaCl at particular pH	HCL—pH 1.0 ± 0.15 NaCl—pH 9.2 ± 0.2	Effective removing mineral deposits and strongly bound protein deposits in membranes	Talebi et al. (2019)
Ultrafiltration	Polysulfone (PS), polyvinylpyrrolidone (PVP), N-methylpyrrolidone (NMP)	Whey	Film prepared using PS, PVP and NMP (1 wt %). Temp.—15 °C TMP—1–6 Bar	Ultrasonic irradiation to prepared film	Power—80–400 W Frequency—20 kHz Time—60 min	Ultrasound effect for membrane cleaning reported to produce the flux recoveries83–91% for membranes	Azami and Amirinejad (2019)
Ultrafiltration	PES membrane	Whey protein	MWCO—10 kDa TMP—1.5 Bar	Enzymes in free & immobilized form as crosslinking agents	Enzymes—β-galactosidase Lignin peroxidase Immobilizing agents—calcium alginate beats Cleaning enzyme membrane—phosphate buffer pH 5.0	Fouling of membranes reduced to a minimum of 9% protein rejection increased to a highest of 85%	Rastogi et al. (2022)

(continued)

Table 16.3 (continued)

Membrane technique	Type of membrane	Product	Operating parameters of Membranes	Methods used to mitigate fouling	Fouling mitigation parameters	Findings	References
Reverse osmosis	PES membrane moulds	Whey	Roughness of membranes—81.1 nm and 152.4 nm Heating temp.—130 °C and 150 °C	Self-cleaning natural leaf—Tropaeolum majus (nasturtium) added to PES membrane	Roughness of membranes—81.1 and 152.4 nm Heating temp.—130 °C and 150 °C	PES membrane with tropaeolum majus exhibited PWF and flux recovery	Madaeni et al. (2020)

MWCO Molecular weight cut—off, *PES* Poly ether sulfone, *PWF* Pure water flux, *TMP* Transmembrane pressure, *UF* Ultra filtration, *US* Ultrasound

increased the concentration of lipoprotein complexes, thus it was able to differentiate the composition of two proteins ά-LA and β-LG and further dilution of 6 × increased the defatting of whey and favorize precipitation of protein. Faucher et al. (2022) studied the recovery of phospholipids (polar lipids) using electrodialysis to defeat the whey. The study resulted in preferential migration of monovalent cations and divalent cations, this further caused a drastic decrease in the ionic strength of the sweet whey, reduction in ionic strength increases the precipitation of protein and leads to increased lipoprotein complexes and 80% recovery of phospholipids, increase in lipoprotein complex favoured higher defatting rate of whey. The study concluded that electrodialysis is considered to be one of the eco-friendly techniques (without the addition of any chemicals) to produce whey protein concentrate (Defatted) and to maintain the functional properties of the treated whey.

16.8 Whey Desalination: Electrodialysis

Desalination of whey and catalytic processes are generally carried out by ceramic or polymeric membranes. These membranes contain certain layers with varying pore sizes and mechanical durability. The roughness of the membrane is decreased by intermediate layers that contain small pores and these pores determine the functional properties of the membrane surface (Dzyazko et al., 2019). These membranes are limited for desalination due to membrane fouling that causes mineral scaling, which further limits the reduction of ionic conductivity of the whey (Merkel et al., 2021). In electrodialysis based on the principle of electrical potential difference, two ion exchange membranes are used: Cation Exchange Membranes (CEM) and Anion Exchange Membranes (AEM). The choice of CEM and AEM is considered to be the most important parameter for increasing the efficiency of desalination (Merkel et al., 2018). These membranes (CEM and AEM) cause the transfer of ions from permeate to the concentrate of the whey that contains charged functional groups, these reactions occur due to electric potential difference. The advantageous part of the desalination of whey using electrodialysis is the inhibition of protein denaturation and loss of lactose content in the permeate, as these proteins and lactose are considered to be the most valuable biological substances present in the whey (Myronchuk et al., 2018).

