Valorisation of Whey and Buttermilk for Production of Functional Beverages – An Overview of Current Possibilities

Running title: Whey and Buttermilk as Functional Beverages

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Received: 23 July 2019
Accepted: 5 December 2019

SUMMARY

Whey and buttermilk are the main by-products of the dairy industry, both having excellent nutritional properties. Buttermilk contains a unique component— the milk fat globule membrane (MFGM). MFGM contains bioactive compounds with positive health effects like antitumor or cholesterol-lowering impact. Whey proteins are found in whey and are a source of bioactive peptides acting positively on coronary, gastrointestinal, immune and nervous system. Yet, buttermilk and whey are insufficiently utilized in functional foods production. Various technological solutions have been studied in order to increase the production of foods based on whey and/or buttermilk whereby the production of beverages appeared to be most acceptable from the economic and technological point of view. Thus, the aim of this paper is to give an overview of current acknowledgments considering the possibilities of creating whey and/or buttermilk beverages.

Key words: whey, buttermilk, beverages, bioactive peptides, MFGM

INTRODUCTION

Whey and buttermilk are the main by-products of the dairy industry. Despite, their potential as raw materials for development of new products is not used at all or very poorly. Whey originates from

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cheese production and contains highly valuable whey proteins, lactose, minerals and vitamin B \(^{(1,2)}\). Whey proteins are rich on essential amino acids which makes their biological value higher in comparison to other proteins of animal and vegetable origin, including egg proteins that were regarded as referent for a long period of time. Therapeutic value of whey is familiar since the era of ancient Greeks and was used for treatments of tuberculosis, hepatitis, diarrhoea, skin eczema, etc. \(^{(3,4)}\)

Buttermilk originates from butter production and contains lactose, proteins, milk fat globule membrane (MFGM), minerals and lecithin. Numerous studies have shown that MFGM contain bioactive compounds with antitumor and cholesterol lowering influence, and which act inhibitory on \textit{Helicobacter pylori} or prevent gastrointestinal infections. Buttermilk is usually being processed by fermentation with lactic acid bacteria, while whey is placed on the market most commonly as pasteurised acid whey. Nevertheless, the quantities of buttermilk and whey which are available on the market are negligible. Both of the previously described by-products represent a very good basis for production of beverages of excellent nutritional value on the one side, and with low energy value on the other side. Such properties are more than welcome in terms of modern consumers’ demands. Thus, the aim of review article would be to highlight the necessity to increase the production of whey and buttermilk based beverages, as well to present current achievements regarding research focused on whey and buttermilk processing into beverages.

COMPOSITION AND CHARACTERISTICS OF WHEY AND BUTTERMILK

\textit{Whey}

Whey is a by-product of milk coagulation by acids and/or renneting enzymes during cheese or casein manufacturing. Thus, it is produced in volumes close (80 - 90 \%) to that of the processed milk used during cheese manufacture and requires therefore proper management \(^{(1)}\). Depending on the processing technique used to separate casein from milk, there are two main types of whey to distinguish – sweet whey with a pH of 5.9 – 6.6 and acid whey with a pH of 4.3 – 4.6. Gross whey composition comprises 80 – 90 \% of the total volume of original milk, having the total dry matter about 6 - 6.5 \%. It contains lactose (~ 70 \%), minerals (~12\%) (both depending on the acidity of the whey), whey proteins (~10\%), vitamins and some fat \(^{(2)}\). Whey also contains small quantities of components like organic acids, non-protein nitrogen compounds (urea and uric acid) and B group vitamins. It's composition and sensory characteristics mostly depend on the production process (acid or sweet), but also on the used milk (cow, sheep, goat etc.), the season and the stage of lactation. The main differences are in the calcium, phosphate, lactic acid and lactate contents, which are higher in acid whey \(^{(3)}\).
Knowing its composition, whey has a very high polluting potential due to a high Biochemical Oxygen Demand (BOD; 40,000-60,000 mg L\(^{-1}\)) and high Chemical Oxygen Demand (COD, 50,000-80,000 mg L\(^{-1}\)) which are mostly conditioned by very high lactose contents in the dry matter \((4,5,6)\). Thus, it has been estimated that the waste load of whey is 100-175 times higher than that of a similar volume of domestic waste water \((7,8)\). Accordingly, adequate whey management is one of the priorities of the modern dairy processing sector.

Throughout the late years of the 20\(^{th}\) century a large number of scientific evidence indicated biological and technological value of whey. Such data were somewhat shocking to dairy processors who treated whey as waste and disposed it for decades. Nowadays it is rather recognised as a highly valuable raw material which can be exploited by the agri-food, biotechnology, pharmaceutical and similar industries \((8,9)\).

**Whey proteins – most valuable bioactive components of whey**

Whey proteins are for sure the unique component responsible for the high nutritional and technological value of whey. They are mainly composed of thermosensitive fractions β-lactoglobulin (β-Lg), α-lactalbumin (α-La), blood serum albumin (BSA) and immunoglobulins. In lower amounts thermo-stable proteose-peptones as well as lactoferrin and lactoperoxidase are present too. Additionally, sweet whey contains caseinomacropeptide (CMP) resulting from chymosin (or pepsin) cleavage of k-casein. It constitutes approximately 20 % of the sweet whey protein fraction and is not found in acid whey unless enzymatic coagulation was included in the process of manufacturing fresh cheese \((4,10)\). Whey proteins are small globular proteins with amino acid profiles quite different from caseins due to high ratios of essential amino acids, smaller fraction of Glutamic acid (Glu) and Proline (Pro), but a greater fraction of sulfur-containing amino acid residues (i.e. cysteine and Methionine) as well as higher contents of the branched chain amino acids (BCAA) which were recognized as important metabolic regulators in homeostasis of proteins, glucose and lipids \((7,8)\). The parameters defining protein quality such as the Protein Corrected Amino Acid Score (PDCAAS) or Biological Value (BV) of whey protein exceeds that of egg protein (the former benchmark), meat, fish, wheat and nuts \((9)\). Accordingly, whey proteins have a superior nutritional value in comparison to common dietary sources of proteins.

