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Whey and its Utilization

Silviya R. Macwan*, Bhumika K. Dabhi, S.C. Parmar and K.D. Aparnathi

Dairy Chemistry Department, SMC College of Dairy Science, Anand Agricultural University, Anand- 388001, Gujarat, India
**Corresponding author*

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A B S T R A C T

Whey is a by-product of the dairy industry, which for years was thought to be insignificant and was either used as an animal feed or it was disposed of as waste. Considering that over 145 million tons of whey is produced worldwide annually, the desire for new methods to utilise whey can be appreciated. Over the last years several studies were carried out concerning the importance of whey is nutritional value and the properties of its ingredients. It is now accepted that main content, whey proteins, have antimicrobial, antiviral and anti-oxidant properties. Due to the substantial difficulties encountered in the treatment of whey as a biological waste and its high potential to be valuable raw material for added value food and bioactive substances production the later tend to be the only accepted and popular trend of dealing with this dairy industry by-product. Productions of whey proteins by ultrafiltration, lactose hydrolysis products, and the use of whole whey or whey permeate as a fermentation feedstock are possible options. A large number of workers have carried out studies on the composition and processing of whey for its use in foods and animal feeds besides studying the nutritive, therapeutic and functional properties of whey.

Introduction

Whey is the liquid fraction that remains following manufacture of cheese, chhana, paneer and casein. The world production of whey is estimated at about 165 million tones. Of which cheese whey contributes about 95 %. In India, the major source of whey is from the production of chhana and paneer. In the absence of systematic survey/statistics the estimated production of whey is about 5 million tons per annum (Gupta, 2008). The utilization was 75% in Europe and probably less than 50% in the rest of the world and as a result a very large amount of material with potential value as a food or feed is wasted (Aneja *et al.,* 2002). Whey is a serious pollutant as it imposes a very high BOD of 30,000- 50,000 mg/lit and chemical oxygen demand of 60,000-80,000 mg/lit. Discarding of whey also constitutes a significant loss of potential nutrients and energy and has been looked upon seriously by the environmentalists and technologists due to its potent polluting strength. In addition the dairy industry suffers from an economic blow due to several treatment costs that are incurred in proper disposal of whey. Although several possibilities of cheese whey utilization have been explored, a major portion of the world cheese whey production is discarded as effluent. Its disposal as waste poses serious pollution problems for the surrounding environment (Marwaha & Kennedy, 1988; Gonzalez-Siso, 1996).

Interestingly, over the last few decades, dairy industries have applied different technologies to process cheese whey resulting in its separation into its principle components, comprising fractions enriched in proteins, lactose and minerals. It was the introduction of new separation technologies such as ion exchange, electrodialysis and membrane filtration, which created possibility of producing a wide range of new products based on whey, including functional food ingredients with added value and products for pharmaceutical and medical industries, Nielsen, *et al.*, (2002). Now-a-days it is no longer considered a waste product but the treasure house of nutritionally rich whey components. Thus its utilization in the human food chain is now being predominantly favoured due to the economic opportunities provided by some of the milk nutrients present in whey (Jelen, 2002a). Productions of whey proteins by ultrafiltration, lactose hydrolysis products, and the use of whole whey or whey permeate as a fermentation feedstock are possible options (Marwaha, 1988). A large number of workers have carried out studies on the composition and processing of whey for its use in foods and animal feeds besides studying the nutritive, therapeutic and functional properties of whey.

Types and Composition of Whey

Whey may be defined broadly as the serum or watery part of milk remaining after separation of the curd, which results from the coagulation of milk proteins by acid or proteolytic enzymes. The type and composition of whey at dairy plants mainly depends upon the processing techniques used for casein removal from liquid milk. The most often encountered type of whey originates from manufacture of cheese or certain casein products, where processing is based on coagulating the casein by rennet, an industrial casein-clotting preparation containing chymosin or other casein-coagulating enzymes (Fox *et al.*, 2000). Rennet-induced coagulation of casein occurs at approximately pH 6.5; this type of whey is referred to as sweet whey (Table 1). The second type of whey, acid whey ($pH < 5$), results from processes using fermentation or addition of organic or mineral acids to coagulate the casein, as in the manufacture of fresh cheese or most industrial casein (Jelen, 2003). In general, whey produced from rennet-coagulated cheeses is low in acidity, while the production of fresh acid cheeses such as ricotta or cottage cheese / paneer or chhana yields medium acid or acid whey.

Whey from cottage cheese manufacture is called “Acid whey” since it is produced during acid coagulation of milk proteins and has a low pH and consequently longer storage life than “sweet whey” which is produced by enzymatic coagulation of milk proteins i.e. during manufacture of cheddar and other kinds of cheese (Chandra, 1980).

The classification of whey based on acidity and pH is given in Table 2.

In the production of cheese or casein, about 80 to 90 % of original milk used yields whey as a by-product, which contains about 50 % of the milk constituents (Jayaprakasha, 1992). Whey retains about 45-55 % of the milk nutrients comprising serum proteins, lactose, minerals and vitamins. The composition of whey varies depending on the type of coagulation. The pH of cheese whey ranges from 5.9 to 6.4 and that of chhana, paneer and Shrikhand whey between 5.3 and 5.6. The Total Solids (TS), fat and protein content of cheese whey is more than that of acid whey, but there is higher amount of calcium, phosphorus and lactic acid in acid whey as compared to sweet whey. The typical composition of sweet whey and acid whey is in Table: 3.

The main components of both sweet and acid whey, after water, are lactose (approximately 70–72% of the total solids), whey proteins (approximately 8–10%) and minerals (approximately 12–15%) Gupta (2000) (Table 3). The main differences between the two whey types are in the mineral content, acidity and composition of the whey protein fraction. The acid coagulation approach results in substantially increased acidity (final pH approximately 4.5), necessary for casein precipitation. At this low pH, the colloidal calcium contained in the casein micelles in normal milk is solubilised and partitioned into the whey. Rennet clotting produces a fragment k-casein molecule, termed glycomacropeptide (GMP), which ends up in whey. GMP constitutes approximately 20% of the whey protein fraction of sweet, rennet-based whey but is not present in acid whey, unless renneting is included in the fresh cheese manufacturing process. Other technological steps used in the pre-treatment of milk before the main processes may also influence the composition of whey such as that from paneer/chhana resulting in acid whey with very low protein content.

