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MINI-REVIEW

Whey-derived valuable products obtained by microbial fermentation

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Abstract Whey, the main by-product of the cheese industry, is considered as an important pollutant due to its high chemical and biological oxygen demand. Whey, often considered as waste, has high nutritional value and can be used to obtain value-added products, although some of them need expensive enzymatic synthesis. An economical alternative to transform whey into valuable products is through bacterial or yeast fermentations and by accumulation during algae growth. Fermentative processes can be applied either to produce individual compounds or to formulate new foods and beverages. In the first case, a considerable amount of research has been directed to obtain biofuels able to replace those derived from petrol. In addition, the possibility of replacing petrol-derived plastics by biodegradable polymers synthesized during bacterial fermentation of whey has been sought. Further, the ability of different organisms to produce metabolites commonly used in the food and pharmaceutical industries (i.e., lactic acid, lactobionic acid, polysaccharides, etc.) using whey as growth substrate has been studied. On the other hand, new low-cost functional whey-based foods and beverages leveraging the high nutritional quality of whey have been formulated, highlighting the health-promoting effects of fermented whey-derived products. This review aims to gather the multiple uses of whey as sustainable raw material for the production of individual compounds, foods, and beverages by microbial fermentation. This is the first work to give an overview on the microbial transformation of whey as raw material into a large repertoire of industrially relevant foods and products.

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Introduction

Whey is the main by-product of cheese manufacture. The total worldwide whey production has been reported as 40.7×10^6 tons per year in 2012 (Prazeres et al. 2012). Currently, about 50 % of the total production is disposed in wastewater treatment plants or used for animal feed, while only 10 % is transformed into whey protein concentrates (WPC), with whey permeate as a remaining by-product (Koutinas et al. 2014).

Whey is the liquid released during cheese-making after coagulating and separating caseins from milk. Whey composition varies according to several factors: (1) type of whey, sweet (obtained when adding rennet) or acidic (as a result of lactic fermentation), (2) the source of milk (cow, sheep, etc.), (3) the feed of the animal used for milk production, (4) the type of cheese processing, (5) the time of the year, and finally, (6) the stage of lactation (Panesar et al. 2007). While sweet whey contains (in %) water (93–94), dry matter (6.0–6.5), lactose (4.5–5.0), lactic acid (traces), proteins (0.8–1.0), citric acid (0.1), and minerals (0.5–0.7) and has a pH value of 6.4–6.2, acidic whey is composed of (in %) water (94–95), dry matter (5.0–6.0), lactose (3.8–4.3), and lactic acid (up to 0.8); pH=5.0–4.6 (www.dairyforall.com). After drying, whey can be directly used in the food industry or can be transformed into several by-products such as lactose, permeate, WPC, whey protein isolates (WPI), whey hydrolysates, and even pure whey proteins such as β-lactoglobulin (BLG), α-lactalbumin (ALA), and immunoglobulins, which are separated and purified. WPC and WPI as well as purified individual whey proteins (obtained by

ultrafiltration process) generate whey permeate as waste (http://www.daviscofoods.com/commodities/whey.html; http://www.arlafoodsingredients.com/products/milk-protein–minerals/whey-protein concentrate). Whey permeate is composed mainly of lactose along with salts and non-protein nitrogen (Espinosa-Gonzalez et al. 2014).

Disposal of whey permeate is critical because of its high biological oxygen demand ($BOD_{5;}$ which is the amount of O_2 (in mg) needed for the biological oxidation of the organic load per liter of whey in 5-days time). In search of new whey applications, an interesting alternative is to transform whey permeate into several value-added products by microbial fermentation. Although whey is a suitable raw material for industrial applications, the main disadvantage is that its sole carbon source, lactose, cannot be fermented by certain industrially relevant microorganisms such as *Saccharomyces cerevisiae*.

Fermentation of raw materials for the production of industrially relevant compounds has been recently reviewed. In this respect, Abdelmoez and Mustafa (2014) and Banat et al. (2014) have reported on the use of crop, animal, dairy and distillery residues, sugarcane molasses, and oil processing residues for biosurfactant production. In addition, the production of oleo-chemicals obtained by vegetable (soybean, canola, palm oil) fermentations and bioethanol production from sugar juice has been reviewed by Zabed et al. (2014). Also recently, the use of LAB as biorefineries for converting plant-derived biomass in different products has been discussed (Mazzoli et al. 2014). This review spans the production of whey-derived products, both individual compounds or fermented foods and beverages using whey and their derivatives as low-cost substrates and microbial fermentation processes. To our knowledge, this is the first report to compile the use of whey fermentation for the synthesis of products with multiple industrial applications.

Individual compounds produced by whey fermentation

The use of whey fermentation for the production of individual compounds such as ethanol, polyhydroxyalkanoates, methane, hydrogen, and films has been reviewed, although individually (Akaraonye et al. 2010; Du et al. 2012; Guimarães et al. 2010; Hassan and Nelson 2012; Karadag et al. 2014). Here, a compilation on the production of diverse individual products (including fuels and food additives) by fermentation using different microorganisms and methods is presented. Compounds produced by whey fermentation and microorganisms involved in such processes are summarized in Fig. 1 and Table 1 and discussed further in more details in the following subsections.

Biofuels

The increasing demand for gasoline and the depletion of fossil fuels as well as security and environmental concerns are the main reasons for the great amount of research seeking for alternative energy resources (Boguta et al. 2014; Raman and Gnansounou 2014). Validation of carbon credits and assurance of economic and political security are issues that are necessary to evaluate for the development of alternative biofuels. These characteristics demand transformative technologies for producing renewable fuels capable of meeting the society's energy needs (Khan et al. 2014).