Dzyazko et al., 2019examined the effect of using an anion exchange membrane in electrodialysis for the desalination of nano-filtered milky whey. The study resulted, in an increase in desalination of 70% at a current density applied at a rate of 625 A/m of the nano-filtered whey permeate and anionic exchange membrane decreased the ionic conductivity up to 35% and this in turn reduced the membrane fouling. Merkel et al. (2018) studied the effect of temperature on the desalination of whey, usually, whey desalination in electrodialysis is carried out at a temperature ranging from 10 °C to 20 °C. Studies have reported that desalination carried at 10–20 °C is very slow, thus, a higher temperature was preferred to increase the desalination rate. In this study, it was concluded temperature above 5 °C and below 60 °C had facilitated

higher efficiency of desalination at a faster rate and higher temperature causes higher dissociation constants that lead to a decrease in the acid contents and also leads to better whey protein concentrates.

16.9 Future Perception

Whey contains multiple kinds of protein with varying molecular weights and functional properties. During membrane processing, protein interaction becomes complex and thus, complexity makes it difficult to separate (Jambrak et al., 2014). New technologies in combination with existing membrane techniques should be developed for a better understanding of the interaction of complex proteins and to improve the performance of whey processing using membranes.

The most commonly used membrane processing technique for whey protein recovery is ultrafiltration. The efficiency of ultrafiltration should be increased to reduce the cost and increase the cleaning of the membrane when used on a large scale, efficiency can be increased by developing new membrane materials, membrane configurations and new membrane modulus based on the type of whey protein fractionation and the fouling behaviour (Corbatón-Báguena et al., 2015).

Developing highly hydrophilic membranes have been reported to have anti-protein fouling, these membranes have a low binding affinity towards macromolecules that are hydrophobic (Macedo et al., 2015). Membrane techniques used for wastewater treatment can be modified according to the processing of whey like membrane bioreactor used for wastewater treatment can be used for whey processing as it helps to produce aggregated protein in large mass and functional peptides of proteins.

To reduce the fouling, feed pre-treatment can be used i.e., increasing the ionic strength, adjusting pH, the addition of metal iron and increasing the protein interactions. These pre-treatments not only reduce the membrane fouling but also increase the efficiency of the permeability flow rate by up to 50% (Chen et al., 2020; Seker et al., 2017).

16.10 Conclusion

This chapter provides an overview of recent studies that focused on membrane materials, their impact on the recovery of whey proteins, operational parameters used for processes, fractionation, desalination and demineralization of whey proteins. Recently, researchers studied integrated membrane processing techniques that increased the separation efficiency according to the pore size, increased the permeability flow rate and increased the quality/mass of the proteins (ά-LA; β-LG; LF; LP; BSA) separated. The overall increase in the recovery of whey protein up to 70–89% was reported in a two-stage membrane separation process and a combination of

two/three membrane techniques. Membrane made of Poly Ether Sulfone (PES) increased the permeability flow rate by 12–2.4 L/m^2 per h compared to that of other membranes. Efforts to control membrane fouling have increased to mitigate the problems in membrane filtration. Ultrasound-assisted method of membrane processing at low frequencies is the best alternative to chemical cleaning agents reducing the fouling rate of the membrane surfaces to a higher rate. Enzyme treatment (TG) to the feed also increased the whey permeability rate and reduced the fouling rate caused by the deposition of proteins. Still, membrane fouling is one of the major limitations that affect membrane processing by reducing process efficiency. Thus, consistent effort in the development of membranes made of material with superior characteristics may further enhance the fractionation of proteins, reduce the fouling rate and increase the recovery rate of whey.

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Chapter 17
Valorization of Whey in Manufacturing of Functional Beverages: A Dairy Industry Perspective

Vatsala Sharma, Ashmita Singh, and Monika Thakur

Abstract Whey is a by-product of the dairy industry that is frequently disregarded, yet it is actually a rich source of nutrients like lactose, minerals, and bioactive peptides. Its potential in the food business is, however, frequently underappreciated. Fortunately, whey protein beverages offer a number of benefits, including high nutritional value, a mild flavour, simple digestion, and distinctive functional qualities. Whey and its components have been increasingly used to make novel and creative whey-based drinks in recent years. The use of whey as a functional and dietary component in the creation of a wide range of beverages, including some with nutraceutical qualities, is explored in this chapter. As the market for dairy and functional foods continues to evolve, it is likely that whey-based beverages made from fruit juices, milk or milk permeates, and other sources will become increasingly popular.