World Whey Production

Dairy industries all over the world generate large amounts of whey per litre of milk processed, depending upon the processes employed, products manufactured. The total world production of liquid cheese whey is in the region of 145 million tons (Affertsholt& Nielsen, 2003). Of this 85 million tons can be considered to be industrially utilised and processed into value added products. The remaining 60 million tons or so is used directly in liquid animal feeds, sprayed on fields as fertilizer or just dumped. Industrially processed fraction, about 50 million tons is processed either into edible lactose or dried directly as cheese whey powder, approximately 30 million tons is processed into protein enriched concentrates, referred to as whey protein concentrates for products containing up to 80% protein and whey protein isolates for products containing > 90% protein. A further 6 million tons are processed into so-called demineralised blends (Clark, 2004). Based on the global distribution of whey utilisation, approximately 60% of whey is industrially utilised, this is built up from 80-90% utilisation in the US and Australasia and only 60% utilisation in Europe. Industrial processing of whey originating from the rest of the world is still at comparatively low levels. In the absence of systematic data, the predicted value for whey production in India is 3.0 million tonnes per annum (Khamrui & Rajorhia, 2000) with major source of whey from the production of chhana and paneer, while about 50,000 tonnes of whey is made available from cheese industry.

The United States of America produces about 5-8 billion pounds (2-6 million tonnes) of acid whey and 45 -I billion pounds (20.5 million tonnes) of sweet whey annually (Clark, 1987). There has been a steady increase in the production of whey powder and lactose over the past three years. Trends in the production of concentrated whey, whey protein concentrate, reduced lactose/reduced mineral whey and whey blends are variable for this period. Current

Australian and New Zealand whey production is about 3634 and 5874 million pounds (1-65 million and 2-67 million tonnes), respectively (Zadow, 1987). On the world scene, the current estimate of whey production amounts to about 224 billion pounds (102 million tonnes) (Hoogstraten, 1987a).

Nutritional Value of Whey

Whey is a rich source of nutritious components and its biological components has proven its effects in treatments of several chronic diseases like cancer, cardiovascular, HIV etc. As it is nutritionally too rich it can also be used in infant, Geriatric and Athletic food.

Research has been unequivocally established the excellent nutritional and functional properties of whey solids, especially the whey proteins (Jelen, 1992, Patel *et al*., 1992). As a first class protein, whey protein is next only to the egg protein in terms of nutritive value, the biological value, protein efficiency ratio (PER) and net protein utilization of egg protein and whey protein being 104 & 100, 3.6 & 3.8 and 92 & 94 respectively. Whey proteins are rich in essential amino acids such as lysine, methionine and cystine (Renner, 1990).

Whey protein has potential as a functional food component to contribute to the regulation of body weight by providing satiety signals that affect both short-term and long-term food intake regulation (Khamrui and Rajorhia, 1998). Because whey is an inexpensive source of high nutritional quality protein, the utilization of whey as a physiologically functional food ingredient for weight management is of current interest. At present, the role of individual whey proteins and peptides in contributing to food intake regulation has not been fully defined. However, whey protein reduces short-term food intake relative to carbohydrate and other proteins.

Whey protein is insulinotropic and whey-born peptides affect the renin-angiotensin system. Whey ingestion activates many components of the food intake regulatory system. Therefore whey protein has potential as physiologically functional food component for persons with obesity and its co-morbidities (hypertension, type II diabetes, hyper- and dislipidemia).

It remains unclear, however, if the favorable effects of whey on food intake, subjective satiety and intake regulatory mechanisms in humans are obtained from usual serving sizes of dairy products. The effects described have been observed in short-term experiments and when whey is consumed in much higher amounts (Luhovy *et al.*, 2007)**.**

Whey proteins have been reported to have one or more biological functions in food. These biological properties have significance in the nutrition and health of people. The health and nutritional value of the components of whey include 1) high quality nutritional source of amino acids; 2) anti-microbial action; 3) growth enhancement of beneficial gut microflora, such as *Bifidobacteria*; 4) immune-enhancing properties; 5) control of specific diseases, including cancer; and 6) antitoxin activity.

Whey proteins

Whey protein are two types major proteins and minor proteins, major whey proteins are Beta-lactoglobulin-65%, Alpha-lactalbumin-25%, serum albumin-8% and minor proteins/peptides are Glycomacropeptide (GMP), Bovine serum albumin, lactoferrin, Immunoglobulin, Phospho lipoproteins. Whey proteins have a Biological Value

(BV), of 110, which is higher than the value for casein, soy protein, beef, or wheat gluten and have a high content of sulfur-containing amino acids such as cysteine and methionine (Fox and MacSweeney, 1998).

Lactose

Lactose, the major component of whey, is probably the least valuable component and most difficult to utilize. Lactose comprises about 70% of the total solids of whey (Jelen, 1992).

Minerals

Whey is a good source of electrolytes including sodium and potassium, which are required during recovering from diarrhea. Minerals such as calcium, magnesium, and phosphorus are present in solution and also partly bound to proteins. Zinc is present in trace amounts (Zadow, 1992). Lactose also promotes absorption of Mg and Zinc ions, which even in trace amount helps in better diarrheal management (Ziegler and Fomon, 1983).

Vitamins

During the manufacturing process, the water-soluble vitamins are transferred into whey in a varying extent: 40-70% of vitamin B12; 55-75% of vitamin B6 and pantothenic acid; 70-80% of riboflavin and biotin; 80-90% of thiamine, nicotinic acid, folic acid and ascorbic acid. In the case of vitamin B12, more of it was transferred into the whey when a rennet coagulation rather than acid coagulation was used (Zadow, 1992).