Enzymatic hydrolysis of whey proteins in the human digestive system, fermentation of milk by starter cultures and hydrolysis by plant or microbial proteases results in releasing bioactive peptides. Once released, bioactive peptides act as signalling molecules and exert various physiological effects on the immune, gastrointestinal, cardiovascular and nervous system \((11,12)\). There is a growing number of scientific evidence confirming valuable influence of whey proteins on human well-being such as
antimicrobial, antioxidant, antihypertensive, antidiabetic and immunomodulatory properties, and may take part in mechanisms of reducing body mass as well as reduce or inhibit allergic reactions \((12, 13, 14)\). The most abundant whey protein \(\beta-Lg\) is a source of multiple bioactive peptides like lactokinins possessing ACE inhibitory ability, \(\beta\)-lactorphin acting as opioid agonist and ACE-inhibiting; and \(\beta\)-lactotensin which was proven to have an anti-stress effect, but also to have the binding ability to neuroreceptors responsible for satiety feeling regulation \((7, 12, 14)\). \(\alpha-La\) was demonstrated to be cytotoxic, to act protective against mucosal injuries and oxidative stress, as well as to act opioid, anti-inflammatory and anti-carcinogenic. Due to high tryptophane content \(\alpha-La\) might be used to improve sleep, mood and cognitive functions. More recently a new genetic variant of \(\alpha-La\) was isolated and identified, the so called HAMLET (human \(\alpha-La\) made lethal to tumor cells) that showed the ability to induce apoptosis of tumor cells while sparing healthy tissues at the same time \((7, 15)\). Among other whey protein fractions, lactoferrine should be mentioned as a source of lactoferricine – a bioactive peptide with confirmed antimicrobial, antiviral and immunomodulatory activity. Also, lactoperoxidase was demonstrated to possess high antimicrobial activity, while products containing this protein are successfully implemented in oral healthcare to promote healing of bleeding gums, gingivitis and oral irritation \((8, 9)\). In addition, whey proteins have excellent functional properties, such as good solubility, viscosity, gelling and emulsifying properties, and their concentrates are widely used in the food industry \((7)\).

**Buttermilk**

Buttermilk is the aqueous phase released during the churning of cream in butter production. In terms of ash and lactose content buttermilk is similar to whey, but contains much higher amounts of proteins \((16)\) (Table 1). However, they largely differ in terms of lipid fractions. Buttermilk contains about 4.6–14.5% fat in the dry matter \((17)\) whereby a specific component derived from milk fat globule disruption during churning is present – the milkfat globule membrane (MFGM). Since MFGM is rich in phospholipids, buttermilk contains up to seven times higher concentrations (about 0.89 mg g\(^{-1}\)) of phospholipids than whole milk (about 0.12 mg g\(^{-1}\)) \((18)\). Annual production of buttermilk in EU28 countries in 2016 accounted for 0.5 million tons \((19)\).

**Table 1. Comparative gross composition of sweet/acid whey and buttermilk \((3, 20)\)**

There are few types of buttermilk that should be distinguished. Most of the studies have been focused on investigating sweet buttermilk originating from churning of uncultured cream. There are also other types of buttermilk that can be produced such as sour buttermilk obtained from churning of cultured cream, or whey buttermilk originating from churning of whey cream during manufacture of whey butter. Whey buttermilk is produced in much smaller quantities compared to the volume of sweet buttermilk.
(21,22), but the potential market for whey buttermilk is huge considering the large volumes of whey produced annually.

Sodini et al. (21) determined the gross composition of sweet and sour cream buttermilk as well as of whey buttermilk. According to their results, sweet cream buttermilk had the highest content of total nitrogen (31.5%) and the lowest content of fat (13.1%). Whey buttermilk had the highest content of phospholipids and lactose. The lowest amount of nitrogen in whey buttermilk might be explained by the lack of casein in whey cream (22).

Libudzisz and Stepaniak (18) discussed the ability of buttermilk to increase the heat stability of recombined milks, mainly due to phospholipid-protein interactions preventing protein coagulation during sterilization. Thanks to casein and considerable amounts of phospholipids, buttermilk is characterised by good emulsifying properties suitable for wide use in the food industry (22,23). In some countries like Russia, Poland, Czech Republic, Finland or Germany, naturally fermented buttermilk is merchandised as a beverage or is sometimes used for animal feed. Problems with increasing the consumption of traditional buttermilk reflect in the short shelf-life (about 1 week at 4–7 °C), in obtaining a uniform quality and in the lack of marketing promotion. Therefore, without putting a special effort to commercialize natural buttermilk, its consumption is destined to decrease (21).

**Bioactive components of buttermilk**

During butter manufacture, milk fat globules are destabilized and disrupted by churning which results in exclusion of the membrane covering the lipid matrix and its recovery in buttermilk along with other milk/cream components contained in the aqueous phase. That way a component known as MFGM is particularly rich in various proteins and phospholipids which possess an outstanding potential for functional and nutraceutical applications. Some of those are related to the prevention or amelioration of widespread chronic diseases such as cancer, obesity, diabetes and cardiovascular disorders (24,25). Buttermilk has received increased interest, as food science and nutrition research turns to develop products with not only increased nutritional value but also health-promoting properties. The MFGM is complex in composition as well as in its supramolecular structure (26). It is mostly constituted of protein fractions (average content about 1800 mg/100 g) followed by polar lipids (about 730 mg/100 g), although some other components like vitamin A, carotenoids, enzymes, adsorbed casein and whey proteins are present too (27). Major MFGM proteins are butyrophilin (BTN), xanthine oxidase/dehydrogenase (XO/XDH), mucin-like glycoproteins MUC1 and MUC15, adipophilin (ADPH), breast cancer type 1 and type 2 susceptibility protein (BRCA 1 and BRCA2). The most abundant polar lipids found in the MFGM isolated from buttermilk are phospholipids and sphingolipids, more exactly
fractions of phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, phosphatidylinositol and phosphatidylserine (20). Both of these have been proven to excrete numerous beneficial effects on the human health, some of which are presented in Table 2.