Ethanol

Ethanol (C_2H_6O) is used as raw material, solvent, and fuel in chemical, pharmaceutical, and food formulations. The worldwide production of ethanol is approximately 87 billion liters, from which 80 % of the total is produced by fermentation (Ariyanti and Hadiyanto 2013). In this sense, ethanol production from fermented whey by non-*Saccharomyces* yeasts has been applied since the 1980s due to whey's high lactose content and low cost (Koushki et al. 2012). Moreover, whey permeate, another whey by-product, is produced in significant amounts in some countries (New Zealand, Denmark, and the USA). Production of ethanol from whey permeate is already an applied technology in New Zealand (Qureshi et al. 2014). Nowadays, eight million gallons/year of ethanol from whey fermentation are being produced by Dairy Farmers of America (USA) and Anchor Ethanol Ltd (New Zealand) (Table 1) (Ling 2008). However, the yield and rate of ethanol production by lactose-fermenting yeast are lower than those of glucose-fermenting *S. cerevisiae*. In this respect, Koushki et al. (2012) showed that *Kluyveromyces marxianus* was a suitable microorganism for producing ethanol from lactose fermentation, showing a maximum alcohol production efficiency of 96.5 %. Ariyanti and Hadiyanto (2013) studied the ability of a strain of *K. marxianus* to produce ethanol during whey batch fermentation. This yeast produced 8.64 g/L of ethanol and consumed almost all lactose present in whey at 16 h of fermentation with a biomass yield of 4.43 g/L. To improve product yield, fed-batch fermentations of whey by a *K. marxianus* strain was applied; ethanol production of 7.96 g/L with a biomass concentration of 13.4 g/L and a maximum growth rate of 0.186/h were reached at 30 °C (Hadiyanto et al. 2014). Another strategy for improving ethanol yield is the use of cell immobilization, which allows protecting cells from inhibitory products and environmental variations, resulting in smaller bioreactor volumes and lower costs. In this respect, Gabardo et al. (2014) showed that immobilized cells of *K. marxianus* in Ca-alginate improved ethanol yield in continuous culture fermentations. The maximum value achieved was found for *K. marxianus* CCT 4086 with a productivity of 6.97 g/L/h; this being one of the highest values reported to date.

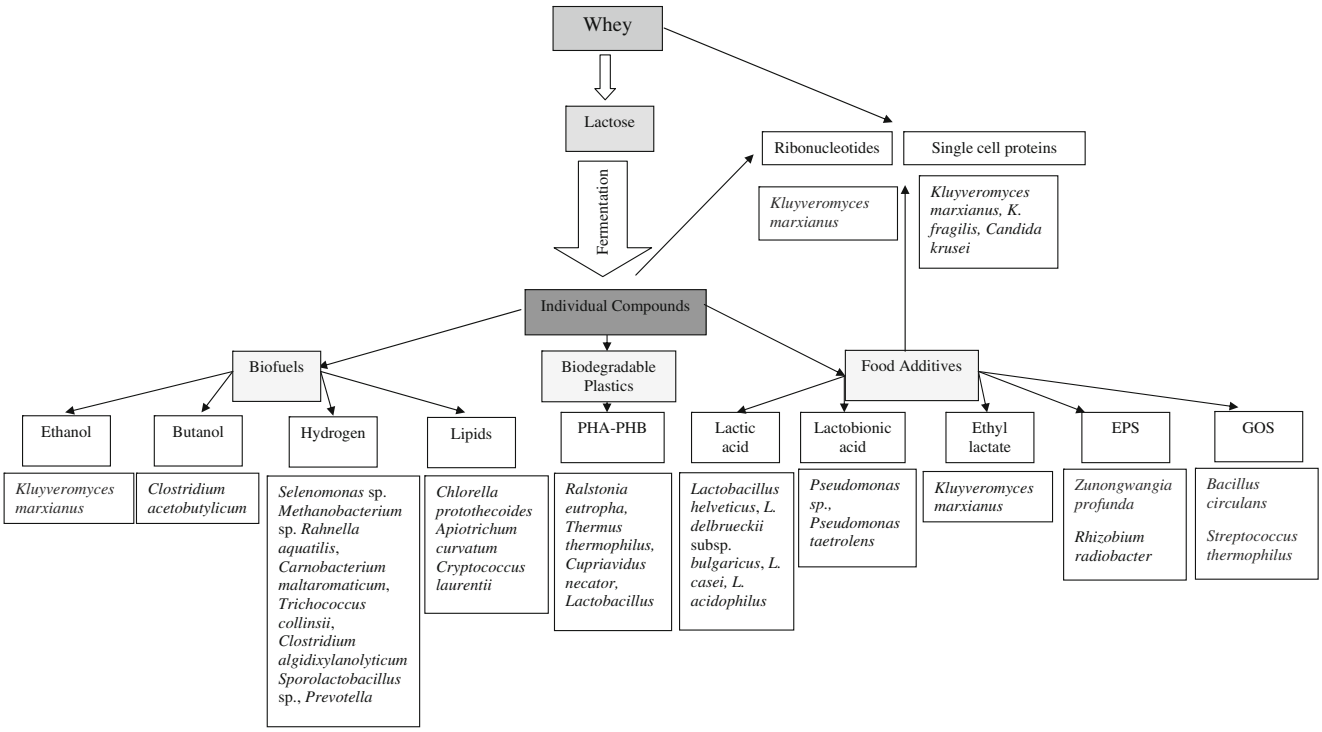

Fig. 1 Individual compounds produced by whey fermentation and microorganisms involved

Butanol

Butanol (C_4H_9OH) has several advantages over ethanol mainly in relation to its easier blending with gasoline and due to its high energy content, low miscibility with water, and low volatility. Moreover, butanol can be used in vehicles with no need of modifying engine technologies (Cascone 2008).

Table 1 Whey-derived products obtained by microbial fermentation and the microorganisms involved

Whey-derived products	Production volume	Involved microorganisms	References
Ethanol	8 billion gallons	Yeast (proprietary)	Ling (2008)
	8.64 g/L	*Kluyveromyces marxianus*	Ariyanti and Hadiyanto (2013)
	7.96 g/L	*Kluyveromyces marxianus*	Hadiyanto et al. (2014)
	6.96 g/L/h	*Kluyveromyces marxianus* CCT 4086	Gabardo et al. (2014)
Butanol	4.93 g/L	*Clostridium acetobutylicum* DSM 792	Raganati et al. (2013)
	7.3 g/L	*Clostridium acetobutylicum* ATCC 824	Ujor et al. (2014)
	72.39 g/L	*Clostridium acetobutylicum* P262	Qureshi et al. (2014)
Hydrogen	3087.57 mL/g	*Selenomonas* sp. and *Methanobacterium* sp. *Rahnella aquatilis*	Ferreira Rosa et al. (2014) Debowski et al. (2014) Romão et al. (2014)
Lipids	3.64 g/L	*Chlorella protothecoides*	Espinosa-Gonzalez et al. (2014)
	2.96 g/L	*Cryptococcus laurentii*	Fernandes Castanha et al. (2014)
Polyhydroxyalkanoates	0.57 g/L	*Thermus thermophilus* HB8	Pantazaki et al. (2009)
	2.40 g/L	*Cupriavidus necator* ($lacZ^+$, $lacI^+$, $lacO^+$)	Povolo et al. (2010)
	4.00 g/L	Mixed culture	Castro-Mayorga et al. (2014)
Lactic acid	33.70 g/L (L)	*Lactobacillus casei* NBIMCC 1013	Panesar et al. (2010)
	24.30 g/L (D)	*Lactococcus. lactis* ATCC 4797	Prasad et al. (2014)
Lactobionic acid	180.00 g/L	*Pseudomonas taetrolens*	Alonso et al. (2013)
	175.00 g/L	*Pseudomonas* sp. LS13-1	Miyamoto et al. (2000)
Exopolysaccharides	17.20 g/L	*Zunongwangia profunda* SM-A87	Sun et al. (2014)
	2.80 g/L	*Rhizobium radiobacter*	Zhou et al. 2014
Galactooligosaccharides	7.70 g/L	*Bacillus circulans*	Das no Rajana et al. (2011)
	53.45 g/L	*Streptococcus thermophilus*	Sangwan et al. (2014)
Ribonucleotides	28.66 mg/g cell dry weight	*Kluyveromyces marxianus* ATCC 85	Húngaro et al. (2013)