Therapeutic Value of Whey

Whey based beverages target a large scale of consumers - from old people to little children. Because of its health benefits, it was used to treat some illnesses, such as tuberculosis and skin and digestive tract diseases, since the time of Ancient Greece. In the 18th century there were specialized institutions built for curing illnesses with whey which designated the start of detailed studies of nutritional and therapeutic properties of whey. These so called "whey cures "were usual in countries like Switzerland, Germany and Austria at that time. Whey was also successfully applied for treatments of diarrhea, bile illness, skin problems, scales in the urinary tract and some intoxication.

Due to high amount of proteins with high nutritional value these beverages are ideal source of energy and nutrients for athletes. Whey proteins are a rich source of branched chain amino acids (BCAA) like isoleucine, leucine and valine. BCAAs unlike other essential amino acids are metabolized directly into the muscle tissue and are first amino acids used during periods of exercise and resistance trainings. (Sherwood *et al*., 2007).

Whey protein fractions include also lactoferrin - an iron-binding protein, Glycomacropeptide (GMP) which derives after cheese making using rennet and is naturally free of phenylalanine and alpha-lactalbumin which is a calcium-binding protein. That way, due to presence of lactoferrin whey beverages can be used as functional food intended to improve iron absorption from food and/or help to keep pathogens from attaching to the intestinal walls. That is very important for nutrition of little children and babies. Furthermore, these beverages may improve absorption of calcium what is very important for older population which is often suffering from osteoporosis. Besides that, a drink with addition of GMP isolate would be a very good source of energy and micronutrients for those suffering from phenylketonuria (Miller, 2005).

Whey possesses potent antioxidant activity mainly contributed by cysteine-rich proteins that aid in the synthesis of glutathione (GSH), a potent intracellular antioxidant, also investigated as an anti aging agent. Detoxifying property contributed by Glutathione peroxidase (GSHPx), which is derived from selenium and cysteine, that converts lipid peroxides into less harmful hydroxy acids and α-lactalbumin, which chelates heavy metalsand reduces oxidative stress because of its iron-chelating properties.

Immunoglobulins and lysozyme in whey provides immunity enhancing benefits to infants and others. Antihypertensive peptides isolated from bovine beta-lactoglobulin, reduces blood pressure. The amino acid precursors to glutathione in whey might increase glutathione concentration in relevant tissues, stimulate immunity and detoxify potential carcinogens.α-lactalbumin, high in tryptophan, an essential amino acid; provides potential benefits include sleep regulation and mood improvement under stress.

Various minerals like, Potassium, involved in the transmission of nerve impulses and muscular contractions; Magnesium, depolarizes the nerve or muscle causing relaxation and help to lower blood pressure and supports an alkaline tissue pH; Calcium, used by the body to maintain an alkaline tissue pH, maintain bone density, cell wall integrity and nerve impulses. Lactoperoxidase, inhibits the growth of iron dependent bacteria. Lactoferrin**,** inhibits the growth of bacteria (including pathogenic bacteria) and fungi, also regulates iron absorption and bio-availability. Whey also possesses the vitamins necessary for its utilization. It contains vitamins A, B1, B2, B3, B5, B6, C, D, and E (Onwulata and Huth, 2008).

Utilization of Whey

In United States of America, only about 50% of the total whey production is "dried or further processed into whey protein concentrate, lactose and other products for use in formulating human food and animal feed products (Jelen& Le Maguer, 1976; Teixeira *et al.*, 1983a; Morr, 1984; Clark, 1987). The remainder of the whey, especially from small cheese manufacturing plants, is treated by private or municipal waste treatment, fed to livestock or spread on agricultural land as fertilizer. Although a large percentage of the whey produced in The Netherlands is reportedly being processed into products other than whey powder, Europe in general utilizes only about 50% of its whey production, mainly as whey powder (Hoogstraten, 1987b).

Processing of whey is limited due to less demand for whey powder and further processed whey products, plus the generally unfavourable economics of whey processing, especially for the smaller sized processing operation (Jelen& Le Maguer, 1976). The whey processing industry has expended considerable effort to improve the utilization of whey powder, lactose, demineralized whey, whey protein concentrate, whey blends and other products. However, processes for manufacturing these whey-based products are economically feasible only for the larger sized processing operations with an adequate supply of consistently high-quality whey, and where there is adequate demand for the resulting products. The predominant driving force favouring whey processing is the tremendous cost associated with whey disposal through municipal or plant waste treatment facilities (Delaney, 1981).

A substantial portion of whey powder, whey concentrate, reduced lactose/ reduced mineral whey, whey protein concentrate and lactose is used in human food product applications, whereas whey blends are used mainly in animal feed products. Whey-based blends are formulated to provide a composition similar to that of non-fat dry milk. Most of the whey concentrate for human food is used in dairy and bakery product applications (Morr, 1984; Clark, 1987). Major portions of whey are used in dairy, bakery, blends, prepared mixes and confectionery applications. Most of the acid whey used in human food product applications is produced as dried and modified whey.

Whey Powder

Whey powder is manufactured by concentrating whey to 40-70% total solids, and spray or roller drying to a moisture content of less than 5%. It is necessary to convert a high percentage of the lactose to its a-monohydrate crystalline form to minimize water absorption and concomitant defects during storage. Lactose crystallization is either completed in the concentrate prior to drying or, as is most commonly done, a two-stage drier is employed that allows for crystallization prior to final drying. Lactose crystallization is also promoted in this hygroscopic product by rapidly cooling it to less than 100°C as it is removed from the drier.

Animal Feed

One of the ways to utilize the whey is in the preparation of formulations of animal feed. About 16 % of the 2.67 million tonnes of whey production in New Zealand and 28% of the 1.65 million tonnes of whey production in Australia is used for pig feed (Zadow, 1987). An additional 5% of total whey production in Australia is utilized as calf milk replacer. The dairy industry in The Netherlands uses about 15000 tonnes of liquid whey and 120000 tonnes of delactosed whey powder for manufacturing calf milk replacer (Hoogstraten, 1987b).

