Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; Sp 10(1): 542-548
Received: 10-11-2020
Accepted: 15-12-2020

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Whey and its valorization using Biorefinary: A review

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Abstract
Whey is a dairy product, which was thought to be less valuable and was used as a feed for animals or as a source of surplus. In view of that, in overall world, more than 145 million tons of whey is produced, every year, the need for fresh ways to use whey can be esteemed. In current years, numerous studies have been shown about the significance of the properties and nutritional value of whey and its ingredients. Whey is disposed of in the trenches without any effective treatment, which raises the environmental issues. Another use of whey has been used to decrease the adverse effect that is produced on the environment. These uses are creation of relatively inexpensive raw materials, generation of energy and the extraction of macromolecules used in the industries. The integral use of waste at the bottom of the biorefinery scheme foreshadows another potential usage of the generated waste. The main purpose of this review is to elaborate the use of biorefinery for commercially available nutrients in the milk whey.

Keywords: Whey, valorization, biorefinary, whey protein, energy generation

Introduction

Milk production and processing have risen all over the world at a much higher rate over 10 percent every four years (FAOSTAT, 2019). A total production of milk is over 801 million of tons and more than 37% of this processed into cheese, chhanna and other blended produces and 30% is used for production of butter. During production of these milk products, the desired end product is only 10-20% of the milk and the left over 80-90% liquid is known as whey (Mirabella *et al.,* 2014).

Whey is a liquid component that is still made from cheese, chhana, paneer and casein. Whey's global output is estimated at 165 million tons, in which 95% contribution is given by cheese whey. In India, the main source of Whey comes from chhana and paneer production. If no survey / statistics are planned, Whey's estimated production is approximately 5 million tons per year (Gupta, 2008).

In the milk processing plants, the whey is released without any effective treatment in the water bodies or in the open land areas, which results in the environmental issues regarding pollution. A substitute to this problem is to generate biogas by anaerobic digestion of the whey for producing the methane and hydrogen. But, this can be done at laboratory scale using a small volume of the diluted whey to decrease the fluid's time to retain in the digestion chamber. However, the process doesn't permit the retrieval of the high value added products of it for example, protein, salts and lactose (Noureddine *et al.,* 2014 and Kargi and Uzuncar, 2013).

The milk whey comprises the proteins having a bioactive property, that's why this portion of the milk whey is studied mostly. When hydrolysis of these proteins is done, they releases the bioactive peptides, which, when intake by the humans, works as adjuncts to decrease the degenerative diseases (Ramos *et al.,* 2015). The exploration of the substitutes to purify and isolate these proteins by means of organic activity has created the interest of their addition in the foods (Gonzalez *et al*., 2014). This is identified very well that, the peptides obtained by the lactic fermentation have amazing properties like antihypertensive, anticancer, antithrombotic, metal carrying and antimicrobial (Kilara and Vaghela, 2018). These compounds are derived by using the biotechnological and chemical synthesis. Biotechnological process is a process used for lactic fermentation using microorganisms of different species which leads to development of various commercial products for example, organic acids, amino acids, bioactive peptides, hydrolyzed proteins and probiotics but chemical synthesis is a costly process (Wakabayashi *et al*., 2006, Elkhtab *et al*., 2017, Tahavorgar *et al*., 2015 and Paul and Somkuti, 2009). Milk whey also comprises of lactoferrin having biological function which depend on the existence of metallic ions in its structure (Marnila and Korhonen, 2009).

Protein derivation from the milk whey aims to utilize them as nutrients and in other cases as bioactive ingredients while preparing the functional foods (El-Salam and El-Shibiny, 2017,

Perreault *et al*., 2017 and Hiss *et al*., 2008). Usage of milk whey is generally done for animal feed and in particular cases concentrated in lactose or production of protein by using the process of ultrafiltration (Aydiner *et al*., 2014). Ultrafiltration is also used to decrease the pollutants emitted by these wastes in the surrounding environment. Protein or lactose is recovered in this breakdown process though it is an expensive process (Das *et al*., 2016 and Patel and Murthy, 2011).
To decrease the undesirable impression of milk whey on the environmental health, an alternative option is biorefinery process. The objective of biorefinery is to recover the high value products by using the combination of physical, biological and chemical techniques. At present, by using these processes the duration of the pollutants is lesser and the generation of waste in the industries leads to negligible. Biorefinery of milk whey integrates the physical, biological and chemical techniques to regenerate the hydrolyzed proteins, lactic acid, lactoferrin, probiotics and bioactive peptides in addition to the description of the concluding leftover to produce the methane using anaerobic digestion. The biorefinery can be synthesized to take the most valuable nutrients that reach technologically treated waste. Therefore, the objective of this review is to discuss the use of biorefinery for the commercially available whey's composition and technological values.