Keywords Whey · Carbonation · Functional beverages · Valorization · Dairy industry

17.1 Introduction

From time immemorial, milk has been recognized as a highly nutritious food. It is considered to be a protective food with many beneficial qualities, making it an ideal choice for those who can afford it. Cow and buffalo milk contain approximately 3.2% and 4.3% protein respectively, in addition to other protective elements such as vitamins and minerals. While many countries in the Far East and Southeast Asia have limited access to milk or milk products in their diets, in India, milk is a preferred food and holds a special place in traditional diets. Due to the advent of new technology, the dairy industry has experienced significant expansion in recent years (Lejaniya et al., 2021). The demand for milk processing, a vital global business

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(Slavov, 2017), has led to an increase in waste generation from operations like whey disposal, dairy sludge, and wastewater. High quantities of nutrients, biological and chemical oxygen demands (BOD/COD), organic and inorganic compounds, and possibly even sterilants, acidic, and alkaline detergents, can all be found in these waste products. The negative impacts of the dairy industry's pollution on the quality of the air, land, and water highlight the need for techniques for the treatment and reuse of industrial dairy waste (Ahmad et al., 2019).

In all the steps for processing of the products (Mirabella et al., 2014):

- Desired end product (10–12%)
- Whey (80–90%)

In this era we all should have the sustainable approach for valorization of the food products, therefore, to encourage the sustainable extraction, there is need for development ways for whey sustainable utilization. The overall management of whey waste from milk processing industries is important and required. The present review extracts the useful information for policymakers and the R&D for valorization of whey in the development of functional beverages with sustainable approaches.

17.2 Whey: A Nutritious Dairy Waste

Whey is appropriately known as a nutritious dairy waste (Fig. 17.1). It has all the required nutritionally & functionally important components as:

17.3 Composition and Properties of Whey

Whey is a by-product of the dairy industry that is produced during the casein and cheese making processes. The liquid that remains after casein proteins in milk are curdled by chymosin (rennet) or a mineral or organic acid is referred to as whey. Depending on the type and quality of the milk, this liquid's normal consistency is thin and watery with a faint yellow/green or bluish colour (Smithers, 2008). Whey

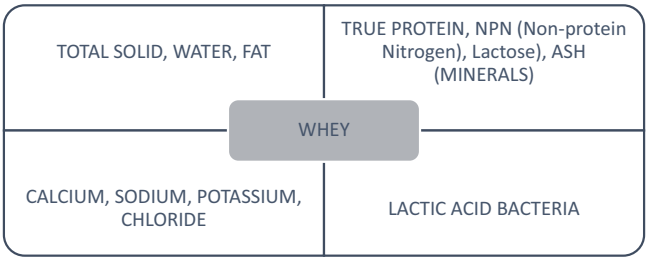

Fig. 17.1 Different components of whey

Table 17.1 Composition of sweet and acid whey

Type of whey (g/L)	Total solids	Lactose	Proteins	Calcium	Phosphates	Lactate	Chlorides
Acid	63.0–70.0	44.0–46.0	6.0–8.0	6.0–8.0	2.0–4.5	6.4	1.1
Sweet	63.0–70.0	46.0–52.0	6.0–10.0	0.4–0.6	1.0–3.0	2.0	1.1

Source: Božanić et al., (2014)

was historically frequently dumped in rivers and agricultural grounds, but researchers recognised its useful potential in the middle of the twentieth century. The majority of the biological elements unique to whey in milk are found there. 100 L of milk require the production of 80–90 L of whey (Tsakali et al., 2010). From an industrial point of view, there are two types of whey:

- Sweet whey: it is produced as a by-product during the making of most cheeses or rennet casein, usually has a minimum pH of 5.6 and contains lower levels of ash and higher amounts of protein compared to acid whey. This yellow liquid makes up about 80–90% of milk's volume fraction and consists mostly of water-soluble milk constituents (Tsermoula et al., 2021).
- Acid whey: it is a valuable dairy by-product of acid casein manufacture, usually has maximum pH of 5.1that contains mostly functional proteins and peptides, lactose, lipids, vitamins, and minerals (Mulvihill & Donovan, 1987). Acid whey has been utilized in the production of drinks such as vinegar beverages, functional beverages and bio-packaging (Rocha-Mendoza et al., 2021).