Whey Disposal

Approximately 8.7 kg of whey is produced per 1 kg of cheese manufactured. Sweet whey and acid whey, by-products from the manufacture of cheese, cottage cheese and industrial casein, are extremely potent pollutants, containing about 35 000 mg/litre biochemical oxygen demand (BOD) and 68000 mg/litre chemical oxygen demand (COD) (Jelen, 1979; Hobman, 1984).

Whey may also be used for farmland irrigation, where it contributes both water and nutrients to the soil which can promote plant growth (Oborn& Piggott, 1968). 3.7 thousand L of whey contains minerals equivalent to 5.9 kg potassium sulphate, 36 kg ammonium sulphate, 12 kg superphosphate and 6.8 kg calcium carbonate. This whey disposal approach works well for near neutral to slightly alkaline soils; in acid soils, the added whey promotes soil compaction, resulting in inhibition of propagation of soil microorganisms that biodegrade the added nutrients from the whey. Spraying is the preferred method for applying whey to the soil since it avoids the formation of stagnant pools that inhibit the ability of the soil microorganisms to biodegrade added nutrients. The amount of whey that can be utilized by spreading on land as fertilizer is limited to about 45-90 tonnes/acre. Exceeding these limits poses a threat to environmental pollution from run-off and off-odour production. Whey reportedly improves soil fertility by providing valuable nutrients, and also by promoting an open, porous structure that increases water infiltration (Gillies, 1974).

Whey can also be treated by waste disposal systems such as outdoor sewage lagoons that utilize aeration and sunlight to assist microorganisms to decompose protein and lactose. Waste water containing whey is difficult to treat by conventional processes. Whey results in a poor settling sludge following primary and secondary waste water treatment. A waste treatment system designed to use primary settling, a two-stage packed tower trickling filter, a final settling, coagulation and sterilization by chlorination and sludge dewatering has been described by Gillies (1974). This trickling filter design was as effective for treating whey as for average industrial effluents. The acidic reaction of whey had no adverse effect on the system. In fact, lowering the pH of whey improved the ability of the process to remove BOD from the waste effluent.

Deproteinized Whey and UF Permeate

Deproteinized whey is normally manufactured by heating whey to temperatures in the range of 70-80°C to denature the proteins, followed by acidification, and removal of the flocculants by decantation, filtration or centrifugation. Whey UF permeate is the major by-product of the UF of whey for manufacturing whey protein concentrate. Deproteinized whey and whey UF permeate contain about 90% of the total solids of the original whey. These solids components, mainly lactose, minerals and non-protein nitrogen, represent a substantial pollution problem for the industry if not utilized (Hobman, 1984). This whey fraction is most often derived as the UF permeate by-product resulting from the manufacture of whey protein concentrate, or from the manufacture of heat-precipitated whey protein, e.g. lactalbumin. Whey UF permeate has great potential as source material for: (Delaney, 1981; Teixeira *et al.*, 1983b; Chiu & Kosikowski, 1985).

Hydrolysed Whey and Whey UF Permeate

Lactose hydrolysis can be catalysed enzymatically by heating in the presence of strong mineral acids, or by treatment with a cationic ion exchanger in the hydrogen form (Jelen, 1979; Zadow, 1984; Harju, 1987). For acid hydrolysis, it is necessary to lower the pH of the lactose solution to 1.2-1.5 prior to heating to approximately 140 °C. Hydrolysis of 50-90% of the lactose increases sweetness and prevents lactose crystallization in concentrated whey syrups. Hydrolysis increases solubility and decreases the viscosity of whey and UF permeate syrups. Whey syrups can be concentrated to 60-70% solids in vacuum evaporators without crystallization problems. However, lactose and lactose hydrolysis products, glucose and galactose, become sticky and hygroscopic in spray driers making it difficult to dry hydrolysed whey and whey permeate syrups. Concentrated syrups are thus preferred over powder forms of lactose-hydrolysed whey and whey permeate, even though problems from crystallization of galactose and non-hydrolysed lactose in these products are sometimes encountered. Crystallization problems are diminished in syrups by hydrolysing about 75% of the lactose. The resulting syrup concentrate is partially acidified and heated at relatively mild temperatures to destroy most microorganisms and to dissolve residual, non-hydrolysed lactose without causing Maillard, non-enzymatic browning. The heated product is partially cooled, seeded with fine lactose crystals and agitated to promote the formation of small crystals that will not settle during storage.

Immobilized enzyme technology has been utilized to improve the economics of lactose hydrolysis (Anon., 1982; Teixeira *et al.*, 1983b; Sprossler& Plainer, 1983). This technology uses lactase enzyme immobilized on porous glass beads or porous plastic beads to hydrolyse lactose in UF permeate. The resulting hydrolysate can be used as a sweetener in food products or as a fermentation substrate for producing baker's yeast.

One striking example has been reported where the dairy industry has incorporated lactose hydrolysis into a multifaceted processing scheme to modify Cottage cheese (acid) whey so that it can be used as an ingredient in ice cream mix (Homer, 1986).

Utilization of Whey Products

In general whey from any source may be used i.e. sweet whey, sour whey or powdered whey, but use of powdered whey makes the final product unnecessarily expensive. Whey or whey derivatives from cheese, casein, paneer etc., can be used for beverage preparation. Whey can be utilized in different ways in food industry, but it is mostly being dehydrated to whey powder or used for manufacture of whey protein concentrates and isolates of lactose or proteins. Whey beverages are considered as thirst quencher, unlike most soft drinks, whey drinks are light and refreshing but less acidic than fruit juices and they are said to be more suitable for health as compared to other drinks (Prendergast, 1985).