Composition of Whey

To show the relative importance of MW elements, Fig. 1 indicate the chemical composition, benefits to health, and importance of each nutrient in different industries (Prazeres *et al.,* 2012).

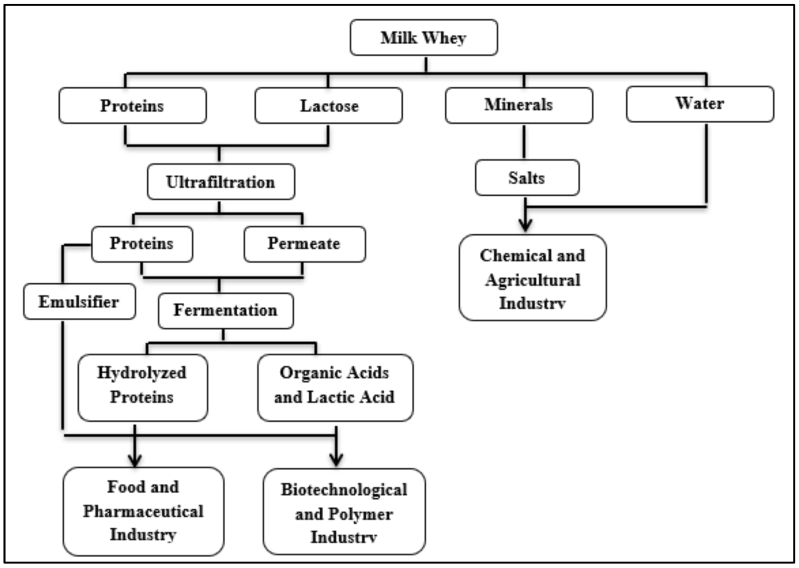

Fig 1: Chemical Composition of milk whey and their potential uses (Mollea *et al.,* 2013)

Cheese is made by using enzymatic treatment or by adding the organic acids. By using enzymatic treatments sweet whey is produced. In this treatment, proteases like chymosins or from any microbial soures are added. This shows the excellent coagulant property and produces high quality cheese at low costs. Some new specific peptidases for coagulation of milk are obtained by variety of fungi, for example, *Rhizomucor, Endothia, Phanerochaete, Mucor, Aspergillus* and *Rhizopus* (Silva and R, 2018). The second option is to use the organic acids for producing the whey acid. Though, the processes of producing whey are different but whey's chemical composition remains same in terms of protein, lactose and minerals (Table 1) (Yadav *et al*., 2015, Jelen *et al*., 2011, Kosseva *et al*., 2009 and Nishanthi *et al*., 2018).

Table 1: Chemical Composition of Whey (g L^{-1})

Components	Acid whey	Sweet whey
Protein	6.0-8.0	6.0-10.0
Lactose	44.0-46.0	46.0-52.0
Total solids	63.0-70.0	63.0-70.0
Sodium	0.21	3.1
Calcium	1.2-1.6	0.4-0.6
Chloride	1.1	1.1
Phosphate	2.0-4.5	1.0-3.0
Lactate	6.4	2.0

To valorize the milk whey, other commercial products can be developed by using the processes like ultrafiltration, demineralization and condensation (Fig. 2.). Using ultrafiltration followed by fermentation, can be used for probiotic production, organic acids, unicellular proteins etc. while demineralized whey is used for infant food and condensed whey is used for animal and human feed (Sesbatian *et al*., 2020).

2.1 Whey proteins

Protein is the component of whey, studied mostly. They are important due to their organic processes used for the derivation of proteins and peptides that are used in the food industries and pharmaceutical industries (Tsakali *et al*., 2010). The protein in whey is 20% of the total protein existing in the animal milk (Mollea *et al*., 2013). These are classified on the basis of their biological properties, molecular weight and solubility (Madureira *et al*., 2007).

Numerous purification methods are used to get them, because of their nutritional value. Therefore, a number of researches have been done during last. Table 2 represents the major characteristics of the whey proteins.