**Table 2. Overview of beneficiary effects of MFGM proteins and polar lipids (20,27,28,29)**

Considering clinical trials, anticholesterolemic effect of sphingomyelin originating from MFGM has been examined since 1979, when Howard and Marks observed a significant decrease in serum cholesterol levels in patients who consumed cream in comparison to those who consumed butter. They concluded that such trends were most probably related to beneficial effects of MFGM which is present in cream, but not detected in butter (29). Ito et al. (30) also conducted a clinical study and confirmed cholesterol lowering effect in hypocholesterolemic mice fed by cream. Noh and Koo (31) found that hypercholesterolemic effect of sphingomyelin relies on its inhibitory action towards cholesterol absorption in the intestinum. More recently, Baumgartner et al. (32) reported that a daily consumption of buttermilk during 12 weeks significantly lowered LDL-cholesterol in individuals taking part in their clinical study. Conway et al. (24) reported that daily consumption of 45 g buttermilk significantly lowered LDL-cholesterol levels in patients with hyperlipidemia. Conway et al. (25) investigated the effects of buttermilk consumption on blood pressure and on markers of the renin–angiotensin–aldosterone (RAS) system in humans. The obtained results were promising and confirmed that buttermilk consumption significantly reduced systolic blood pressure, the mean arterial blood pressure and plasma levels of the angiotensin I-converting enzyme compared with the placebo.

Thus, similarly to whey, buttermilk is also a nutritionally valuable coproduct with numerous potentially beneficial effects on human well-being. The production of buttermilk beverages should therefore be one of key points when considering the future of milk based beverages.

**PRODUCTION OF WHEY AND BUTTERMILK BEVERAGES**

*Whey beverages*

Production of whey based beverages appears to be the most economical and simplest solution for whey utilization in human nutrition. The need for development of whey based beverages is closely related to nutritional and functional properties of whey proteins, as well as to fulfilling the expectations of modern consumers who demand innovative products with enhanced functionality (33). Up to now, numerous research studies have been focused on optimising whey beverage production from native or from processed whey (e.g. powdered, de-proteinated, diluted) (34). Along with technological
development, the application of alternative food processing methods such as membrane processes, high intensity ultrasound or supercritical carbon dioxide technology is being investigated too. By applying non-thermal processing methods, hurdles like the low shelf life due to microbiological deterioration, occurrence of undesired sediments or whey protein denaturation might be overcome, but also the improvement of the existing products could be achieved \((9,35,36,37)\). Over the past two decades, numerous whey based beverages and similar products containing isolated whey components (mainly whey proteins) were placed on the market as refreshing, value added and/or functional foods \((33)\).

**Non fermented whey beverages**

Although the easiest solution for designing a functional whey beverage seems to be using native sweet or acid whey as a basis, recently it has been suggested to use de-proteinised whey or whey permeate remaining after ultrafiltration \((33)\) in order to avoid undesirable sediment formation and blurring. Among the most frequently examined combination is for sure the addition of orange juice to whey which is often accompanied by adding CO\(_2\). Chatterjee et al. \((38)\) tested the production of a refreshing beverage consisting of pre-concentrated whey and orange juice in various ratios. The mixture of orange juice and concentrated whey in ratio 3:2 appeared to be the optimal formulation evaluated by the best sensory properties. The shelf life at room temperature was determined to 11 days, while it lasted up to 3 months under cold storage conditions. Kumar and Bangaraiah \((39)\) also tested multiple formulations of orange juice and whey for sensory properties, chemical composition and shelf life. Thereby two formulations (70 % whey and 30 % orange juice; 65 % whey and 35 % orange juice) were evaluated by the highest sensory scores, while all of the produced beverages showed good microbiological stability and the potential to be stored up to 2 months at room temperatures. Sady et al. \((40)\) studied the production of whey-orange juice beverage and compared it’s properties to the same beverage produced without whey. The beverage containing whey was characterized by higher contents of proteins, ash, glucose, lactose and vitamin B, but contained less sucrose, fructose and vitamin C. Also, no considerable difference was detected in the polyphenolic content and the sensory evaluation of colour desirability. Pareek et al. \((41)\) investigated the production of refreshing carbonated beverage consisting of orange juice and whey in different ratios \((70:30, 60:40\) and 50:50\). The mixture containing 70 % orange juice and 30 % whey was the most acceptable one and was characterised by a general increase in nutrients in comparison to the standard orange juice. Very often the addition of tropical fruits or berries to whey was investigated too. For instance, Jaworska et al. \((42)\) added blackcurrant to acid whey and compared the characteristics between the pure blackcurrant juice and whey-blackcurrant beverage. The blackcurrant-whey beverage had higher amounts of ash, proteins and vitamin B\(_2\) while pure blackcurrant juice showed slightly higher
antioxidant activity and received higher scores at sensory evaluation. Baccouche et al. (43) investigated the addition of prickly pear juice to acid whey. According to the obtained results the beverages were physically stabilized by adding sugar and pectin to the heat-treated whey. Singh et al. (44) studied the production of whey-guava beverage consisting of approximately 68% whey and 20% guava pulp. The beverages were processed by pasteurisation at several temperature/time regimes and were cool stored up to 90 days. The best beverage appeared to be the one pasteurized at 65°C/25 min and cool stored for 45 days. Chavan et al. (45) optimised the production of a whey-mango beverage by mixing whey powder, whey protein concentrate or fresh whey with mango pulp or mango powder. The produced beverages were analysed for chemical composition, sensory properties and microbiological parameters. The obtained results showed that regardless of prior processing (drying, concentrating) whey could be successfully utilized for beverage production, although a significant increase in acidity was detected in all samples. The beverage made from whey protein concentrate and mango powder showed good overall acceptability after 30 days of storage at refrigeration temperature. Valadao et al. (46) examined the possibilities of using Riccotta cheese whey in sports drink production and found that tangerine, passionfruit and strawberry-passionfruit recipes achieved the best sensory scores. Janiaski et al. (47) optimised the production of strawberry enriched fermented and non-fermented whey beverages. The non-fermented whey-strawberry beverages were recognized as not enough acidic and viscous, having a more intense artificial strawberry aroma.