The components of milk, such as serum proteins, lactose, minerals, and vitamins, make up around 50% of whey. While acid whey contains more calcium, phosphorus, and lactic acid than sweet whey, sweet whey has higher quantities of total solids, fat, and protein. The main ingredient in both forms of whey is lactose, which makes up approximately 44–52% of their total solids. Whey proteins make up 6–10% of their total solids, and minerals make up 12–15%. Mineral content, acidity, and the makeup of the whey protein fraction serve as the main distinguishing factors between acid and sweet whey (Macwan et al., 2016). An overview of the sweet and acid whey composition is given in Table 17.1.

While the amounts of protein in sweet and acid whey are very similar, the amount of free amino acids can differ depending on how much casein is hydrolyzed when making cheese. When compared to milk, sweet whey has up to 10 times as much free amino acids than acid whey. Whey proteins are renowned for having superior functional qualities and being more easily digestible than casein. They are added to a number of foods to enhance their nutritional value, including infant formulae and dairy-based goods. Immunoglobulins, glycoproteins, and enzymes with antibacterial capabilities are also present in whey and may aid to lessen allergic reactions. In order to maximise whey use, researchers have been concentrating on whey protein powders. Because whey proteins are present, concentrated whey powders have great nutritional and functional qualities. The source and conditions of processing can, however, affect how these powders behave. The impact of

processing techniques like heating, acidification, or salting, as well as the inclusion of other components like caseins, minerals, and organic acids, can influence the surface and structural characteristics of whey proteins.

The main properties of whey include:

1. *High protein content:* Whey is recognized for its significant protein content, which typically ranges from 20% to 25% by weight. This protein source is considered a high-quality one, as it comprises all the essential amino acids required by the body.
2. *Low in fat and lactose:* Whey protein generally has low levels of fat and lactose, making it a favourable option for people who are lactose intolerant or aiming to reduce their fat intake.
3. *Mineral-rich:* Whey is an abundant source of essential minerals such as calcium, potassium, and phosphorus, which are fundamental for the proper functioning of the body.
4. *Easily digestible:* Whey protein is easily digestible and absorbed by the body, making it an ideal option for people who require a rapid source of protein.
5. *Immune-boosting properties:* Whey protein contains immunoglobulins, which are antibodies that can boost the immune system.
6. *Muscle-building properties:* Whey protein is frequently used by athletes and bodybuilders to enhance muscle growth and improve overall performance.
7. *Appetite suppression:* Scientific studies have found that whey protein can aid in suppressing appetite and reducing calorie intake, making it helpful for weight loss efforts.
8. *Antioxidant properties:* Whey protein comprises antioxidants such as glutathione, which can help to safeguard against oxidative stress and promote overall health.

17.4 Utilization of Whey

Approximately 50% of whey production in the United States is processed into whey protein concentrate, lactose, and other products for human food and animal feed, while the rest is disposed of through waste treatment, livestock feed, or fertilizer use on agricultural land, particularly from smaller cheese manufacturing facilities. Similarly, in Europe, only about half of the whey produced is utilized, primarily as whey powder. The economics of whey processing are unfavorable, particularly for smaller operations, resulting in limited processing due to lower demand for whey powder and processed products. Despite efforts to improve the utilization of whey powder, lactose, and other products, manufacturing processes remain economically feasible only for larger-scale operations with a steady supply of high-quality whey and sufficient demand for resulting products (Hoogstraten, 1987).

According to Delaney (1981), the main benefit of digesting whey is to minimise the high costs involved with disposing of it through municipal or plant waste treatment facilities. Whey blends are mostly used in animal feed products, whereas whey powder, whey concentrate, reduced lactose/reduced mineral whey, whey

protein concentrate, and lactose are frequently employed in human food product applications. Blends made from whey are designed to resemble non-fat dry milk in composition. Dairy and bread goods employ the most whey concentrate in human food applications, although large amounts are also used in prepared mix, confectionery, and blend applications. According to Jelen and Le Maguer (1976), the majority of acid whey used in human food applications is created as dried and modified whey.

17.5 Whey Valorization

Researchers are drawing attention to the potential for valorization of whey waste, which is being managed sustainably. Mazaheri Assadi et al. (2008), Carlini et al. (2015), Macwan et al. (2016), Panghal et al. (2017), Yadav et al. (2014), and Tostivint et al. (2017) are just a few research that have discussed the advantages of whey valorization. In order to achieve its sustainable development goals, the dairy sector waste has received special attention from governments and organisations around the world (United Nations, 2015). Sustainability in terms of the environment, the economy, and society are being investigated when it comes to whey valorization using green extraction technology. Whey valorization is more advantageous and cleaner than other approaches of managing food waste.