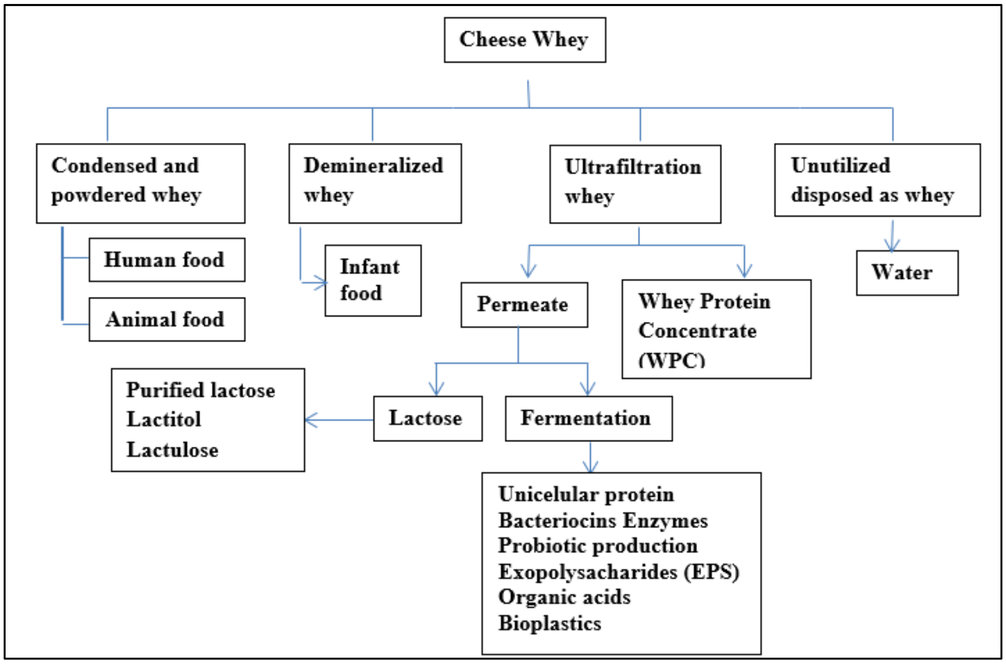

Fig 2: Different products obtained from milk whey

Table 2: Major characteristics of whey proteins

Protein	Concentration (g/l)	Molecular mass (Kg/mol)	Isoelectric point	Number of amino acids
Lactoperoxidase	0.03	78	9.6	612
Lactoferrin	0.1	77	7.9	700
Bovine serum albumin	0.4	66	5.1	582
Immunoglobulin G	0.7	150	5.8	*
α-lactalbumin	1.2	14	4.4	123
β-lactoglobulin	3.2	18	5.4	162

*Variable value

2.2 Lactose
The most abundant carbohydrate found in the milk whey is Lactose as compare to other nutrients. Lactose in the milk whey is found in 5- 6% concentrations and is generally recovered to be used as food stabilizer and also as a source of carbon in microbial cultural media. Lactose is derived from milk whey by using diafiltration, ultrafiltration or reverse osmosis. The efficiency of recovery of pure lactose by using these methods is upto 98%. Though using membranes in the refining process is costly that's why only certain industries with this type of technology provide additional value to milk whey decade (Nath *et al*., 2016, Smithers, 2015 and Pescuma *et al*., 2010).

2.3 Whey minerals
Some of the minerals present in the milk are retained in the milk whey. Sodium (Na), magnesium (Mg), Zinc (Zn), Calcium (Ca), Phosphorus (P) and Pottasium (K) are most abundant, Cooper (Cu) and Iron (Fe) are found in low concentrations. Other minerals are found less than 40 mg L^{-1}. Ca, Mg and P are vital nutrients of the milk because of their association with retaining casein micelles in the curd while cheese is manufactured (Crowley etal., 2019 and Gupta and Prakash, 2017).

Technical Uses of Whey
Milk whey for animal feed
A large amount of whey is produced in the industries, which make handmade cheese. This whey is used for food purpose for domestic animals such as cattle, pig and sheep. This source of food is cheaper than regular feed because it helps in weight gain to the animals. However, farmers should have to collect the fresh milk whey from the industries, to avoid any microbial contaminations which can affect the health of animals. Although the whey produced from every industry is not useful for animal feeding particularly in summers and rainy season (Westhoek *et al*., 2011).