Butanol can be obtained by acetone–butanol–ethanol (ABE) fermentation. This type of fermentation is commonly carried out by *Clostridium* strains during the last stage of batch fermentation. At the beginning of this process, cells produce hydrogen, carbon dioxide, acetic acid, and butyric acid, which lower medium pH, generating a shift to solvent production in which cells are change to endospores. The bottleneck for butanol production by fermentation is the culture media (Yu et al. 2007). Lignocellulose was commonly used as substrate, although needs for pre-treatments are required (Raganati et al. 2012). Inversely, whey is an inexpensive substrate that can be fermented by *Clostridium* strains. Whey fermentation using batch or continuous reactors by different *Clostridium* strains favored butanol over acetone production. In this regard, Raganati et al. (2013) found that the strain *Clostridium acetobutylicum* DSM 792 displayed a butanol productivity of 2.66 g/L/h, generating 4.93 g/L of butanol with 82 % selectivity over other solvent production in continuous culture fermentations. More recently, Ujor et al. (2014) observed that *C. acetobutylicum* ATCC 824 could produce 7.3 g/L butanol in batch cultures using dairy waste. Moreover, a fermentation system strategy with simultaneous product removal using a pervaporation membrane and a highly concentrated lactose whey medium was applied to give a butanol productivity of 0.43 g/L/h, this value being 307 % times higher than that achieved in a non-product removal system when fermenting 60 g/L whey permeate (Qureshi et al. 2014).

Hydrogen

Hydrogen represents a good alternative as energy source since it is clean and environmentally friendly (Show et al. 2012). Hydrogen, generally produced by chemical processes, can also be obtained from photosynthesis or by fermentation. Microorganisms responsible for hydrogen production can be strict anaerobes, thermophiles, rumen bacteria, facultative anaerobes, or mixed cultures of several strains (Vardar-Schara et al. 2008). Fermentation has the advantage over photosynthesis in that it produces a higher hydrogen yield and it is light independent, more stable, and technically simpler (Ust'ak et al. 2007). Hydrogen can be produced by fermenting carbohydrate-rich raw materials; glucose, for example, can be transformed into hydrogen through propionic acid, butyric acid, or ethanol-type fermentations (Kapdan and Kargi 2006). Cheese whey containing high (4.5–6.0 %) lactose content has a theoretical H_2 yield of 8.0 mol/mol of substrate (Davila-Vazquez et al. 2011). Hydrogen production by whey fermentation has been applied using different strategies such as continuous reactors including an anaerobic digester, continuous stirred tank reactor, anaerobic fixed bed reactor (AFBR), dark fermentation, etc. These technologies showed higher stability and H_2 yield compared with batch fermentation. Recently, Ferreira Rosa et al. (2014) studied the effect of different hydraulic retention times and type of inoculums (sludge from swine and from poultry) on H_2 yield in AFBR. Results showed that the highest H_2 yield (1.33 mol/mol lactose) was achieved when applying the highest hydraulic retention time (4 h), independently of the amount of inoculum used, which was composed of *Selenomonas* sp. and *Methanobacterium* sp.

Cold-tolerant bacteria (psychrophilic) often have enzymes with low optimal temperatures, making it possible to produce metabolites in cold climates. Debowski et al. (2014) studied the possibility of producing H_2 using the psychrophilic bacteria *Rahnella aquatilis*, *Carnobacterium maltaromaticum*, *Trichococcus collinsii*, and *Clostridium algidixylanolyticum* isolated from underground water (3.7–5.0 m) and lake water (17 m). Batch fermentations using cheese whey as substrate were done at 20 °C during 50 days. The effectiveness of H_2 production was between 1587.47 and 3087.57 mL/g biomass for *R. aquatilis* isolated from demersal lake water. On the other hand, Fernández et al. (2014) evaluated the production of H_2 using a packed bed reactor with polyurethane foam acting as support material using a mixed microbiota in non-sterile conditions from wastewater. The system was fed with whey at varying organic loading rates (ORL). Highest H_2 yields were observed with the highest ORL (18.8 COD/L) used, indicating that whey could inhibit the growth of non-hydrogen-producing organisms such as *Sporolactobacillus* sp. and *Prevotella*. Dark fermentation has also been applied to generate hydrogen; Romão et al. (2014) studied the effect of initial pH and addition of different concentrations of ferrous sulfate and ammonium sulfate to whey on the production of hydrogen on batch culture under anaerobic conditions at room temperature and in the absence of light. The optimum H_2 yield (4.13 mol/mol lactose) and productivity (86.31 mmol H_2/L/day) were achieved at initial pH 7.0 using 0.6 g/L$FeSO_4$ and 1.5 g/L $(NH_4)_2SO_4$.

Lipids

Triacylglycerides (TAG) generally serve as energy storage and can be easily converted into biodiesel through transesterification reactions (Sharma et al. 2012). Algae accumulate lipids for resisting adverse environmental conditions. TAG are produced mostly under stress conditions such as nutrient starvation (nitrogen and/or phosphorus), osmotic, radiation, pH, temperature stresses, and presence of heavy metals and other chemicals (Wang et al. 2009). Microalgae are easily integrated into a biorefinery for obtaining lipids with high productivity and can be grown with minimal impact on freshwater resources in inexpensive media including wastewater steams; moreover, they are biodegradable and relatively harmless to the environment if spilled (Menetrez 2012). *Chlorella protothecoides* is one of the best microalgae lipid producer and is able to grow in whey permeate using glucose, from previously hydrolyzed lactose, as

substrate for lipid synthesis. In a recent study conducted by Espinosa-Gonzalez et al. (2014), the authors reported that a strain of *C. protothecoides* was able to produce 42 g of lipids (dry weight bases) grown in whey permeate in batch culture, while 20.5 g was obtained using fed-batch cultures. Productivity was higher (approximately 50 g) when simultaneous saccharification and fermentation processes in batch mode using immobilized enzyme were applied (Espinosa-Gonzalez et al. 2014).