Several difficulties occur during the processes of whey beverage production. First of all, the high water content makes fresh whey very susceptible to microbial spoilage whereby heat treatments are needed. On the other hand, whey proteins are heat sensitive and start to denature at temperatures above 60 ºC whereby a certain amount of present proteins precipitate after the usual thermal treatment of whey (72 ºC/15 - 20 sec.). Therefore a lot of efforts are made in studying the implementation of ultrasound or membrane processes like microfiltration instead of thermal treatments. Ultrasound can also improve solubility of whey proteins (Rezek-Jambrak*et al*., 2008) and in that case the amount of sediment formed during storage of whey beverages can be reduced. Besides that, acidification of whey down to pH < 3.9 causes whey proteins to become heat resistant and do not precipitate even during UHT sterilization treatments (Jelen, 2002b).

Relatively high content of minerals in the dry matter of whey presents the next problem in whey beverage production because these minerals are responsible for undesired salty-sour taste of whey. This problem is especially encountered in acid whey due to higher amount of lactic acid whereas the content of minerals (mostly Ca-phosphates and Ca-lactates) is also higher due to better solubility. That causes abundant acidity and appearance of clots in the final product and also formation of higher amounts of sediment during heat treatments (Tratnik, 2003).

However, despite of all difficulties, fresh whey processing has proved to be the most economical technological solution. Therefore many efforts have been made in development of beverages with addition of fruit concentrates in order to produce a drink with acceptable sensory properties especially flavour (Koffi *et al*., 2005). These whey beverages are classified into two categories viz. alcoholic beverages and non-alcoholic beverages

Alcoholic Beverages

Since lactose is the main constituent (70%) of whey dry matter, whey is a very good material for production of alcoholic beverages. Alcoholic whey beverages are divided into beverages with low alcohol content (≤ 1.5%), whey beer and whey wine.

Production of whey beverages with low alcohol content includes deproteinizing whey, whey concentration, fermentation of lactose (usually by yeast strains *Kluyveromyce sfragilis and Saccharomyces lactis*) or addition of sucrose until reaching the desired alcohol content (0.5 - 1%), flavoring, sweetening and bootling (Sienkiewicz & Riedel, 1990). Thereby, a certain amount of lactose is being transformed to lactic acid which gives a refreshing sour taste to the end product, while the rest ferments to alcohol. Some of noted beverages belonging to this category are "Milone" obtained by fermentation with kefir culture and whey sparkling wine "Serwovit" produced in Poland.

Whey beer can be produced with or without addition of malt; it can be fortified with minerals or can contain starch hydrolyzates and vitamins. Some of problems observed in such type of beverages are loss of beer foam, undesirable odour and taste due to low solubility of whey proteins and inability of beer yeasts to ferment lactose because of presence of milk fat.

Whey wine contains relatively low alcohol amount (10-11%) and is mostly flavored with fruit aromas. Production of whey wine includes clearing, deproteinazation, lactose hydrolysis by β-galactosidase, decanting and cooling, addition of yeasts and fermentation, decanting, aging, filtering and bottling (Palmer and Marquardt, 1978).

Patel (2012) had developed the self-carbonated probiotic whey beverage using the chhana whey. The beverage was prepared by inoculating the *Kluveromyces marxianus* and *Lactobacillus helveticus* MTCC 5462 cultures and addition of sugar (7%) then it was incubated at 25°C till acidity reaches 0.5% L.A. and stored at refrigeration temperature for self-carbonation.

Non-alcoholic Beverages

Non-alcoholic beverages can be further classified into fruit and flavouring based beverages and Milk based beverages.

(a) Fruits and flavouring based beverages

Processing of whey to beverages began way back in the 1970, and one of the oldest whey beverages is Rivella from Switzerland. It is a clear, amber coloured liquid with a refreshing acidic flavour. Until today a large scale of different whey beverages has been developed, which are produced from native sweet or acid whey, deproteinized whey, fresh diluted whey, fermented whey or powdered whey.

Jayaprakasha*et al.*, (1986) suggested a process for whey beverage using deprotenized and clarified whey from cheese, Chhana or acid casein. The process consisted of adding 6 to 13 % sugar, 0.02 to 0.4 % citric acid and flavour at 0.15 to 0.45 ml per litre of whey followed by in-bottle pasteurization or sterilization with or without carbonation. Beverage with 10 % sugar and orange flavour received highest score.

Reddy *et al.*, (1987) developed an

acceptable beverage, deproteinised cheddar cheese whey, lemon juice (8%) and sugar (14%). The shelf life of the product was up to 15 days at room temperature (18-25 °C) without much damage to the organoleptic quality.

Gagrani *et al.,* (1987) added different fruit juices like orange, pineapple and mango at the rate of 10%, 15%, and 15% respectively to whey for the manufacture of fruit based beverage. The reported mango based beverage to be superior than others with respect to colour, flavour, mouthfeel and physical characteristics like sedimentation, turbidity, viscosity etc.

Acid whey has been found most suitable for blending with fruit juices to produce cloudy fruit drinks (Tuohy *et al.,* 1988). A pH of less than 4.0 is recommended to prevent protein coagulation during pasteurization. The pH reduction can be achieved by addition of lactic acid or other organic acids. The pectin esterase is inactivated at 85-95 °C/15-40 sec to prevent loss of cloudiness, which is required in fruit juices such products possess a shelf life of 2 weeks at refrigeration temperature.

Gandhi (1989) patented acid whey-lactic fermented noncarbonated beverage produced from cheese whey or paneer whey, heated (85 to 90 °C / 20 min) and cooled to 40 °C inoculated with *L. acidophilus* culture (@ 2%). The final product had 0.8 to 0.85 % (LA) acidity.

Jayaprakasha (1992) prepared whey drinks from deproteinized and clarified cheddar cheese whey with the addition of sugar(10%), citric acid (0.4%) and orange, pineapple, mango or raspberry flavouring and in – bottle pasteurization or sterilization with or without carbonation.

Singh *et al*., (1994) developed an acceptable beverage from paneer whey by blending mango, pineapple, lemon and banana pulp or juice. Pulp of 4% and juice content ranged from 5% for lemon to 20% for pineapple and banana while the whey content ranged from 73 to 87%. Another soft beverage was also prepared from a mixture of paneer whey (3parts) and banarsisurkh variety of guava extract (1part), sugar (8%) and lemon colour.