Whey Protein Concentrate (WPC)
WPC is produced by application of ultrafiltration processes. The protein content in WPC varied from 34% to 80% (Barile *et al*., 2009). WPC is mainly used by athletes for intake as it increases the muscle mass (Baldasso *et al*., 2011). The protein concentration of whey protein is varied due to its variable chemical composition. The production of WPC by ultrafiltration starts with pretreatment of whey by skimming, bactofugation and pasteurization followed by reverse osmosis to remove the ions and salts. After this pretreatment the concentrated whey of 20% protein content is obtained. It is the base to produce the WPC of different protein concentration (Bacenetti *et al*., 2018). However, the disadvantage of using ultrafiltration is, use of membrane is uneconomic. The physicochemical properties such as temperature, pH and ions affect the life of membrane. This is the reason behind the pretreatment of whey before concentration process (Ganju and Gogate, 2017).

Separation of Lactoferrin from Milk whey
The purification of whey is must, for using the milk whey in pharmaceuticals and nutritional industries. Chromatographic methods are used to recover the proteins. For separation and

purification of proteins according to specific functional groups, affinity chromatography is used (Karakus *et al*., 2016). First time, Lactoferrin was isolated in the secretion of mammary gland by Sorenian *et al*., 1939. Afterwards, It was recognized that Fe^{3+} ions are present in this protein, which provides various biological functions, having anticancer and antimicrobial properties (Yao *et al*., 2013).

Currently, a number of methods are used to separate or obtain the lactoferrin for example ion exchange and solid phase separation (Sebastián *et al*., 2020), Dye affinity chromatography (Baieli *et al*., 2014), Cation exchange (Billakanti and Fee, 2009) and adsorptive membrane chromatography (Plate *et al*., 2006).

Production of Lactic acid using fermentation

Production of lactic acid using fermentation of the residues rich in simple carbohydrates became an interesting topic for recent researches. For growing different lactic acid bacteria (LAB), Whey is an ideal substrate, yielding 70-90% lactic acid (Abdel-Rahman *et al*. 2003 and Ghaffar *et al*., 2014). The disadvantages of lactic acid production by fermentation is, the costly recovering it using conventional methods for example, simple extraction or distillation (Komesu *et al*., 2017). A number of researches have been conducted to reduce the cost of recovering lactic acid. The different methods for example, solvent extraction, membrane extraction, ultrafiltration, drying, microfiltration, diffusion dialysis, distillation, chromatographic methods, adsorption, reverse osmosis, conventional electrodialysis have been used (Bedas *et al*., 2017, Choi *et al*., 2002, and Sun *et al*., 2006). However, some of these methods are more effective and cheaper than electrodialysis and these were conventional electrodialysis and microfiltration (Delgade *et al*., 2018 and Chen *et al*., 2016).

Production of Bioactive Peptides

Lactic acid bacteria play an important role not only in production of lactic acid but also for generation of peptides. The peptides are released by the protcolytic system of LAB (Hernandez *et al*., 2011). The amino acids which constitute the peptides are the reason behind their biological activity. Therefore, there are many researchers, who have been put efforts to study on different beneficial biological activities of these peptides on humans with their antimicrobial, immunostimulatory, antihypertensive, opioid, antithrombotic and mineral transporting activity (Smacchi and Gobbetti, 2000). The great interest of these molecules mainly for food and drugs industries since these can be the basis of food or medicines production (Pintado *et al*., 2001).

3. Environmental impacts of whey

Milk whey has a high concentration of contaminants having a high quantity of chemical and biological oxygen demand (COD & BOD). Organic load in the milk whey is mainly caused by fat, protein and lactose (Table 1). This organic matter is considered as highly polluting, almost 60-80 times more than that of domestic wastewater, though 99% of the organic matters are biodegradable.

If the milk whey is delivered into the water bodies without any effective treatment, it could arise serious environmental issue. The organic matter in the milk whey effortlessly absorbed in the environment, which results in the exhaustion of oxygen. The septic conditions as well as strong odors are discharged, which turns the water bodies into the habitat and breeding ground for the disease born insects and pests (Shete and Shinker, 2013). Lactose, a major component of the milk whey, is reported as a sponsor of sewage fungus in the water bodies, which are greasy trails and are unwanted and harm the water bodies. One more environmental impact of milk whey in waterways is because of its good nutritional value and its capability to enhance the eutrophication (Curtis *et al*., 1939).