In addition, the so-called oleaginous microorganisms such as *Apiotrichum curvatum* and strains from the genus *Cryptococcus* are capable of producing lipids during their growth in whey. In this sense, Fernandes Castanha et al. (2014) showed that *Cryptococcus laurentii* had a maximum lipid productivity of 0.00822 g/L/h with high content of 16- and 18-carbon chain saturated and monosaturated fatty acids, considered suitable for biodiesel production.

Bioplastics

Plastic production, predominantly derived from petroleum, is of 200 million tons per year. Due to the fast fossil fuel resources depletion, the production of plastics is becoming very expensive. For this reason, there is need to produce plastics from sustainable raw materials and environmentally friendly conditions. Bioplastics can be produced using starch, sugar, or cellulose by microbial fermentation.

Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are biodegradable polymers produced through fermentation processes using a variety of microorganisms such as *Ralstonia eutropha*, *Alcaligenes latus*, *Aeromonas hydrophila*, and *Pseudomonas putida*. PHAs are accumulated by microorganisms as water-insoluble inclusions in the cytoplasm and are used as intracellular carbon and energy materials under nutrient-stressed conditions (Sudesh et al. 2000). PHAs are classified according to their chain length in short-chain-length (composed of three to five carbon atoms), medium-chain-length (having between 6 and 14 carbon atoms), and long-chain-length (carrying more than 14 carbon atoms) PHAs. Poly3-hydroxybutyrate is the most common and intensively studied PHA (Chen et al. 2009) and can be accumulated in more than 80 % of the cell dry weight (CDW) in strains of *Ralstonia eutropha* (Du et al. 2012). The strain *Thermus thermophilus* HB8 is able to produce PHA during its growth in whey-based media using lactose as substrate. This strain accumulated up to 35 % of its biomass with PHA when grown in 25 % (*v*/*v*) of whey during 24 h. *T. thermophilus* HB8 produced a novel heteropolymer consisting of a short-chain-length 3-hydroxyvalerate (3HV; 38 mol%) and the medium-chain-length 3-hydroxyheptanoate (3HHp; 9.89 mol%), 3-hydroxynanoate (3HN; 16.59 mol%), and 3-hydroxyundecanoate (3HU; 35.42 mol%) (Pantazaki et al. 2009). Although whey is a non-expensive and suitable growth substrate, not all PHA-producing strains can grow in media containing only lactose as sole carbon source. In this respect, Povolo et al. (2010) constructed a recombinant strain of *Cupriavidus necator* (by cloning the *lacZ*, *lacI*, and *lacO* genes from *Escherichia coli*) able to produce PHA in whey. This strain accumulated 22 % of its dry cell weight in PHA. More recently, Duque et al. (2014) produced PHA by using a mixed microbial culture in a three-stage fermentation process with a feedstock of a mixture of whey and sugarcane molasses achieving a maximum PHA content of 65 %.

PHAs are biodegradable plastics that could be used for food packaging carrying antimicrobial agents; in this sense, Castro-Mayorga et al. (2014) obtained a PHA able to carry silver nanoparticles, known to have antimicrobial properties, by fermenting a whey-simulated medium. The production of PHA in whey has been also demonstrated in some lactic acid bacteria (LAB) strains belonging to the genera *Lactococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus*, with *Lactobacillus* being the species which gave the highest yield (36 % of their CDW). However, their production is considered low in comparison to those of *Alcaligenes* and *Azotobacter* species for which productions higher than 55 % of their CDW were reported. LAB are usually co-cultivated with PHA producer strains to produce lactic acid and further used for the synthesis of PHA (Mazzoli et al. 2014). The disadvantage with respect to petrol-derived plastics is the production costs and the low productivity; however, the high versatility of these biopolymers makes them a good alternative for producing them in low volume especially for medical or biomedical uses (Keshavarz and Roy 2010).

Polylactic acid

Polylactic acid (PLA) are produced using mixtures of D (−) and L (+) lactic acids in different ratios to obtain bioplastics with distinct characteristics (Gupta et al. 2007; John et al. 2006). The polymers are obtained by ring-opening polymerization from *l*,*l*-lactide and *d*,*d*-lactide, glycolide, ε-caprolactone, trimethylene carbonate, 1,5-dioxepan-2-one, and other cyclic analogues (Södergårda and Stolta 2002). These polymers are used in medicine for suture, soft issue anaplerosis, fracture fixation, oral implant, drug delivery microsphere, etc. (Ikada and Tsuji 2000). PLA has a moderate production cost of $2.1/kg (Endres and Siebert-Raths, 2011). Lactic acid is produced by Hitachi (http://www.hitachi.com/businesses/infrastructure/product_site/ip/process/pla.html) and Cargil (Vink et al. 2003) by fermentation of mainly corn, molasses, and other farm products. This compound is used for production of bioplastics by polymerization or used directly in the food and pharmaceutical industries.

Food and pharmaceutical additives

Lactic acid

Nowadays, lactic acid ($C_3H_6O_3$) is mainly (90 %) produced by fermentation using selected bacteria and fungi since individual lactic acid isomers can be produced by this method. In contrast, a mixture of both isomers is always obtained when chemical methods are applied. L (+) lactic acid is used for the production of polymers suitable for fibers and orientated films; also, it is employed as emulsifier and food preservative, being used in its acid form or as a calcium or sodium salt (John et al. 2006). On the other hand, D (−) lactic acid is not used individually.

LAB are able to grow in whey and whey permeate and to produce lactic acid as a main product of lactose fermentation. The production of lactic acid by *Lactobacillus* species such as *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus casei*, and *Lactobacillus acidophilus* using whey as growth substrate has been extensively studied (Abdel-Rahman et al. 2013; Fakhravar et al. 2012). However, some LAB strains are not able to grow in whey at high extent since their complex nutrient requirements are not fulfilled; for this reason, some authors have added yeast extract to whey or developed methods in which cells are maintained in non-proliferating states (Mazzoli et al. 2014). Lactic acid can be produced as a racemic mixture or as separate isomers depending on the LAB strain chosen. In this respect, González et al. (2007) showed that *L. helveticus* HD100 produced a racemic mixture of D- and L-lactic acid when grown in whey. On the other hand, Panesar et al. (2010) demonstrated that *L. casei* NBIMCC 1013 produced only L-lactic acid when grown in whey cultures. These authors observed that lactic acid production was maximum using a 20-h-old culture (2 %, *v*/*v* inoculum) grown at 37 °C and pH 6.5 without agitation, reaching a lactose conversion of 95.6 % and a lactic acid concentration of 33.7 g/L after 36 h of incubation. Oppositely, *L. lactis* ATCC 4797 produced only D-lactic acid (12.5 g/L) when grown in whey permeate containing 35 g/L lactose in a bioreactor. The lactic acid yield was improved by adding casein hydrolyzate to whey, enabling to reach a concentration of 24.3 g/L of D-lactic acid (98 % pure) after 40 h (Prasad et al. 2014).

