Acid whey: A dairy by-product with potential benefits

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ABSTRACT

The rising demand for dairy products has fueled the expansion of the dairy industry leading to a buildup of waste. Acid whey is obtained through the acidification of whey, a major by-product of cheese and yogurt manufacture, whose production has accelerated globally in recent years mainly because of the increased preference of health-conscious consumers looking for high-protein and low-fat products. Enormous costs required for appropriate disposal, along with the environmental hazards associated with their direct release into water bodies owing to their high biological oxygen demand (BOD) and chemical oxygen demand (COD), have necessitated their management. Besides, these have been noted as reservoirs of important nutrients, including carbohydrates, proteins, lipids, minerals, and essential vitamins that can be utilized for maintaining good health and ameliorating several disorders, hence further entailing their valorization. Moreover, their low cost makes them an attractive source of nutritional and functional components. Notably, the physical and chemical properties of acid whey have been noted to vary marginally depending on their source of origin. Acid whey has indeed been studied to improve gut, respiratory, nerve, muscle, bone, and skin health and portray anti-cancer, anti-microbial, anti-inflammatory, and antioxidant effects Therefore, the use of acid whey for consumption either directly or as a supplement through food and nutraceutical products may not only solve the problems associated with their clearance and management but also aid in issues related to food and nutritional security.

Keywords—acid whey; dairy; health benefits; nutrients; waste management

#  INTRODUCTION

 Industrial wastes are a potent problem worldwide because of their long-term influence on both human health and the environment. The food-processing industry produces giant volumes of waste, both solid and liquid, from the production, preparation, and consumption of food. Amongst others, whey constitutes one of the major end products of the dairy industrial sector. In fact, whey was discovered around 3000 years ago when the stomachs of calves were used for storing and transporting milk. The milk coagulated during storage and shipping, resulting in curds and whey, through the action of the naturally occurring enzyme chymosin (rennet) found in the stomachs of the calves. This ignited the start of the cheese and yogurt industries. Nowadays, whey is a significant co-product of the modern industry of cheese and casein. Whey volumes are rising at about the same rate as milk volumes, on an average at 42% per year worldwide [1]. Increased quantities of milk are channeled into the manufacture of greater amounts of cheese, casein, and other milk products, resulting in a significant increase in whey quantity. Unfortunately, several dairy companies have not yet realized the true worth of both whey and a whey-based by-product called acid whey [2]. Today, most dairy farm industries continue to treat acid whey as waste. They are usually disposed of either in waste streams or sold for very little or no profit to farmers. Whey including acid whey has historically been viewed as a waste stream and an annoyance by both casein and cheese producers. Spraying these by-products in fields and their disposal in water bodies and sewage streams is often difficult on account of problems associated with pollution and cost. The disposal of acid whey in large volumes imposes costs on the dairy manufacturers and results in environmental damages in the form of unproductive land and water pollution, resulting in fish kills. In addition to that, the water, energy, and carbon footprints related to wasted acid whey account for about 320 million tons of water, 2 billion kilowatts of energy, and 500 thousand tons of greenhouse gas emissions each year in the United States [3]. Contemplating the issues related to the production of dairy wastes and their potential nutritional properties owing to their dairy origin, these by-products and wastes can be utilized as food and nutritional supplements, especially for use in developing and underdeveloped nations. 11% of the world's population is food insecure. Food waste has a significant ethical dimension due to the high prevalence of food insecurity [4]. Noteworthy, dairy waste utilization can aid in combating food insecurity. Controlled food supplies and the valorization of wastes, including those produced by dairy industries, and may help battle the above problem. Moreover, while maintaining current levels of production, reducing food waste and managing dairy waste have been noted as potential methods of meeting global food needs [5].

# ACID WHEY

 The manufacturing of various dairy products like soft cheeses, yoghurts, cream cheeses, and caseinates produces an acidic by-product known as acid whey [6]. The main source of whey in India is from the processing of chhana and paneer, where acid-whey is eliminated during the concentration step involved in the production of the former. It contains valuable active ingredients that possess several health benefits. Importantly, acid whey dispersion in huge volumes by the dairy industry has led to increasing research on ways to find its value and systematic techniques for utilizing it. Several industries are finding ways to use acid whey on-site. Moreover, academic communities are aiming to further investigate the practical uses, composition, utilization, and health benefits of this by-product. The production, processing and applications of acid whey are displayed in figure 1.