Some authors have studied the addition of herbs or spices in order to design a novel functional whey beverage. Yadav et al. (48) studied the production of whey beverage enriched by banana juice and Mentha arvensis extract addition. The optimal addition of Mentha extract was estimated at max. 2%, while the shelf life was determined to be 15 days. Similarly, Kumar et al. (49) developed a beverage from the ripe pineapple juice, whey and Mentha arvensis extract in the amount up to 3%. The produced beverages were subjected to analysis of storability at refrigerated, of BOD at room temperature, while the eventual changes were determined at 15 day-intervals up to 2 months. The most acceptable beverages were the ones containing between 0 and 1 % of Mentha arvensis, while in samples containing higher concentrations a decrease in overall sensory quality was observed. Satpute et al. (50) also incorporated Mentha arvensis extract into whey, but also beetroot pulp was added. There were 4 different types of beverages produced which were analysed for chemical, sensory and microbiological parameters. Among all of the prepared beverages, the one consisting of 80 % whey, 20 % beetroot and 6 % Mentha arvensis extract was evaluated as the best one. Alane et al. (51) optimised preparation of a whey-based mango herbal beverage with addition of ginger extract in amounts from 0.5 – 5 mL (v/v). Thereby the highest overall acceptability achieved the sample containing mango 10 g, sugar 8g, whey 82mL, 0.5 % ginger extract and 0.05 % guar gum. However,
the microbiological quality was not satisfying for neither of the produced beverage samples during the tested storage period.

Regardless of the components chosen to create a refreshing whey beverage, the occurrence of sediment, salty sour taste and odour of cooked milk are still not avoided. Adding sufficient quantities of fruit base is critical for reaching the desired sensory quality, but on the other hand certain components of the fruit dry matter tend to precipitate and adversely influence beverage appearance. Thus, other solutions such as fermentation or non-thermal processing methods need to be reconsidered for application in whey beverage processing.

**Fermented whey beverages**

Fermented beverages have been recognized by consumers all over the world for their therapeutic value. Considering the fact that whey contains almost 70% of lactose present in starting milk, fermentation to yogurt-like drinks appears to be a meaningful way of whey utilization. Since fermentation process is accompanied by a decrease in pH due to transformation of lactose to lactic acid, sweet whey appears to be a better choice for fermented beverage production. Whey fermentations usually employ starter and/or probiotic cultures able to metabolise lactose such as *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus reuteri*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, *Propionibacterium freudenreichii* ssp. *shermanii*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Enterococcus faecium*, *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus paracasei* (33). Sohrabi et al. (52) studied the production of whey beverage fermented by a commercially available yoghurt culture DELVO®-YOG TY-17A. Thereby whey protein concentrate was reconstituted and enriched by adding vitamin E prior to pasteurization and fermentation. At the same time, identical beverage was produced but without fermentation. Both of the produced drinks were analysed for chemical, microbiological and sensory parameters. Although there was no considerable difference in the chemical composition, fermented beverage achieved significantly higher overall acceptability and sensory scores. Very often probiotic whey beverages are investigated and are generally considered as one of the target segments of developing whey utilization to minimally processed functional products with added value (53). Among the most important factors is the chosen probiotic strain since it determines the unique flavour and texture of the fermented beverage. *Lactobacillus rhamnosus* was frequently used, but since it lacks on enzyme β-galactosidase, it does not have the ability to metabolize lactose. Therefore, it is often necessary to hydrolyse lactose prior to the fermentation process or to use a proper co-culture. Pescuma et al. (54) studied the fermentation of whey protein concentrate by the strains *Lactobacillus acidophilus* CRL 636, *Lactobacillus delbrueckii* subsp.
bulgaricus CRL 656 and Streptococcus thermophilus CRL 804, as single or mixed cultures. Fermented whey was then mixed with peach juice and calcium lactate and stored for 28 days at 10 °C. According to the obtained results, mixed cultures and single culture Streptococcus thermophilus CRL 804 showed a good surviving potential during the tested storage period. Also, all of the tested strains degraded β-lactoglobulin (41–85 % after 12 h incubation), which is of a great importance since β-Lg B is one of the major milk allergens. Koc et al. (55) fermented whey by probiotic strains Lactobacillus plantarum, Lactobacillus brevis and their combination. Fermented whey was supplemented by different fruit concentrates (lemon, mango, pineapple, apple, grape) and sucrose in order to mask the bitter flavour and achieve acceptable sensory characteristics. According to the obtained results, beverage inoculated with Lb. plantarum and enriched by pineapple concentrate was the most preferred one. Seyhan et al. (56) studied the production of fermented whey beverages from whey powder fortified with soy isoflavones or phytosterols and inoculated by probiotic strains Lactobacillus acidophilus La-5 or Lactobacillus casei LBC-81. The addition of nutraceuticals did not change the basic composition of the produced beverages, but the phytosterols-fortified beverages were significantly more acceptable in terms of sensory quality and would be suitable for industrial scale production. Similarly, Schlabitz et al. (57) studied the shelf life of probiotic fermented products produced from different mixtures of whey and powder milk. The beverages were produced by inoculation with probiotic strains Lactobacillus acidophilus LA-5, Bifidobacterium animalis subsp. lactis BB-12 and Streptococcus thermophilus. Those were fortified by adding prebiotics, strawberry pulp and strawberry flavour. 11 formulations were developed and were analysed for chemical, microbiological and sensory parameters. The obtained results confirmed the possibility of producing a probiotic fermented beverage containing up to 70 % Ricotta cheese whey. Yasmin et al. (58) found that fermented whey beverages supplemented with probiotics plays an important role in lowering cholesterol levels in rats. Faisal et al. (59) investigated the production of probiotic fermented whey beverage enriched with orange powder. Beverages were produced using the fresh Cheddar cheese whey supplemented by orange juice powder and orange flavour and fermented at 42°C by a combined thermophilic probiotic culture consisting of strains Lactobacillus acidophilus La-5, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus and Bifidobacterium sp BB-12. The best ranked beverage consisted of 1 litre cheese whey, 0.70 g stabilizer, 8% sugar, 1% orange powder and 0.40 mL flavour. The authors concluded that addition of orange flavour and sugar into whey fermented by probiotic strains might be a successful method for utilizing cheddar cheese whey into beverages with acceptable sensory characteristics. Skryplonek and Jasińska (60) and Skryplonek (61) studied the potential of acid whey to be used in fermented drink production. Acid whey was inoculated with yoghurt culture and by probiotic strains Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp. lactis BB-12. For further supplementation buttermilk powder, sweet
whey powder, condensed milk, UHT milk and skim milk powder were tested. The obtained results showed that acid whey could also be used as a raw material to manufacture probiotic fermented beverages and could also provide sufficient levels of bacteria required to ensure health benefits to consumers.