17.5.1 Whey Based Beverages

Whey-based beverages first came into being in the early 1970s, and ever since then, a wide range of them have been developed and sold on the market. These drinks can be produced with sweet or acid whey, de-proteinized whey, or whey powder along with a variety of ingredients. Whey makes up the majority of the liquid component of these drinks. Fruits, cereal crops and their products, different vegetable protein isolates, CO_2, chocolate and cocoa, vanilla extracts, and aromatizing chemicals are a few examples of these additives.

The high water content of fresh whey, which renders it susceptible to microbial deterioration, is just one of the difficulties involved in producing whey beverages. Usually, heat treatments are needed to keep food from spoiling, but because whey proteins are heat sensitive, they can precipitate out at temperatures higher than 60 °C. Researchers are looking into alternate procedures like microfiltration and ultrasound to get around this problem. Whey proteins can be made heat-resistant by acidifying it to a pH below 3.9, which can also prevent them from precipitating during UHT sterilisation procedures. Particularly in acid whey with higher lactic acid concentration and better solubility, the minerals contained in whey's dry matter might result in an unfavourable salty-sour taste and increased acidity in the finished product. Despite these difficulties, the most economical method of processing fresh whey is

still being developed by researchers, and fruit concentrates are being added to whey drinks to improve flavour.

17.5.2 Classification of Whey Based Beverages

The Whey based beverages are broadly classified into two categories: Fermented and Non fermented. The non-fermented whey-based beverages have been categorized as mentioned in (Table 17.2).

17.5.2.1 Fermented Whey Beverages

Traditional methods for food preservation include fermentation, which also enhances the nutritional value and flavour of dairy by-products. While yeast species like *Kluyveromyces* are utilised for alcoholic fermentation, LAB cultures are frequently used as starter or probiotic cultures in the case of whey fermentation. Due to its high lactose content, whey is an excellent growth medium for LAB cultures. According to the starting cultures utilised in the process, there are two types of whey fermentation, as depicted in (Fig. 17.2).

Lot of research has been conducted on the formulations of Whey beverages under these two categories as alcoholic and non-alcoholic beverages mentioned in (Table 17.3).

Table 17.2 Non-fermented whey based beverages

Type of whey beverage	Description
Plain whey beverage	Includes the mixing of whole whey along with sugar flavour (either natural or synthetic and colour. These may or may not be carbonated
Whey based fruit beverage	Whey based natural fruit juice/ pulp drink concentrated fruit based whey beverages fruit flavoured drinks
De-proteinated whey beverage	Whey permeate, a by-product of ultrafiltration used to prepare whey protein, has the potential to be a superior raw material for the production of a variety of carbonated and non-carbonated beverages. Because they contain a lot more electrolytes compared to conventional sports drinks, beverages made with whey permeate are a great option
Whey protein enriched whey based beverages	Whey protein can be added to whey-based beverages to increase their protein content. This is accomplished by combining hydrolyzed lactose, pineapple, orange, and passion fruit juices with premium whey protein
Whey based soups	Whey-based soup powders can be made in a number of ways, including by boiling vegetables in concentrated whey, mixing veggies in whey, frying seasonings, and then spray drying them

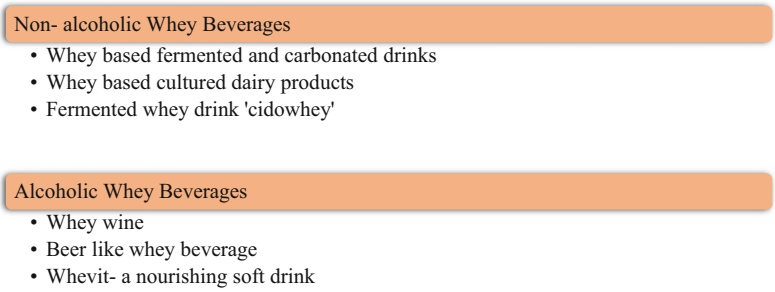

Fig. 17.2 Fermented whey beverages

Whey is a fascinating product because of its components. The properties, functions, and chemical structure of whey make it an excellent foundation for developing a range of new products or as an alternative compound to more traditional ones. There are numerous possibilities for utilizing whey, rather than disposing of it as waste.