Macedo *et al.* (1998) manufactured cultured whey milk beverage using buffalo milk cheese whey, cow skim milk and soy milk with six combinations of *Lactobacillus casei subsp. shirota, L.acidophilus, B. longum and B. adolescentis.* The result indicated that the mixture of 35, 35 and 30%, respectively of skimmed cow milk, buffalo cheese whey and soy milk was suitable as a low cost substrate for the growth of co-culture *L. casei subsp. Shirota and B.acidolescentis,* resulting in a product of good taste and flavor with a high number of these desirable organisms.

Iniquez and Vera (1999) developed a formulation for flavoured beverage using buffalo milk whey, and concentrated pineapple juice or orange (4.5 - 5.0%) shelf life of the beverage was 7-8 days at 4 °C. Shukla *et al.,* (2000) developed a ready to serve beverage from whey by the addition of 10% sugar and 30% litchi juice.

Khamrui (2000) used cheddar cheese whey for the development of ready to serve kinnow juice beverage. The process consisted of mixing kinnow juice concentrated 23 °C Brix by reverse osmosis, with concentrated fresh cheddar cheese whey (45%) total solids along with other ingredients sugar 7%, Pectin 0.05% and CMC 0.15%. The formulation on reconstitution with three times water gave a good beverage.

Besides that, addition of many other fruits like concentrates of apple, pear, peach, apricot and cherry has also been applied. The addition of berries which are known as a good source of iron and antioxidants have proved to be very useful. That is especially important in production of whey beverages with improved nutritional value. Best example for supporting this thesis is Brazilian group of scientists who have developed a whey drink flavoured by addition of strawberry concentrate and fortified with ferrous bisglycinate. They have proved that long-term consumption of this drink had an impact on reduction in the prevalence of anaemia in children and adolescents (Miglioranza *et al*., 2003).

Duric *et al.,* (2004) studied the effect of quality of whey fruit juice (orange, pear, peach and apple) blends. The pH of the beverage was adjusted to 3.5 using citric acid. The quality of blends mostly depended on the sucrose content and dry matter content. They concluded that peach–whey beverage containing 6% of dry matter and 2% of sucrose as well as having pH 3.5 was of best quality.

Deosarkar (2004) manufactured beverage from lactose hydrolyzed permeate with mango pulp as flavor. The level of sugar was added at 5%, guar gum at 0.1% and sodium benzoate at 0.03%. The pH of the beverage was maintained at 4.0 using citric acid solution (20%) and pasteurized (72 ºC/15 sec) product was packed in preformed polyethylene pouches and stored safely up to 35 days under refrigerated storage conditions (7±1 °C).

There are even some indications that fermentation of whey using yoghurt culture (*Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*) produces a more intense yoghurt flavor compared to the one obtained when skim milk is fermented. This suggests the possibility of producing beverages from whey with similar sensory profiles to those of fermented milk drinks or with some flavor attributes of drinking yogurt, following manufacturing procedures conventionally used for milk (Gallardo-Escamilla *et al*., 2005).

Drgalic *et al.,* (2005) studied survival and growth of probiotic strains *Lactobacillus acidophilus La-5*, *Bifidobacterium bifidum Bb-12* and *Lactobacillus casei Lc-1* in reconstituted whey for 28 days of cool storage. All strains have shown good survival during storage time of fermented beverages. The beverage fermented by probiotic strain *Bb-12* obtained lower sensory score than the other two beverages fermented by strains *La-5* and *Lc-1*.

Brandao *et al.,* (2006) developed fermented symbiotic drink using whey, soy milk and whey protein concentrates in different combinations. Four formulae were developed containing whey, soy milk and sugar (A1), soy milk, whey protein concentrate, sugar and inulin as a dietetic soluble fiber (A2), soy milk and sugar (A3), soy milk, whey, inulin, sugar and whey protein concentrate (A4) and inocuated with *L. acidophilus bacteria.* The differences were observed in the fat and protein content. The results demonstrated that formula A3 was accepted more in comparison to other formulae.

Sahu *et al.,* (2006) developed whey based mango- herbal (lemongrass) beverage with different concentration of lemon grass distillate by making use of paneer whey. In the preparation of beverages the proportion of mango pulp (12%), sugar (8%), water (48%), paneer whey (32%) were kept for constant while the volume of lemongrass distillate was varied from 0 -2.5 percent. The beverage prepared with the addition of

1.5 percent lemongrass distillate was found to be the acceptable formulation. Stored beverage having the properties of 14.93° B TSS, 3.98 pH, 23.01 percent total sugars, 5.02 percent reducing sugars, 0.211 percent acidity and 1.5 percent lemongrass distillate obtained the average sensory score of 8.33 at the end of 60 days of storage period which was highest among all other combinations of lemon grass distillates.

Dubey *et al.*, (2007) prepared whey-guava beverages using paneer whey. The volume of guava pulp (25%), sugar (10%) and paneer whey (65%) were kept constant while the pasteurization temperatures and timings were varied from 60°C – 70°C for 15-35 minutes. However, whey-guava beverages pasteurized at 70°C for 35 minutes was found to be best in terms of sensory quality after 45 days and pH, acidity, protein, total sugars and reducing sugars found to be high than that of the other samples.

Choudary and Sandey (2009) developed whey based mango- herbal (cardamom) beverage (WBMH) with different concentration of cardamom extract by using chhanawhey. Mango pulp of langra variety was used in the preparation of beverage. The level of sugar (8%) and mango pulp (12%) was kept constant while, the volume of cardamom extract was varied from 0 to 3%. The sterilized beverages were kept in refrigerated condition for a storage period of one month. The beverage prepared with the addition of 2% cardamom extract was found most acceptable in sensory characteristics up to 30 days at refrigeration temperature.