Lactobionic acid

Lactobionic acid (4-*O*-β-galactopyranosyl-D-gluconic acid) is a disaccharide form of gluconic acid and galactose attached via an acetal linkage. This acid is synthesized by oxidation of the free aldehyde group of glucose on the lactose molecule to the carboxyl group by using chemical synthesis, specific enzymes, or microorganisms as biocatalysts. The production of lactobionic acid has been proven in different microorganism species such as *Zymomonas mobilis*, *Sclerotium rolfsii*, *Myriococcum thermophilum*, *Pseudomonas graveolens*, and *Acetobacter orientalis* (Gutiérrez et al. 2012). Lactobionic acid is considered a prebiotic as bifidobacteria and lactobacilli strains are able to metabolize it as growth substrate (Adebola et al. 2014). In addition, this acid is used in calcium food fortification since it is able to form salts with different minerals (Seiki and Saito 2012). Lactobionic acid is also employed in the pharmaceutical and cosmetic industries, being used in tissue engineering and nanomedicine due to its biocompatibility, biodegradability, and chelating, amphiphilic, and antioxidant properties. Interestingly, this acid is included in organ transport solutions to provide osmotic support and prevent cell swelling. In cosmetics, it is used as an anti-aging and keratinizing agent (Green et al. 2008; Gutiérrez et al. 2012).

Lactobionic acid is usually produced by chemical catalysis; however, this method generates side reaction products such as undesirable organic acids (Chia et al. 2008). An interesting alternative to its chemical synthesis is microbial fermentation using whey as substrate. Some *Pseudomonas* strains have been reported to produce lactobionic acid when grown in whey. In this respect, Alonso et al. (2013) demonstrated that *Pseudomonas taetrolens* was able to produce 180 g/L of lactobionic acid with a 90 % yield when cultivated in fed-batch culture under co-feeding conditions using highly concentrated whey supplemented with yeast extract and peptone at a feeding rate of 4.2 mL/h. The authors showed that a moderate agitation of 350 rpm had a better lactobionic acid yield than at higher agitation rates (1000 rpm), although its growth rate and pH shift were lower. In a previous work, Alonso et al. (2011) reported that the production of this acid was not cell growth-associated and that 1.12 g/L/h of lactobionic acid was reached in batch cultures by shifting from uncontrolled pH above 6.5 during growth to a constant pH of 6.5 during cumulating production. On the other hand, Miyamoto et al. (2000) reported that *Pseudomonas* sp. LS13-1 could produce 175 g/L of lactobionic acid when grown in reconstituted dry whey (spray-dried whey containing 75 % of lactose) at a concentration of 207 g/L and three intermittent additions of 69 g/L in a fed-batch culture at pH 5.5 for 180 h. Hua et al. (2007) studied the conditions for maximum lactobionic acid production by a strain of *Microdochium nivale* grown in whey permeate and obtained a lactobionic acid yield of 98 %.

Exopolysaccharides

Exopolysaccharides (EPS) can be classified into hetero- or homopolymers according to their monomer composition, with molecular weights ranging from 10 to 1000 kDa. Some bacteria are able to produce EPS, which can be either secreted to the environment or loosely attached to the cell wall (Nwodo

et al. 2012). Bacterial EPS have multiple industrial applications due mainly to their emulsifying, thickening, and water holding capacities. Alginate, for example, is used for microencapsulation as well as for making dental impressions and for anti-reflux therapies. Dextran produced by *Leuconostoc mesenteroides* strains and xanthan synthesized by *Xantomonas campestris* are widely used in the industry, with the former being used as plasma substitute while the latter EPS is used in foods, cosmetics, and paints among other applications. However, the main disadvantage of bacterial EPS synthesis is its high production costs. In this respect, the use of whey as growth medium for EPS production could lower the costs, making the process more competitive. Several authors have shown that several bacterial strains could produce EPS when grown in whey or whey derivatives. Sun et al. (2014) reported that the strain *Zunongwangia profunda* SM-A87 could grow in whey and produce the highest amount of EPS (17.2 g/L) reported for a marine bacterium; this polymer displays antioxidant activity and is of industrial interest since it has optimum biosorption capacities for Cu(II) and Cd(II) and has good rheological properties to enhance oil recovery (Liu et al. 2011). Also, the strain *Rhizobium radiobacter* S10, isolated from kefir grains, is able to produce 2.8 g/L of a $3.03-10^6$ Da polymer with interesting thickener or stabilizer properties during its growth in whey (Zhou et al. 2014).

Galactooligosaccharides

Galacto-oligosaccharides (GOS) are oligomers of monosaccharides consisting of chains of galactosyl–glucose having a degree of polymerization between 2 and 10. These compounds are non-digestible and consequently low-caloric; they are considered as prebiotics as they stimulate the growth of beneficial gastrointestinal microbiota. GOS are biotechnologically produced by the enzyme β-galactosidase from different microorganisms (Gobinath and Prapulla 2013).

Ranjana et al. (2011) proposed a method for using lactose from whey to produce GOS by using the β-galactosidase from *Bacillus circulans* in batch reactor and in recycle membrane reactor; the authors observed better results with the latter method (33 % higher production) obtaining 77 % of pure GOS. Sangwan et al. (2014) reported that the *S. thermophilus* β-galactosidase could produce GOS in an appreciable amount (53.45 g/L) using lactose-supplemented whey as substrate. This amount was obtained using a total of 30 % lactose concentration and 10 U/mL of enzyme after 5 h at 40 °C and pH 6.8.

Ribonucleotides

Ribonucleotides such as 5′-guanosine monophosphate and 5′-inosine monophosphate are compounds used as flavor enhancers in the food industry, while in pharmaceutical preparations 5′-nucleotides are employed in the synthesis for antiviral and anticancer drugs (Makendran 2012). In addition, ribonucleotides are known to increase immune response in infants. These compounds are normally present in human milk and are at much lower concentration in cow's milk; for this reason, some authors studied the effect of supplementing infant formula with ribonucleotides and observed a positive effect on the immune response (Schaller et al. 2004). Húngaro et al. (2013) studied the ability of the strain *K. marxianus* ATCC 8554 to produce ribonucleotides during its growth in whey. This strain was able to produce a maximum of 28.66 mg ribonucleotides/g cell dry weight when grown at 30 °C and pH 5.0. Ribonucletide production from whey is a good strategy for reducing pollution since it reduces 98 % of its BOD.