Recently, some studies focused on production of kefir-like whey beverages. Pereira et al. (62) studied fermentation of ultrafiltered whey protein concentrates by kefir grains and/or selected probiotic strains. The fermented drinks were acceptable relating to their physicochemical and sensorial properties, and contained satisfactory counts of microorganisms after 14 days of refrigerated storage. Magalhães et al. (63) compared kefir produced from milk to the one produced from whey by using kefir grains. Lactose hydrolysis, ethanol levels, formation of organic acids and volatile compounds were determined during fermentation were compared among samples of milk kefir and whey kefir. The obtained results indicated that kefir grains utilized lactose from whey and deproteinised whey and produced similar amounts of ethanol, lactic acid and acetic acid to those obtained during milk fermentation. Thus, whey could be a valuable substrate for production of kefir-like beverages.

**Application of non-thermal processing methods in whey beverages production**

In recent years, lots of efforts have been put into optimizing application of non-thermal processing methods into whey processing. In order to partially or entirely prevent the undesired advents such as sediment formation, poor mouthfeel, salty sour taste, etc., different attempts were made including for example the application of membrane processes or whey treatment by high intensity ultrasound. Barukčić et al. (36, 64) examined the possibility of applying microfiltration and ultrafiltration in order to achieve adequate microbiological stability without whey protein denaturation and sediment formation. Promising results were obtained by combining microfiltration with a 0.5 µm membrane and a subsequent ultrafiltration with a 0.2 kDa membrane. Both of the used membranes were ceramic and the process was performed at 20 °C. Thereat optimal microbiological quality was obtained and the nutritional quality was almost entirely preserved meaning that whey proteins were maintained in the native state. Such findings were better in comparison to those achieved by the usually applied pasteurization at 73 °C / 20 s which opened new possibilities of producing minimally processed whey based beverage of excellent nutritional quality. Ultrafiltration or reverse osmosis allow whey concentration and could solve the problem of the poor mouthfeel inherent to whey beverages (65).

Accordingly, Dilipkumar and Yashi (66) investigated a production of refreshing fruit beverage using the native, pre-filtered and ultrafiltered (UF) acid whey as basis. Thereby, the addition of mango, pineapple and orange juice in different amounts (18, 20, 22, 24 %) was tested. The best properties and overall acceptability among consumers was recorded for the beverage consisting of UF whey basis enriched by the addition of 22 % of pineapple juice and carbonated. Since the implementation
of membrane processes eludes great nominal financial expenses, they are mainly used for the production of more cost-effective products such as whey protein concentrates or isolates. In order to establish a production process with no waste products, many authors have proposed the utilization of the remaining UF permeate. Beucler (67) investigated the application of whey permeate for production of carbonated beverage enriched by addition of different flavours and vitamins. Amaral et al. (37) investigated the application of supercritical carbon dioxide for application as alternative technology to thermal processing during whey-grape juice. They found no differences in juice characteristics (acidity, total solids, antioxidative activity) processed by conventional heat treatment and by the supercritical carbon dioxide technology. High intensity ultrasound was also investigated for purposes of preventing sediment formation or reducing its amount, for improving the fermentation process or for partial substitution of pasteurization (35, 68). Barukčić et al. (68) investigated the influence of high intensity ultrasound application on the quality and on fermentation process of reconstituted sweet whey. In the first stage of the study, whey was subjected to treatments with different power inputs (480 W, 600 W) over 6.5, 8 and 10 min at constant temperature (45 °C, 55 °C). Treated whey samples were analysed for microbiological quality, particle size distribution, protein content, acidity, electrical conductivity, viscosity and sensory properties. All of the analysed parameters were compared to the control sample (pasteurized) and to fresh whey. Whey thermosonation by nominal power of 480 W for 10 min at 55 °C resulted in better microbiological quality and sensory properties in comparison to whey pasteurization. Hereafter, influence of high intensity ultrasound on whey fermentation with yoghurt culture YC-380 and with monoculture Lactobacillus acidophilus La-5 (both Christian Hansen, Hersholm, Denmark) was investigated. Different ultrasound treatments were applied for culture activation prior to or after the inoculation, and treatment with nominal input power of 84 W over 150 s resulted in the highest increase of the viable count during the activation process. Jeličić et al. (35) studied the application of a combination of high intensity ultrasound with nominal inlet power of 400 W with moderate heat (55 °C) in whey processing. The applied treatment resulted in a better reduction of total bacteria, coliform bacteria and yeasts and moulds number in comparison to conventional pasteurization process. Also, sensory properties were improved regarding mouth feel, absence of sediment and unchanged colour in all of the ultrasound-treated whey samples. Thus, along with development of new processing technologies whey utilization could be improved too. There are numerous scientific studies that have proven the feasibility as well as the importance of producing whey based beverages and similar products, not only for resolving the question of environmental pollution, but also for its outstanding nutritional value.
Buttermilk beverages