Land use of milk whey for example nitrogen and phosphorus fertilizers has been practiced for decaded in USA, Australia and Canada, along with the other countries (Ghaly *et al*., 2007). This trend may appear to be ongoing because whey is reported as an appropriate source of potassic superphosphate, which results in improved soil properties. Though, high application rates can result into the reduced plant growth and and adverse effect on the soil properties (Watkins and Nash, 2010). It has been resulted that $1mm^3$ irrigation of whey added 400-600kg of salt/ha, which leads to high salinity levels and decreased crop produces (Ghaly *et al*., 2007). It is suggested that to overcome the problem of salinity and to get the adequate irrigation quality, a ratio of 1:20 of whey and pure water can be used.

The whey nitrogen leads to the biological changes like aerobic nitrification, which lead in production of highly leachable nitrates and nitrites that result in polluting the groundwater. Denitrification (decrease in nitrate bacteria in the N2O and N2) occurs in polluted areas and where the right source of carbon is found, and leads to a loss (approximately 5 - 20%) of whey nitrogen used. Since N2O is an important greenhouse gas, it poses some of the environmental consequences of using the earth.

Biorefinery CW: Valorization

Currently, agricultural waste treatment technologies aim to reduce environmental pollution (Mohan *et al*., 2016). The reason behind consideration of whey's use in biorefinery systems as an important topic is the pollution caused when it is released into the water bodies and land without any effective treatment. Kamm and Kamm, 2004, have described three categories of biorefineries as Category I, II and III. The category I of biorefinery produces only one final product, if more than two different products are produced than it is category II of biorefinery. Category III of biorefinery, combine various technologies to generate their own energy.

Nemeth and Kaleta, 2015, considered the ergoestrol production of milk whey by using the biorefinery program. Vitamin B12 and propionic acid were produced by using the residues leftover from their designs. This study concluded that this process developed a positive relationship of zero waste theory with the obtained value added produces. Other conclusions given by Domingos *et al*., 2018, demonstrate the possibility of production of high concentrated polyhydroxyalkanoates (PHAs) by fermentation of cheese whey. These types of plastics are sustainable and biodegradable and used for manufacturing of biopolymers.

Sampio *et al*., 2019 calculated the capacity of production of bioethanol of milk whey and using the Kluyveromyces lactis as an alternate of the treatment given to the leftover residue of the dairy industry and to produce value added product. DE Giorgi *et al*., 2018, explored the primary situations for the whey's use in industries as substrate for possible biotechnological treatment of the leftover residue by producing *Pseudomonas taetrolens.* A multi feed biorefinery design was developed by Sanchez *et al*., 2016 for producing bioethanol using lignocellulosic waste and simultaneously for the treatment of agricultural and industrial waste. This process is more complicated as compared to simpler once which uses the simple lignocellulosic biorefineries.

Challenges and opportunities in whey valorization

From ancient to present the usage of whey has been changed, in 17th century whey was used for medicinal purpose. In present time, it is used for many types of useful and valuable compounds. Though, since the middle of 20th century it has been considered as pollutant, but technical knowledge of Whey structures, their properties, and their useful properties has grown. A whey biorefinery parallel to the dairy industry will be more useful for producing a high value added product from the generated waste of dairy industry. Advancement in the technologies results in the less expensive processes and manufacturing of products efficiently as whey as components. Whey and its components such as lactose, proteins, lipids and peptides, have been used commercially in bio-medical products, food, pharmaceuticals, specialized formulations and cosmetics, and a market for the biological, functional and nutritional quality of the components of the whey. It should be noted that, protein of the whey is the most widely used component.

Currently, the whey industries are facing new and more challenges and chances. One such case in point is to extend the existing dairy industry, in which production of whey is a part. More examples include, to decrease the manufacturing cost of whey components, acid whey production, market struggle with other products and opportunities in the area of active peptides of whey. The main challenge to treat this type of waste is to integrate the different procedures to produce a new component. The waste valorization is the complete utilization of the waste and end up with the energy production using an ecological process.

Conclusion

Whey, because of its ingredients is an interesting product. The functions, characteristics, structure of whey make it a good base for producing new value added products and an ideal alternate of traditional bases. There are so many options to utilize whey in replacement of treating it as a waste. Where whey is considered as a problem for environment, this problem can be solved by the biorefinery processes by producing value added products. This paper discussed the whey, its components, and impact on environment, valorization of whey and challenges and opportunities in whey industries.

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