17.5.2.2 Carbonated Beverages

The soft drink industry is dominated by Carbonated Beverages (CBs), which have exhibited remarkable growth potential globally. The market for CBs is expected to exceed US $410 billion by 2023, with an annual compound growth rate of 2.8%, encompassing soft drinks, energy drinks, and other beverages. Success factors include the olfactory experience of CBs, branding, and marketing strategies. Recently, carbonated beverages (CBs) have dominated the soft drink market and have showed great development potential on a worldwide level. By 2023, the market for CBs, which includes soft drinks, energy drinks, and other beverages, is expected to reach more than US $410 billion, with an annual compound growth rate of 2.8%. The olfactory experience, marketing tactics, and branding are few examples of the variables that contribute to CBs' success. Companies have created new flavours that prioritise consumer well-being in response to the industry's changing difficulties and consumer health concerns. Abu-Reidah (2020) offers additional information on this pattern.

Companies have begun to provide new flavours in carbonated beverages that address consumers' worries about their health and well-being in order to satisfy the industry's increasing difficulties. From sparkling water to soda pop, cola, and beer, carbonated beverages are a common component of contemporary cuisine. They have distinctive qualities including carbonation, high sugar content or artificial sweeteners, and acidity. When a bottle is opened, the effervescence and bubbles are the result of the carbonation process, which involves the dissolving of CO_2 gas in liquid under pressure. Due to the conversion of CO_2 with HCO_3 and H+ ions as well as additions like citric acid and phosphoric acid, colas, sodas, and beers have a pH level

Table 17.3 Research trials for the production of whey-based beverages

Research trials for the production of whey-based beverages	References
Alcoholic beverages	
By fermenting lactose using yeast strains like *Kluyveromyces fragilis* and *saccharomyces lactis*, beverages with a low alcohol content can be made, with an alcohol level of 0.5–1%	Sienkiewicz and Riedel (1990)
The product "Milone" is made by fermenting whey with kefir culture, and polish sparkling wine "Serwovit" is produced there	Macwan et al. (2016)
Alcoholic drinks can be made with whey as the primary ingredient (up to 70%), such as whey beer and whey wine. Malt can be used in the production of whey beer, and it can also be supplemented with minerals, vitamins, and starch hydrolyzates. Clearing, de-proteinization, and lactose hydrolysis are frequently performed during the production process	Palmer and Marquardt (1978)
Self-carbonated probiotic whey beverage (*Kluveromyces marxianus* and *lactobacillus helveticus* cultures) + 7% sugar (incubation at 25 °C until the acidity reached 0.5%) the final product is stored at refrigeration temperature to carbonate	Patel (2012)
Whey beverage involved adding sugar (6–13%) + citric acid (0.02–-0.4%) + flavouring. There is in-bottle pasteurization with or without carbonation	Jayaprakasha et al. (1986)
Deproteinized cheddar cheese whey was used to make a pleasant whey beverage. It was also flavor-boosted with 8% lemon juice and 14% sugar. The shelf life of this beverage is 15 days when kept at room temperature, which is between 18 °C and 25 °C	Reddy et al. (1987)
Production of fruit-based beverages by adding different fruit juices	Gagrani et al. (1987)
The best method for producing fruit drinks with a hazy appearance is said to be blending fruit juices with acid whey	Tuohy et al. (1988)
De-proteinized and clarified cheddar cheese whey, 10% sugar, 0.4% citric acid, and your choice of orange, pineapple, mango, or raspberry flavourings were used to create the drink	Jayaprakasha (1992)
A beverage produced from buffalo milk, cow skim milk, and cultures of lactic acid bacteria (LAB)	Macedo et al. (1998)
Ready-to-serve kinnow juice beverage using cheddar cheese whey	Khamrui and Rajorhia (1998)
Whey was combined with ferrous bisglycinate and strawberry concentrate to produce a beverage that decreased the prevalence of anaemia in children and teenagers. The mixture has also been supplemented with a range of fruit concentrates, including those rich in iron and antioxidants including apple, pear, peach, apricot, cherry, and berries	Miglioranza et al. (2003)
Whey fruit juice blends on beverage, added blends of orange /pear/ peach/, apple + pH to 3.5 using citric acid	Duric et al. (2004)
Beverage using lactose-hydrolyzed permeate + mango pulp	Deosarkar (2004)
Beverage added yogurt cultures	Gallardo-Escamilla et al. (2005)
A drink that has undergone fermentation utilising a combination of probiotic microorganisms, specifically *lactobacillus acidophilus* La-5, *Bifidobacterium bifidum* Bb-12, and *lactobacillus casei* Lc-1	Drgalic et al. (2005)