Non-fermented buttermilk beverages

According to its composition, buttermilk has a great potential for utilization in producing innovative food products based on protein and carbohydrate dairy raw material. Knowing these facts Meshram (69) studied the production of buttermilk based fruit beverages containing certain amounts of mango, orange and banana. Composition of an acceptable beverage formulation mostly depended on the type and the amount of fruit juice content which ranged from 10 % for mango to 35 % for orange, while the buttermilk content of the beverages ranged from 62 % to 83 %. Among all tested beverages, mango buttermilk beverage was found to be most acceptable. It contained 12 % mango juice, 81 % buttermilk, 7 % sugar, 0.05 % pectin, 0.12 % CMC, and pH was 4.25. Buttermilk can also be very well utilised in milkshakes, since it contains considerable amounts of phospholipids which decrease surface tension on “liquid-air” boundary and assist foamy structure creation at mechanical beating of buttermilk (70). Buddhadasa et al. (71) investigated the addition of fruit juices or pulps to production of buttermilk based beverages. Soursop (Annona muricata) is a fruit is rich in vitamin B, potassium, fructose and vitamin C, and is also proven for anticancerogenic properties as well as for broad spectra of antimicrobial activities against bacterial and fungal infections, and is used for depression and stress. According to the obtained results, the addition of 13 % soursop and 12 % sugar was recognized as optimal for the production of a functional buttermilk beverage. Mudgil and Barak (72) optimized buttermilk production enriched by 4% soluble fibre (partially hydrolysed guar gum) and improved thereby its physicochemical and sensory characteristics. Fibre fortification of buttermilk considerably reduced the phase separation which is a serious problem in reference to consumer acceptance. Kiruthika et al (73) investigated the production of buttermilk based beverage supplemented with millet - Bengal gram flour paste and spices (cumin, salt, ginger extract, Asafoetida, green chilli extract, curry leaves, coriander leaves). All beverages were tested for physico chemical, microbiological and sensory properties during a storage period of 7 days. The optimal ratio of buttermilk and paste was determined at 80:20, while the produced beverage was described as superior in comparison to sole buttermilk due to better sensory properties and richer nutritional composition.

Similar to whey, there have also been made some efforts in developing carbonated beverages. Shaikh and Rathi (74) investigated the development of fruit-flavoured carbonated buttermilk beverages. According to the obtained results, sensory, physicochemical and nutritional parameters were improved by addition of mango, pineapple and orange fruit fresh juices. The best combination, evaluated from the sensory panel, was the fruit-flavoured carbonated buttermilk beverage prepared by the addition of 12 % sugar and 24 % pineapple juice.
Fermented buttermilk beverages

Cultured buttermilk is a by-product in butter production from cultured cream, but can also be produced by fermentation of sweet buttermilk. Sweet buttermilk used for buttermilk production should be of the highest quality from the compositional and microbiological points of view.

Starter cultures for the production of fermented buttermilk usually contain strains of *Lactococcus lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris* and *Leuconostoc mesenteroides* subsp. *cremoris* (75). These species produce mainly lactic acid and are often referred to as acid producers, in contrast to *Leuconostoc* spp., which ferment citric acid and produce important metabolites, such as CO$_2$, acetaldehyde and diacetyl which are referred to as aroma and flavour compounds (22). The most important is to achieve the balance between the aroma and intensity. Wróblewska et al. (75) reported that max. 20% of aroma-producing bacteria would be recommended for use in order to achieve diacetyl at concentrations of 2–5 mg kg$^{-1}$, which are responsible for the characteristic ‘buttery’ flavour and aroma. Therefore, supplementation of milk with citrate or citric acid is often recommended. Another very important carbonyl-flavouring compound produced by lactic acid bacteria in buttermilk is acetaldehyde that is undesirable and if present in excess, responsible for the flavour defect described as ‘green’ or ‘yogurt’-like. In starters for the production of buttermilk, it is better to use *Leuconostoc mesenteroides* subsp. *cremoris* rather than *Lactococcus lactis* subsp. *lactis* for flavour production. Generally, in order to obtain buttermilk with desirable organoleptic properties, the optimum ratio of diacetyl and acetaldehyde needs to be around 4:1 (18). The important sensory characteristics of buttermilk resulting from mesophilic fermentations are the typical consistency, which is due to the coagulation of milk proteins by lactic acid, and the aroma and flavour produced by the fermentation of citric acid and lactose. Fermented buttermilk is characterized by thick, smooth, fairly viscous properties. The texture also depends on whether or not polysaccharide producing strains are included in the starter culture and on the concentration of total solids (18).