Naik *et al.,* (2009) prepared Whey Based Watermelon Beverage (WBWB) by blending watermelon juice (15%), sugar (7%) and different concentration of Betel leaves distillate (0, 1, 2, 3%) into chhanna whey (78-75 %).The beverage was In-bottle sterilization was done at 121 °C for 10 minutes and the pH was maintained at 5.1 and Bottles were cooled at room temperature and then stored under refrigerated conditions (7±1 °C).The prepared beverage during storage study showed that there is an increasing trend in the TSS, acidity, and reducing sugar and a decreasing trend in the pH and ascorbic acid but total sugar has non-significant effect during storage.

Divya and Archana (2009) prepared whey guava beverage by heating the whey at 45 ºC and addition of sugar (10%), guava pulp (25%), guar gum (0.05%), sodium alginate (1%) and potassium meta bisulfate (100 ppm). The mixture was filtered, bottled and crown corked and the beverage was in bottle pasteurized at 70 ºC for 35 min and cooled and stored.

Cruz *et al*., (2009) developed the butter cheese whey and acerola juice beverage. This beverage contains good amount of vitamin C due to presence of vitamin C rich Brazilian fruit acerola. Combination of 30:70 mixture of Butter cheese whey and acerola juice was found to be more acceptable. The beverage was prepared by heating the whey to 72 ºC for 15 sec and addition of sugar (12%), diluted acerola juice (1:3) and pectin (0.5 %). The mixture was homogenized, filtered, crown corked, in bottled pasteurized (85 ºC for 10 min), cooled (25 ºC) and stored at 5 ºC.

Madhavi (2009) developed synbiotic whey drink using whey, sugar, inulin and orange fruit juice. For beverage preparation probiotic culture of *L. rhamnosus* MTCC 5462was used. The beverage was made using fermented whey, 10% sugar, 3% inulin and 10 % orange fruit juice. The product will remain acceptable up to 28 days.

Yadav *et al.*, (2010) prepared the whey based banana herbal beverage using banana juice (10%), sugar (8%) and *menthaarvencis* extract (2%). The whey was heated to 45 ºC and other additives are added and after proper mixing and filtration, filtrate was bottled and crown corked. Shelf life of 15 days at refrigerated temperature was achieved by in bottle pasteurization in boiling water for 30 min.

Pandian *et al.*, (2011) developed mango flavoured sweetened whey drink using whey, sugar (12%) and mango pulp (4, 5 and 6%). The whey was heated to 45 ºC and sugar and mango pulp was added and after proper mixing and filtration, bottles were filled and crown corked. The beverage was pasteurized at 63 ºC for 30 min.

Milk based whey beverages

Wroblewska (1977) developed a cultured whey-milk beverage namely “Rewit” from milk (2% fat), rennet whey and using starters (*Streptococcus* and *Lactobacillus*). “Rewit” contained 30 to 50ml concentrated apple or orange juice. Sabikhi and Thompkinson (2007) developed whey based Lassi using whey and buffalo milk. Combination of CMC, tri sodium citrate and pectin was used as stabilizer in processing. Fermentation was carried out using the lactic acid bacteria. The Lassi was UHT treated and packed in tetrabricks under aseptic condition which gave the shelf life of 150 days at 30 ºC.

Utilization of Whey for Biomass/Lactic Acid Production

Since lactose is the major component of whey solids, in addition to water-soluble vitamins, minerals and proteins, numerous biotechnological processes have been developed to utilise whey to make useful products of industrial importance, such as lactic acid. Lactic acid or α-hydroxy propionic acid, as an unnamed component of soured milk has been known since the days when man first herded animals. Lactic acid and its derivatives are widely used in the food, pharmaceutical, leather, and textile industries. Recently, there has been an increased interest in lactic acid production, since it can be used as a raw material for production of polylactic acid, a polymer used as a specialty medical and environmental-friendly biodegradable plastic. Of the 80,000 tonnes of lactic acid produced worldwide every year, about 90% is made by LAB fermentation and the rest is produced synthetically by the hydrolysis of lactonitrile (Panesar *et al.*, 2007)

Whey from dairy industry although low in sugar content constitutes an economical raw material for lactic acid production (Shahbaz *et al.*, 1996).

Currently, a high fraction of generated Cheese Whey is managed by membrane processes, mainly, ultrafiltration. The obtained permeate has a low protein content and an elevated lactose and mineral salts concentration, both aspects are advantageous in lactic acid fermentation. As a consequence, several works have been carried out aimed at obtaining lactic acid after ultrafiltration of Cheese Whey. Highest lactic acid production rate was obtained with *L. helveticus* cultivated in whey permeate, with corn steep liquor (CSL) as the nitrogen source. Lactic acid productivity of 9.7 g/L/h using *L. helveticus*strain milano has been obtained in continuous fermentation of whey-yeast extract permeate medium. In a work with *L. salivarum*YE supplementation was replaced by *in situ* treatment of fermentation medium with proteolytic microorganisms. (Mollea *et al.*, 2013)

However, expensive culture media, which contain natural complex organic nitrogen sources such as yeast extract, malt extract and/or polypeptone, are necessary for the cultivation of lactic bacteria because nutritional requirement of lactic bacteria is very complicate. *Lactococcuslactis* especially has numerous growth requirements. The growth mechanism and the nutritional requirement of lactic acid bacteria have not been clarified yet. Recently, many workers have been studying the effects of nutrients (Amrane *et al.*, 1993; Ledesma *et al.*, 1977) on the growth and product formation of lactic bacteria. Among the nutrients contained in the culture medium for lactate fermentation, the nitrogen source such as yeast extract seems to be very important for cell growth and acid production. Some workers have reported the promotion effect of yeast extract on the growth of lactic bacteria (Aeschlimann *et al.*, 1990; Orberg *et al.*, 1984). However the growth promotion effect of these complex nitrogen sources have not been completely clarified yet (Tanaka *et al.*, 1995). Growth medium of milk whey for the production of lactic acid by *Lactobacillus acidophilus* was developed and different conditions were optimized for lactic acid production. Maximum yield of lactic acid (4.4 %) was recovered from shake medium in the presence 0.1% NaCI. 0.1 % $MgS0_4.7H_2O$. 0.1 % $NH_4H_2P0_4$, 2% molasses. 0.1 % yeast extract and 2.0% corn steep liquor after 48 hours incubation at 35 ºC and pH 5.5 on orbital shaker (Shahbaz*et al.*, 1996).