(continued)

Table 17.3 (continued)

Research trials for the production of whey-based beverages	References
Rabadi, prepared by fermenting pearl millet (*Pennisetum typhoideum* L.) (PM) flour with fermented whey	Poonia and Kumari (2018)
Whey-based mango-herbal (cardamom) beverages (WBMH) using chhana whey+ mango pulp+ cardamom extract (0–3%) + with constant levels of sugar (8%) + mango pulp (12%)	Choudary and Sandey (2009)
Preparation with whey-guava beverages	Divya and Archana (2009)
A beverage using butter cheese whey and acerola juice with a 30:70 mixture	Cruz et al. (2009)
A synbiotic (pre + probiotic) whey drink	Madhavi (2009)

of roughly. In order to give beer a sweet flavour, sugars and artificial sweeteners are added. Additionally, beer's fermentation process adds carbs, raising the osmolality and caloric content of the drink (Johnson et al., 2010).

Companies have begun to provide new flavours in carbonated beverages that address consumers' worries about their health and well-being in order to satisfy the industry's increasing difficulties. From sparkling water to soda pop, cola, and beer, carbonated beverages are a common component of contemporary cuisine. They have distinctive qualities including carbonation, high sugar content or artificial sweeteners, and acidity. When a bottle is opened, the effervescence and bubbles are the result of the carbonation process, which involves the dissolving of CO2 gas in liquid under pressure. Due to the conversion of CO2 with HCO3 and H+ ions as well as additions like citric acid and phosphoric acid, colas, sodas, and beers have a pH level of roughly 3. Sugars and artificial sweeteners are used to provide a sweet flavour. Been developed to increase the production of milk and milk-based products (Lejaniya et al., 2021). Milk processing is one of the world's most important industries (Slavov, 2017). Over the previous 30 years, milk output has climbed from 530 million tonnes in 1988 to 843 million tonnes in 2018, an increase of more than 59% (Chandran et al., 2020; FAO, 2019; Patange et al., 2022). It has climbed up further by 1.3% in 2019 and India being the leading producer with an annual In recent years, the dairy industry has grown tremendously as new technologies have been developed to increase the production of milk and milk-based products (Lejaniya et al., 2021). Milk processing is one of the world's most important industries (Slavov, 2017). Over the previous 30 years, milk output has climbed from 530 million tonnes in 1988 to 843 million tonnes in 2018, an increase of more than 59% (Chandran et al., 2020; FAO, 2019; Patange et al., 2022). It has climbed up further by 1.3% in 2019 and India being the leading producer with an annual In recent years, the dairy industry has grown tremendously as new technologies have been developed to increase the production of milk and milk-based products (Lejaniya et al., 2021). Milk processing is one of the world's most important industries (Slavov, 2017). Over the previous 30 years, milk output has climbed from 530 million tonnes in 1988 to 843 million tonnes in 2018, an increase of more than 59% (Chandran et al., 2020; FAO, 2019; Patange et al., 2022). It has climbed up

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17.6 Processing Techniques for Whey Utilization in Beverage Manufacturing

Beverage manufacturing is one of the major areas where whey utilization is being explored. Here in (Table 17.4), some sophisticated whey processing techniques used in beverage manufacturing are given:

Table 17.4 Whey processing techniques and whey products

Whey processing techniques	Whey products	References
Ultrafiltration/Centriwhey	Whey protein concentrates	Panghal et al. (2018)
Ultrasound/microfiltration	Whey protein beverages	Macwan et al. (2016)
Fermentation	Whey beer and whey wine	Gandhi (1989)
Pasteurization/sterilization	Deproteinized whey beverage	Jayaprakasha et al. (1986)
Reverse osmosis	Ready-to-serve whey juice beverages	Khamrui and Rajorhia (1998)
Distillation	Whey based herbal drinks	Sahu et al. (2006)
Microfiltration and fractionation	Whey protein concentrates	Tunick (2008)
Membrane separation and demineralization technologies	Demineralized whey products	Jelen (1992)
Ion exchange or electrodialysis	Reduced mineral whey and lactose	Morr (1992)
Chromatography/dialysis	Whey protein isolates	Mehra et al. (2021)
Ohmic heating	Sweet whey	Costa et al. (2018)
Nanofiltration	Whey syrups	Ramos et al. (2016)
Fermentation	Bioethanol production	Christensen et al. (2011)
Fermentation	Whey based sports drink	Abella et al. (2016)
Cold plasma technology	Flavoured whey beverage	Silveira et al. (2019)