Bassi et al. (76) evaluated three fermented milk beverages supplemented by sugar and strawberry puree post-fermentation. The base was composed of 70 % of milk, with whey and buttermilk added in quantities of 30 % and 0 %, 15 % and 15 %, and 0 % and 30 %, respectively. The chosen starter culture developed well in all beverage formulations reaching pH 4.7 – 4.9 within 180 min of fermentation. Lactic acid bacteria in the products were above 8 log CFU mL$^{-1}$ throughout the storage period, while there were no considerable differences in the pH, acidity and viscosity. The authors concluded that buttermilk and whey could be interesting ingredients for fermented beverage production, because the consumers liked all the products equally. Mudgil et al. (77) studied a production of cultured buttermilk beverage supplemented by *Aloe vera* juice. The produced beverage was analyzed for viscosity, phase separation, acidity, pH and sensory properties. There were no significant differences in acidity of the developed beverages, but it was noticed that *Aloe vera* juice
decreased the phase separation and increased the viscosity of the beverage. Samples with 10 % *Aloe vera* juice obtained the highest scores in the sensory evaluation and had improved nutritive and physicochemical properties as well. Sheth and Hirdyani (78) discussed a development of a buttermilk based fermented drink fortified with barley and fructooligosaccharide (FOS) as functional ingredients. Barley was pre-cooked, added to buttermilk and fermented, after which FOS, different flavours (rose, khus, chocolate and salt-jeera) and colours were added. A panel of 24 trained members evaluated the beverages for colour and appearance, mouthfeel, texture, taste, after taste, and overall acceptability. According to the obtained results, there was no significant difference among drinks with different flavours, but salt-jeera flavour was the most appreciated by all panel members, followed by rose. No after taste or bad mouthfeel was reported in any of the beverages.

**WHEY AND BUTTERMILK BEVERAGES AS HEALTH PROMOTING FOODS**

Taking into consideration a constantly increasing number of data published within studies focused on investigating properties of whey and buttermilk as well as options for their utilization, it is obvious that both of these by products have more than a promising potential for functional food production. Considering the production costs as well as the effects processing technologies such as drying at high temperatures on nutritional properties, production of beverages seems to be the logical solution for utilization of whey and buttermilk., especially since it has been extensively researched over the past 10 years (Table 3). In addition, functional beverages are popular among various consumer groups either as meal replacements or for purposes like rehydration after exercise, refreshment and rehydration during exposure to high environmental temperatures, easy available and *ready-to-eat* healthy snacks, etc. Those are mostly consumed by athletes, health conscious working population and consumers eager on including healthy alternatives into the everyday diet (80). Compared to non-diary beverages, whey and buttermilk are considered as a pool of bioactive proteins, peptides and/or lipids with numerous health promoting activities such as antimicrobial, anticancerogenic, antihypertensive, antiulcerative, anti-tumor, anti-diabetic, cholesterol-lowering and Immunomodulatory. Besides, they are also rich on B-group vitamins, minerals, especially calcium, sodium and potassium, and lactose – a disaccharide with relatively low glycemic index (26). Therapeutic value of whey is known for centuries and has already been exploited for healing diarrhoea, bile illness, arthritis, various intoxications, anaemia, liver and skin diseases in specialized institutions that were intensively established in Switzerland, Germany and Austria during the 18th century (81).
Nowadays, there is an increasing occurrence of diseases related to stress, unhealthy diet and/or busy lifestyle such as atherosclerosis, obesity, diabetes, osteoporosis, etc. Knowing the aforementioned properties, buttermilk and whey based beverages might be an excellent choice for overcoming these disorders. Some clinical trials have already demonstrated their effectiveness in reducing and controlling blood pressure and serum cholesterol levels (24, 25, 32, 82), regulation of blood sugar levels in diabetes type 2 patients (83), reduction and control of body mass (84, 85), supporting recovery of children suffering from malnutrition (86), etc. Accordingly, whey and buttermilk are much more than by-products of dairy industry and their valorisation into functional beverages with exceptional nutritional value should be one of target points in creating the future of dairy products.

CONCLUSION

Whey and buttermilk based beverages target a large scale of consumers - from little children to old people. Their nutritional properties as well as potential health promoting effects meet the requirements of modern consumers who are increasingly becoming aware of the great influence food has on humans. Beverages containing a certain amount of fruits or carbonated beverages have gained the most attention up to now, and were evaluated as acceptable to the consumer. Besides, very large interest has also been detected for fermented beverages, especially if those contain probiotic strains. Thus, there are many options for creating whey or buttermilk based beverages that are delightful to the consumer. However, the main problem seems to be in the lack of information that dairy industry has put on disposal to the consumers considering the advantages of these products. In turn, there is insufficient interest for such beverages, which makes the reviewing of their production rather risky from the dairy industry point of view. So, future perspective lies in putting more efforts into marketing and informing activities in order to draw the consumers’ attention to the outstanding nutritional and functional properties of whey and buttermilk.