16 isolates of Lactic acid producing bacteria isolated from various biological sources were inoculated in whey water (1% inoculum) and kept at 37 °C in the shaker at a speed of 150 revolutions per minute for 36 h. Lactic acid production was estimated after 36 h and the strains 4a, 12a and 15b showed lactic acid production of which 12a produced the highest concentration. The amount of Lactic acid produced by 12a was 0.62 g L-1 under unadjusted condition which is comparable to previously reported strains in enriched medium (Shiphrah*et al.*, 2013).

Table.1 Typical composition of whey

Components	Sweet whey	Acid whey	Chhana/ paneer whey
Total solids (%)	7.0	7.0	6.5
Fat (%)	0.3	0.1	0.5
Protein (%)	0.9	1.0	0.4
Lactose (%)	4.9	5.1	5.0
Ash (%)	0.6	0.7	0.5
			(Gupta, 2000)

Table.2 Types of whey based on acidity and pH

Type of whey	Titratable acidity (%)	pH
Sweet whey	0.1 – 0.20	5.8 – 6.6
Medium acid whey	0.20 – 0.40	5.0 – 5.8
Acid whey	0.40 – 0.60	4.0 – 5.0
		(Bund and Pandit, 2005)

Table.3 Composition of different types of whey

Composition	Sweet whey		Medium acid whey			Acid whey	
	CM cheddar cheese whey	**BM cheddar cheese whey**	**Paneer whey**	**CM chhana whey**	**BM chhana whey**	**Casein whey (HCl)**	**Shrikhand whey**
Solids (%)	6.35	6.87	6.06	6.25	6.40	5.95	6.96
pH	6.10	6.40	5.60	5.50	5.70	4.60	NA
Lactose (%)	5.00	5.01	5.03	4.86	4.90	4.30	NA
Protein (%)	0.90	0.98	0.30	0.72	0.89	0.70	0.38
Fat (%)	0.06	0.34	0.13	0.10	0.10	NA	0.43
Ash (%)	0.59	0.54	0.60	0.57	0.44	0.72	NA
Lactic acid (%)	0.13	0.14	0.21	0.19	0.20	0.25	0.24
Calcium (ppm)	448	501.5	710.65	526	446.3	1,298	NA
Phosphorus (ppm)	486	441.5	560.5	493.7	533.3	1,770	NA
CM : Cow milk; BM : Buffalo milk; NA : Not available							
(Khamrui and Rajorhia, 1998)							

Continuous mix batch bioreactors were used to investigate the effects of various concentrations (0, 5, 10 and 15 g/L) of two nutrients (Yeast extract and Lactamine AA) on the growth of *Lactobacillus helveticus* (ATCC 15009) and the production of lactic acid from cheese whey. The results from this study showed that yeast extract was superior to Lactamine AA in its influence (improved yield and conversion efficiency) when used as nutrient during batch fermentation experiments of lactic acid production from cheese whey (Ghaly *et al.*, 2003).

Industrial fermentation of lactic acid may be limited by the availability of micro and macronutrients, which are required by lactic acid producing microbes for cellular growth and maintenance. Micronutrients are predominantly metallic ions, which are required in trace quantities as cofactors in enzymatic reactions, whereas macronutrients include nitrogen, phosphorus, potassium, sodium and sulfur and are needed mainly for the synthesis of cellular material (Amrane and Prigent, 1998). Roy *et al.,* (1987) and Aeschlimann and Von Stocker (1990) showed the need for a complex of nutrients for *Lactobacillus helveticus*for the growth and product formation and the need to supplement cheese whey with some commercially available growth supplements. Several Studies showed that lactic acid productivity of most *Lactobacilli* is significantly improved by the addition of yeast extract, amino acids, protein concentrates, hydrolysates, vitamins and inorganic compounds such as $(NH_4)_2HPO_4$ and $(NH_4)_2HPO_4$ (Amrane and Prigent, 1998; Demirci *et al.,* 1998; Champagne *et al.,* 1992; and Cheng *et al.,* 1991). Other studies showed the need to supplement cheese whey with some commercially available growth supplements such as corn steep liquor, yeast extract, casamino acids, peptone, neopeptones, cane molasses and trypticase (Cheng *et al.,* 1991; Gupta and Gandhi, 1995; and Roy *et al.,* 1986, Ghaly *et al.,* 2003).

Some studies report the use of mixed cultures in lactic acid production with synergistic effects. Other research groups tried to improve LA production using r

E.coli harbouring an inducible expression plasmid containing D-lactate dehydrogenase encoding gene of *Lactobacillus plantarum* or using metabolic engineering of LAB, fungal or yeast systems, but all of these strategies, if compared with the use of mixed cultures, involve higher costs due to genetic engineering studies and the need of sterilization (Mollea *et al.*, 2013).

LAB have been immobilised by several methods on different supports (calcium alginate, k-carragenane, agar and polyacrylamide gels) and the immobilised systems have been investigated for lactic acid production from whey. A two-stage process was used for continuous fermentation of whey permeate medium with *L. helveticus* immobilised in k-carrageenan/ locust bean gum, which resulted in high lactic acid productivity (19–22 g L−1 h−1) (Mollea *et al.*, 2013).

*L. casei*was immobilized in Ca pectate gel. A high lactose conversion (94.37%) to lactic acid (32.95 g L−1) was achieved and the cell system was found highly stable, no decrease in lactose conversion to lactic acid was observed up to 16 batches (Mollea *et al.*, 2013).

Whey is a very interesting product due to its components. Their properties, functions and chemistry structure make whey a great base for the creation of a series of new products or an ideal alternative compound to more traditional ones. In terms of whey utilization there are so many things that can be done instead of treating whey as a waste.

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