Single-cell proteins

Whey is an attractive low-cost medium to produce yeast or microbial biomass since by applying a simple wastewater treatment process, the COD is reduced. In this respect, Yadav et al. (2014) analyzed the possibility of producing single-cell proteins (SCP) by fermenting whey with *K. marxianus* and *Candida krusei* strains at high temperature and low pH (40 °C, pH 3.5) in order to avoid contamination. The maximum biomass yield obtained was 0.3 g/L/h with 34 % COD removal at 6 h of incubation. Ghaly et al. (2005) developed a mathematical model for improving SCP yield by a *K. fragilis* strain when growing in cheese whey. The model was able to predict the effluent cell and substrate concentration with R^2 close to 0.99. The maximum yield (0.74 cell/g lactose) was obtained at 12 h of retention time with 3 (vol/vol/min) air flow rate and 600 rpm mixing speed combination. On the other hand, Schultz et al. (2006) observed that during SCP production of *K. marxianus*, the amount of eight out of ten free essential amino acids increased in fermented whey compared with non-fermented whey, exceeding the World Health Organization guidelines for valine, leucine, isoleucine, threonine, phenylalanine, and tyrosine. Moreover, whey fermentation reduced 80 % of its oxygen demand.

Whey-fermented foods and beverages

Whey is currently used as supplement in bakery (bread and crackers) and in the dairy (cheese, cream, and butter) industry. In a bakery, whey is used to substitute flour or fat, giving these foods a creamier flavor and improved (brownish) color. Whey is added to cream and butter, giving these products a saltier taste. In addition, some cheese producers put whey cream back into the cheese to increase cheese fat (http://www.ehow.com/list_6874297_list-foods-contain-whey.html). In

this review, the use of whey as growth substrate for the elaboration of fermented foods and beverages is presented and shown in Fig. 2.

Bread

Soukoulis et al. (2014) formulated a probiotic bread by applying a film-forming solution (edible film) based on a binary blend containing 0.5 % (*w*/*v*) sodium alginate and 2 % (*w*/*v*) WPC inoculated with the probiotic strain Lactobacillus *rhamnosus* GG. Edible films are thin layers of biopolymers that can be consumed and are usually applied on the food surface by spraying or brushing. These layers can prevent spoilage by raising the thermo-dynamical or physical barrier and thus retarding water vapor, oxygen, and solute mobility. The addition of whey to the film composition improved the survival of the *L. rhamnosus* strain during drying and storage due to the ability of this microorganism to interact with whey proteins, reducing in this way heat, osmotic, and oxidative stresses. Interestingly, the film did not modify the textural properties of the bread crust.

Baby foods

Recently, Rasane et al. (2014) developed a cereal baby food with low content of phytic acid. Phytic acids are antinutrient compounds present in several cereals that can bind minerals, making them unavailable for absorption in the human body. Fermentation by LAB can activate phytase enzymes, improving protein and mineral absorption. Furthermore, cereals are less nutritive than milk and milk-derived products due to their less digestibility and amino acid balance. These authors formulated a baby food consisting of cereals, whole milk powder, WPC, and sugar. To reduce the phytic acid content and to improve the digestibility and palatability as well as to obtain an adequate nutritive quality, germination and fermentation with strains of *L. casei* and *L. plantarum* were applied. The fermented food showed high protein, vitamin, and minerals as well as good quantity of carbohydrates and fat.

Fermented infant formula

The Committee on Nutrition belonging to the European Society for PaediatricGastroenterology, Hepatology and Nutrition (ESPGHAN) defines fermented infant formula and follow-on formula as those that have been fermented with LAB but do not contain viable bacteria in the final product. Bacterial elimination from the formula can be achieved by means of heat, homogenization, pasteurization, sterilization, and/or spray-drying (Labuschagne et al. 2012). Infant fermented formulas are generally mixtures of milk and whey in 50:50 and 40:60 ratios, respectively. These formulas have demonstrated to be microbiologically safer and to have positive effects on the immune system of a baby who cannot be breastfed (Joosten and Lardeau 2004). Furthermore, Campeotto et al. (2011) reported that a fermented infant formula with *Bifidobacterium*

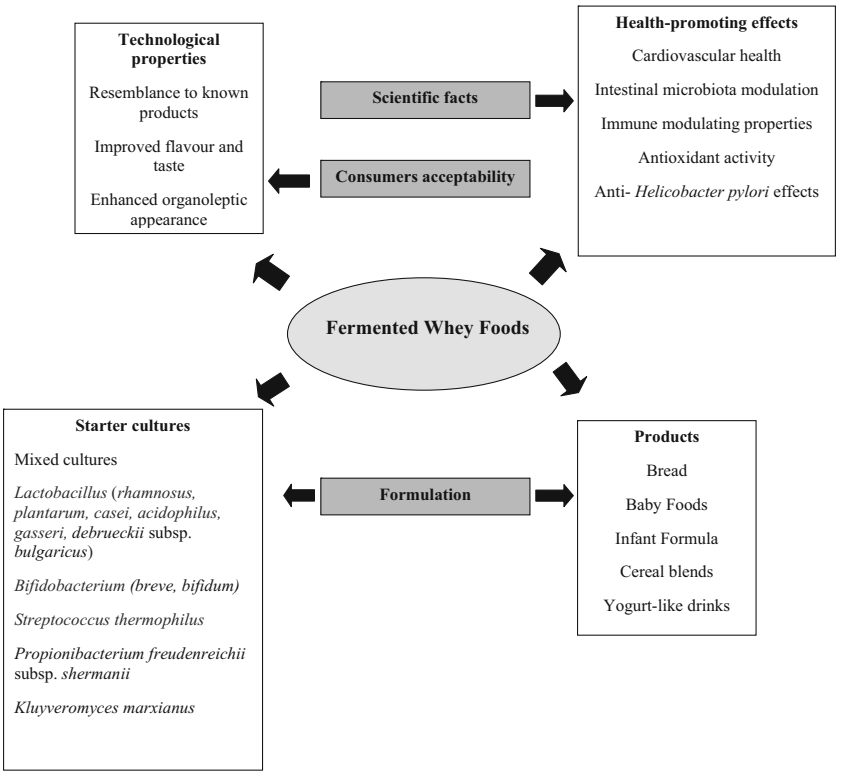

Fig. 2 Fermented foods and beverages using whey as substrate. Technological properties, health-promoting effects, and microorganisms involved

breve and *S. thermophilus* strains has a positive effect on a pre-term infant's immune system. This formula was fermented at 37 °C for 8 h, with the bacteria being heat-inactivated at the end of the fermentation. Pre-term babies of gestational ages between 30 and 35 weeks were fed for 2 weeks with fermented and non-fermented formulas; results indicated that the former ones had benefits on inflammatory and immune markers related to gastrointestinal tolerance compared to those receiving non-fermented formula.