17.7 Market Potential of Whey Beverages

Due to their nutritious and useful blend of fruit bases, vitamin components, dairy-based calcium, and whey proteins, numerous functional beverages containing whey have become more and more popular in the food market. These drinks are available in a variety of formats, including powdered drinks, milk-like drinks, dietetic drinks, and drinks with lactose hydrolyzed liquids. Additionally, there are many different kinds of whey-containing drinks on the market, including fortified whey drinks, de-proteinated whey drinks, fermented dairy drinks.

including whey, and flavoured milk-like products containing whey or whey components. Whey has a bright future in the beverage industry given the rising demand for cold beverages and fruit juices. The Indian whey protein market is segmented by product type and application. Based on application, the market is segmented into sports and performance nutrition, infant formula, and functional or fortified food (Fig. 17.3). It comprises the majority of the electrolytes included in oral rehydration solution (ORS), which is frequently used to treat dehydration, and can be consumed as a beverage to replace lost organic and inorganic salts in the extracellular fluid.

17.8 Future Scope

As Hippocrates, a Greek physician who lived in 460 B.C., recommended whey for a variety of diseases, the use of cheese whey in human nutrition may be traced back to ancient times. Today, advances in flavours, functional trends that are diverging, and growing concentration all have an impact on the beverage sector. Whey is still employed in beverage production despite manufacturing difficulties because of its solubility range, bland flavour that serves as a carrier for scent, buffering ability that

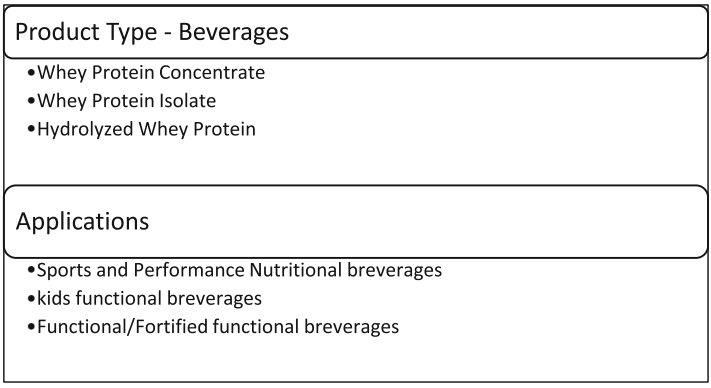

Fig. 17.3 Market potential of whey based beverages

supports microbial survival, ability to improve mouthfeel, and ability to resolve cloudiness in tropical fruit liquids. However, there are difficulties such whey protein coagulation, lactose crystallisation, and a high mineral concentration that results in unpleasant flavours. Overall, the nutritional, biological, and functional qualities of whey-based functional beverages make them a potential beverage ingredient.

17.9 Conclusion

In today's highly competitive beverage market, traditional whey drinks may be struggling. In order to appeal to modern consumers, a beverage must meet certain key criteria, such as being enjoyable to drink, effectively quenching thirst, being reasonably priced, and having a positive association with health. However, due to the distinct flavor of whey and the costs associated with processing it, the future success of whey beverages may primarily rely on their unique nutraceutical qualities.

Consumer preferences, such as convenience, practicality, taste, nutritional value, and variety, all play a role in the market size of functional drinks. Milk, in its various forms such as drinkable yogurt and flavoured milk, is increasingly being seen as a functional drink. Whey-based drinks are likely to play an important role in this trend. Whey drinks containing added carbohydrates and salts could be formulated as sports drinks, which can help with muscle recovery and cramping, increasing lean muscle mass, overcoming adenine nucleotide depletion, acting as a neuro-stimulant, replenishing glycogen, and more. Whey and whey-based products offer a wide range of applications and functionalities in the formulation of different beverages.

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