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Table 1. Comparative gross composition of sweet/acid whey and buttermilk (3,20)

<table>
<thead>
<tr>
<th>Constituent %</th>
<th>Sweet whey</th>
<th>Acid whey</th>
<th>Sweet cream buttermilk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>6.00</td>
<td>6.40</td>
<td>9.80</td>
</tr>
<tr>
<td>Fat</td>
<td>0.05</td>
<td>0.05</td>
<td>0.59</td>
</tr>
<tr>
<td>True protein</td>
<td>0.60</td>
<td>0.60</td>
<td>3.73</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.50</td>
<td>4.60</td>
<td>4.81</td>
</tr>
<tr>
<td>Ash (minerals)</td>
<td>0.50</td>
<td>0.80</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Table 2. Overview of beneficiary effects of MFGM proteins and polar lipids (20, 27, 28, 29)

<table>
<thead>
<tr>
<th>Component</th>
<th>Potential health effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid binding protein (FABP)</td>
<td>Cell growth inhibition</td>
</tr>
<tr>
<td></td>
<td>Anticancer factor</td>
</tr>
<tr>
<td>Butyrophillin (BTN)</td>
<td>Suppression of multiple sclerosis</td>
</tr>
<tr>
<td></td>
<td>Influences pathogenesis of autistic behaviour</td>
</tr>
<tr>
<td>Xanthine dehydrogenase/oxidase (XDH/XO)</td>
<td>Bactericidal agent</td>
</tr>
<tr>
<td></td>
<td>Anti-inflammatory action</td>
</tr>
<tr>
<td>Breast cancer type 1 susceptibility protein (BRCA1)</td>
<td>Inhibition of breast cancer</td>
</tr>
<tr>
<td>Breast cancer type 1 susceptibility protein (BRCA2)</td>
<td></td>
</tr>
<tr>
<td>Sphingolipids and metabolites</td>
<td>Shift in tumor type from malignant to benign</td>
</tr>
<tr>
<td></td>
<td>Anticholesterolemic</td>
</tr>
<tr>
<td></td>
<td>Protection of the liver from fat- and cholesterol induced steatosis</td>
</tr>
<tr>
<td></td>
<td>Suppression of gastrointestinal pathogens (Campylobacter jejuni, Listeria monocytogenes,</td>
</tr>
<tr>
<td></td>
<td>Clostridium perfrigens, E.coli, Salmonella enteritidis)</td>
</tr>
<tr>
<td></td>
<td>Neonatal gut maturation</td>
</tr>
<tr>
<td></td>
<td>Myelination of the developing central nervous system</td>
</tr>
<tr>
<td></td>
<td>Endogenous modulators of vascular function</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>Restore normal memory on a variety of tasks</td>
</tr>
<tr>
<td></td>
<td>Positive effects on Alzheimer patients (daily intake 200 mg)</td>
</tr>
<tr>
<td></td>
<td>Improve exercise capacity of exercising humans</td>
</tr>
</tbody>
</table>
Phosphatidylcholine | Support liver recovery from toxic chemical attack or viral damage  
| Protects the human GI mucosa against toxic attack  
| Reduction of necrotising enterocolitis  

Mucins and glycoproteins | Inhibition of *Helicobacter pylori*  
| Protective effect against rotavirus infections  

MFGM hydrolysates | Antimicrobial activity against pathogens  

**Table 3.** List of some recently reported studies on development of beverages based on whey or buttermilk  

<table>
<thead>
<tr>
<th>Beverage formulation</th>
<th>Outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice and whey in different ratios</td>
<td>Best sensory and microbiological properties for formulations 70% whey/30% orange juice and 65% whey/35% orange juice</td>
<td>(39)</td>
</tr>
<tr>
<td></td>
<td>Whey orange juice characterised by higher content of proteins, ash, vitamin B in comparison to orange juice</td>
<td>(40)</td>
</tr>
<tr>
<td></td>
<td>Beverage containing 70% whey, 30% orange juice and CO₂ achieved best sensory scores and had the best nutritional composition</td>
<td>(41)</td>
</tr>
<tr>
<td>Different formulations of guava pulp and whey</td>
<td>The best beverage was obtained by pasteurisation at 65°C/25 min and cold storage for 45 days</td>
<td>(44)</td>
</tr>
<tr>
<td><em>Mentha arvensis</em> extract addition to whey</td>
<td>Optimal beverage contained banana juice and max. 2% <em>Mentha arvensis</em> extract, shelf life of 15 days</td>
<td>(48)</td>
</tr>
<tr>
<td></td>
<td>Best formulation consisted of 80% whey, 20% beetroot and 6% <em>Mentha arvensis</em> extract</td>
<td>(49)</td>
</tr>
<tr>
<td>Whey fermentation by probiotic strains <em>Lactobacillus plantarum</em>, <em>Lactobacillus brevis</em> and their combination and supplementation by fruit concentrates (lemon, mango, pineapple, apple, grape) and sucrose</td>
<td>Optimal beverage inoculated with <em>Lb. plantarum</em> and enriched by pineapple concentrate</td>
<td>(55)</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>5 mixes of probiotic drinks formulated from sweet whey and black mulberry (BM) juice</td>
<td>viability of probiotic strains <em>L. rhamnosus</em> GG and <em>Bifidobacterium animalis ssp. lactis</em> Bb-12 remained high up to 14 days of cold storage, sensory properties of sample 25% whey and 75% BM juice obtained the highest scores</td>
<td>(79)</td>
</tr>
<tr>
<td>addition of CO₂, mango, pineapple and orange fruit fresh juices to buttermilk</td>
<td>Best formulation was obtained by adding 12% sugar and 24% pineapple juice to buttermilk</td>
<td>(74)</td>
</tr>
<tr>
<td>cultured buttermilk beverage supplemented by <em>Aloe vera</em> juice</td>
<td>Samples containing 10% <em>Aloe vera</em> juice obtained the highest sensory scores and had improved nutritive and physicochemical properties when compared to sole buttermilk</td>
<td>(77)</td>
</tr>
<tr>
<td>therapeutic buttermilk formulation by fermentation and incorporation of Moringa Pod Powder (MPP)</td>
<td>Buttermilk fermented with mesophilic/thermophilic <em>dahi</em> culture, supplemented by 1.92% MPP was found optimal due to satisfying shelf life of 20 days at refrigeration and high content of calcium, iron and vitamin A</td>
<td>(87)</td>
</tr>
</tbody>
</table>