Cereal blends

Arora et al. (2011) formulated a probiotic food composed of raw and germinated pearl millet flour, whey powder, and tomato pulp (2:1:1, *w*/*w*/*w*, respectively) inoculated (5 %*v*/*v*) with a *L. acidophilus* strain at a cell density of 10^6 cells/mL and fermented at 37 °C for 12 h. The authors observed that fermentation increased the content of thiamine, niacin, total lysine, protein fractions, sugars, soluble dietary fiber, and in vitro availability of Ca, Fe, and Zn of food blends compared to unfermented cereal blends.

Fermented drinks

Whey is an excellent source of lactose and also a good source of vitamins, proteins, minerals, and lipids. Whey proteins have a high nutritional value, exceeding 15 % that reported for egg proteins; moreover, whey has a high content of essential branched chain amino acids (Smithers 2008), which provide metabolic energy to the muscles and promote the synthesis of Ala and Glu during stress. The amino acids Leu, Lys, Trp, and Ile play a role as regulators of glucose and protein metabolism, also being important for weight control (Pescuma et al. 2010). These properties make whey a good raw sustainable material for developing new health-promoting foods. Due to its liquid nature, whey is often used for producing different kinds of beverages (i.e., sport and energy drinks) including those obtained by fermentation (fermented milks).

The consumption of dairy beverages is often associated with yogurt; for this reason, the acceptability of a new whey drink would be associated to the resemblance with0020that drink. In this respect, Castro et al. (2013) studied the acceptance of a strawberry-flavored dairy beverage supplemented with whey. Beverages were prepared by mixing 0, 20, 35, 50, 65, and 80 % of whey, strawberry pulp, and sugar, adjusting the remaining volume with milk. The formulation was fermented using a mixed culture containing strains of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* until a pH of 4.7 was reached. The fermented beverage with best acceptance was that containing 45 % of whey as assessed by a mathematical model (Weibul distribution) based on the analysis of 55 randomly selected dairy consumers. Maity (2008) formulated a beverage by fermenting whey with the strains *L. rhamnosus* NCDO 243, *B. bifidum* NCDO 2715 and *Propionibacterium freudenreichii* subsp. *shermanii* MTCC 1371 and studied its properties and acceptability during storage. The beverage showed good acceptability and stability. Moreover, cell free extracts of the fermented drink showed to be active against the pathogenic and deteriorating bacteria *E. coli, Staphylococcus aureus*, *Shigella dysenteriae*, and *B. cereus*. Kücükcetin et al. (2012) studied the effect of adding different whey concentrations to Aryan, a Turkish salt-containing yogurt. These authors analyzed the general appearance (graininess, visual roughness, serum separation, particle size, and rheological properties) and observed that the increase in whey concentration caused a decrease in graininess and visual roughness and an increase in serum separation and flow behavior index. On the other hand, Saeed et al. (2013) selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* to produce a *wheygurt* (fermented whey using the yogurt starter culture) with acceptable characteristics. The authors found that a 2 % (*v*/*v*) inoculum of the LAB starter culture was able to produce an acceptable drink that was relatively stable during storage. Martínez Rodríguez et al. (2013) formulated a fermented whey drink with passion fruit pulp free of lactose. Here, whey lactose was hydrolyzed by a commercial β-galactosidase enzyme previously to fermentation with the MY800 DANISCO thermophilic culture until a pH of 5.8 was reached; after cooling, passion fruit was added to the fermented drink. The lactose-free fermented drink containing 10 % of pulp was considered a very good product by 59 untrained tasters.

Buttermilk is a popular fermented beverage in India, which is known to be refreshing and thirst quenching. Recently, Ghanshyambhai et al. (2014) studied the acceptability of buttermilk when adding acid whey. The beverage was prepared by fermenting separately double-toned milk (buffalo skim milk, powdered skim milk, and water) and acid whey and mixing them at the same concentration. The beverage showed acceptable sensory qualities and shelf-life (5 days) under refrigeration conditions.

Conti et al. (2012) studied the ability of a microbial community (composed of *L. helveticus*, *L. paracasei*, *L. fermentum*, *L. gasseri*, *L. parabuchneri*, *L. casei*, *L. panis, Pichia kudriavzevii*, and *S. cerevisiae*) to degrade whey proteins by fermentation and produce a raw material for formulating a functional whey drink; after fermentation, whey was enriched with low molecular weight peptides. In this respect, Pescuma et al. (2008, 2010) studied the ability of the yogurt strains *L. debrueckii* subsp. *bulgaricus* CRL 454 and CRL 656 and *S. thermophilus* CRL 804, as well as *L. acidophilus* CRL 636, to degrade whey proteins during fermentation of and WPC35% (WPC35). The main whey proteins, ALA and BLG, could be partially degraded by the strains after 24 and 12 h of fermentation, respectively, releasing small-sized peptides and essentials amino acids. This

feature was of health interest as BLG, which is poorly digested and highly allergenic, is the main allergenic protein present in milk, especially for children under 3 years old. The authors formulated a functional fermented whey beverage composed of WPC35 and peach juice, which was stable, from technological and microbiological points of view, when kept at 10 °C for 28 days.

Besides yogurt, kefir is also a common dairy drink in many Eastern European countries. Kefir is a refreshing beverage naturally carbonated with acidic and yeasty flavor and creamy consistency. This drink is fermented with kefir grains, which are symbiotic associations of bacteria and yeast in which proteins and polysaccharides are also present. Sabokbar and Khodaiyan (2014) developed a drink composed of whey and apple juice fermented by kefir grains and studied the optimal incubation temperature and the grain quantity able to release the higher amount of phenolic content and antioxidant activity. It has been established that some peptides obtained by hydrolysis of the whey proteins ALA and BLG have antioxidant activity; in addition, apples contain many kinds of polyphenol components. In this regard, the authors found that a concentration of 7.6 % (*w*/*v*) of kefir grains and a temperature of 24.8 °C were optimal for obtaining the maximum antioxidant activity. De Bassi et al. (2012) formulated a beverage consisting in a mixture of 70 % milk supplemented with whey and buttermilk in concentrations of 30 and 0 %, 15 and 15 %, and 0 and 30 %, respectively. The bacterial performance was adequate as a final cell count of 8 Log cfu/mL and a pH value of 4.7–4.9 (similar to a regular yogurt) after 3 h of incubation were obtained. After fermentation, sugar and strawberry were added; the final drink had good acceptance independently of the amount of whey and buttermilk employed. This is an interesting drink since both whey and buttermilk (by-product of cream when preparing butter) are by-products of the dairy industry, which are commonly discarded. On the other hand, Dragone et al. (2008) developed an alcoholic drink by fermenting whey with *K. marxianus* in a continuous reactor maintained at pH 4.0 and 30 °C for 2 months. The fermented product was distilled, and volatile compounds were analyzed by gas chromatography. Most of the volatile compounds found were similar to those of other alcoholic drinks; the relation between iso-amyl alcohol/2-methyl-1-propanol and 2-methyl-1-propanol/1-propanol, which is the parameter used for alcoholic drink quality, was acceptable, indicating that this new drink could be adequately produced. On the other hand, the effect of whey and whey proteins on yogurt-like probiotic products regarding the probiotic microbial growth and survival during storage was analyzed. Results indicated that WPC enhanced the buffering capacity of yogurt, thus preventing death of the microorganism. Lourens-Hattingh and Viljoen (2001) have observed that whey could improve the growth of *L. acidophilus* and survival of bifidobacteria in fermented beverages.

Health-promoting effects of fermented whey products

The health-promoting effects of whey have been claimed for centuries; historical records indicate that whey was used for medicinal purposes during the seventeenth and eighteenth centuries in Europe. It has been reported that whey proteins and peptides have multiple bioactive properties such as increase in physical performance, recovery after exercise, weight management, anticancer effect, wound care and repair, management of infections, infant nutrition, and healthy aging (Smithers 2008). A large literature exists on the health-promoting effects of whey and whey proteins; however, just a few reviews compile the health benefits of fermented whey (Marsh et al. 2014; Uchida et al. 2007). Fermentation is known to give added value to foods by enhancing their shelf-life and improving their nutritional qualities and sensory characteristics (Marsh et al. 2014). For these reasons, several authors aimed to study the health-promoting effect of fermented whey products. In this regard, Sachdeva et al. (2014) overviewed the studies on *Helicobacter pylori* eradication by administrating fermented milks to patients with peptic ulcer and observed that whey proteins could be responsible for the anti-*Helicobacter* properties of fermented milk-based probiotic preparations. Oh et al. (2014) studied the effect of fermented Mailliard reactions on cardiovascular health. Fermented Mailliard reactions were prepared by mixing WPC or sodium caseinate and lactose, heating, and further fermenting with the human isolates *L. gasseri* H10 and H11 and *L. fermentum* H4 and H9, which had excellent proteolytic activity and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (>20 %). Results indicated that *L. gasseri* H11 had the greatest activity for thrombin and 3-hydroxy-3-methylglutaryl-CoA reductase inhibition in Maillard-reacted WPC (42 and 33 %, respectively), while hydrolysates of Maillard-reacted sodium caseinate fermented by *L. fermentum* H9 displayed the highest reduction rate for micellar cholesterol solubility (52 %). Further analysis by size exclusion chromatography demonstrated that protein hydrolysates obtained by fermentation were responsible for these effects.

On the other hand, it has been demonstrated that the administration of a cell-free fermented whey product with the strain *B. breve* C50 could modulate the intestinal microbiota of healthy adults, while whey itself had no effect (Granier et al. 2013). Beaulieu et al. (2007) analyzed the immunomodulating properties of a malleable matrix composed of fermented whey proteins by LAB. The matrix, composed of a mixture of fermented whey by LAB, capsular EPS, vitamins, minerals and peptides obtained during fermentation, could stimulate the immune system. An increase in the polymorphonuclear cell counts and intracellular glutathione levels with respect to control trials in an animal model was observed.

The effect of whey protein hydrolysates obtained by fermentation using selected LAB on BLG allergenicity was also investigated. In this respect, Pescuma et al. (2009, 2011, 2015) claimed that the strains *L. acidophilus* CRL 656 and *L. delbrueckii* subsp. *bulgaricus* CRL 656 and CRL 454 could cleave the main epitopes of BLG and that the peptides released by *L. delbrueckii* subsp. *bulgaricus* CRL 656 were less immunoreactive than those obtained by trypsin degradation. In addition, these authors showed that the peptides obtained with these LAB strains were different from those achieved by using digestive enzymes in an in vitro simulated gastrointestinal model (Pescuma et al. 2015).

Subrota et al. (2013) studied the antioxidant effect of a fermented soymilk supplemented with WPC and observed that the antioxidant activity increased while the polyphenol content decreased when whey proteins were present. Moreover, De Simone et al. (2009) analyzed the peptide extracts of Mozzarella di Bufala waste whey and found that some bioactive peptides had antioxidant properties and modulatory effect on the cell cycle using $CaCo_2$ cell lines.

On the other hand, Zhao et al. (2014) studied the effect of fermented whey by a *L. casei* strain on the anti-alcoholic liver activity of chronic alcohol-induced mice and observed that the pathological characteristics of livers of alcohol-receiving mice were improved by the administration of fermented whey. Kume et al. (2012) studied the effect of an enteral formula containing whey peptides and fermented milk on inflammation produced by concanavalin A-induced hepatitis. C57BL/6 mice were fed with a regular diet or enteral formula and intravenously administered with concanavalin A; inflammatory cytokines were analyzed in plasma, liver, and spleen. Results showed that inflammatory cytokine levels were lower in mice receiving the whey peptide-containing formula.

Conclusions

Whey, which was for a long time considered mainly as waste, has been valorized as raw material for producing a wide range of products comprising from fuels to functional foods. The reuse of whey is of industrial interest not only because of its high nutritional value and its inexpensive feature for producing value-added products but also because of its high pollutant capacity. In this review, we aimed to summarize all products that have been obtained by microbial fermentation of whey, their utilities, and health-promoting benefits. Fermentation is a rather cheap process in comparison to chemical synthesis, and in addition, it does not produce toxic side-products, which are normally found in chemical processes. A rather broad kind of products can be obtained by whey fermentation, taking advantage mainly of its high lactose concentration. Some of these comprise biofuels and bioplastics and aim to replace petrol. Others, like lactic acid and lactobionic acid, can be used in the food and pharmaceutical industries. Furthermore, whey can be directly fermented to produce new foods, some of them with functional health-promoting properties. As a disadvantage, a rational selection of the microorganism used for whey fermentation should be considered as not all industrially relevant bacteria are able to grow on lactose. Overall, we can conclude that whey has been valued in the recent decades, mainly thanks to the new separation and concentration technologies which allowed separation of several whey components. Moreover, the research on whey proteins and their encrypted peptide sequences with bioactive properties as well as the recognition of the high content of branched chain amino acids of whey proteins highlights the importance of recovering this by-product for their application in the food and pharmaceutical industries. Whey conception has definitely evolved from waste to valuable raw material for producing valuable-added products. To our knowledge, this is the first work thoroughly encompassing the use of microbial whey fermentation for traditional and novel applications.

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Ethical statement The authors state that principles of ethical and professional conduct have been followed in this research and in the preparation of this article.

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