 **Figure 1: Production, Processing and Applications of Acid Whey**

***(Adapted from* [7])**

 Based on the processing conditions, whey is classified as acid whey or sweet whey [8, 9]. In contrast to sweet whey, which commonly has a pH of 6 to 7 and is collected after casein coagulation (with rennet or enzymes including chymosin in the making of hard cheese), acid whey is a residue of acid coagulation [10]. It is processed using either *Lactobacilli* activity or the addition of organic acids such as citric, acetic, lactic, or mineral acids such as hydrochloric or sulfuric acid [11]. In fact, acid whey is a residue of the manufacturing of acid-coagulated cheeses (cottage cheese, ricotta cheese, etc.) and Greek yoghurt. Acid whey has a high content of lactic acid and minerals. Its composition is determined by various factors, including the source of the milk, the type of cheese or yoghurt being produced, and the type of thermal treatment performed on the milk [12]. Even acid whey collected from the same source and processed in the same way can have varying protein levels depending on the season [13]. Previous research has reported that the composition of acid whey shows differences depending on the source of origin. In general, Greek yoghurt acid whey (GAW) has been shown to contain lower ash, total solids, lactose, protein, and lactic acid content than cottage cheese acid whey (CAW). Nonetheless, both variants have similar lipid profiles owing to their production from skim milk.

## **Industrial Production of Acid Whey**

 The expansion of Greek yoghurt manufacturing has increased the production of acid whey. Demand for Greek yoghurt in the United States has surged since 2009, owing to health-conscious consumers seeking high-protein, low-fat, on-the-go goods. In fact, Greek yoghurt currently controls more than half of the US yoghurt market and was the most popular type of yoghurt purchased in 2019. During the manufacture of Greek yoghurt, 100 units of milk are turned into 33 units of yoghurt and 67 units of acid whey. In 2015, 771,000 tons of Greek yoghurt and roughly 1.5 million tons of acid whey were produced [14]. In contrast, during the manufacturing of acid-coagulated cheese, 1 kilogram of cheese yields 10 litres of acid whey. About 5-8 billion pounds (2-6 million tons) of acid whey and 45 billion pounds (20.5 million tons) of sweet whey are produced annually in the United States of America. The European Union has the world's largest cheese market, resulting in a massive production of acid whey. The output of acid whey from cheese alone is estimated to be 40 million tons per year in the European Union [15]. In addition, the cottage cheese industry is expected to rise because of the increased demand for high-protein, convenient snacks and creative dairy products, leading to generation of an excess of acid whey. The forecast value for whey production in India is 3.0 million tons per year, with the main source of whey being chana and paneer production, while the cheese industry provides approximately 50,000 tons of whey [16].

## **Utilization of Acid Whey**

## Global whey output is estimated at approximately 165 million tons, around 95 percent of which is cheese whey. The lower pH of acid whey allows certain individual whey proteins to be insoluble, making processing via membrane filtration difficult. Because of its unique qualities, acid whey has been employed in the creation of beverages such as vinegar beverages, with a focus on health benefits. Indeed, CAW has been used in the manufacturing of functional beverages and in bio-packaging [15]. Moreover, the production of glycine, betaine, and trehalose for use in the food, agricultural, and pharmaceutical industries has also been carried out using GAW [17]. Nonetheless, enormous quantities of acid whey remain unutilized. According to reports, the usage of 75% in Europe and possibly less than 50 percent in the rest of the world results in the waste of a very large amount of material with potential value as food or feed. Discarding whey also represents a substantial loss of potential nutrients and resources, and the polluting effects of the same have been a strong concern for environmentalists and technologists. Furthermore, proper disposal of the above leads to an economic strain on the dairy industry because of the heavy treatment costs. Furthermore, GAW and CAW have high biological oxygen demands (BOD), ranging from 52,400 to 62,400 mg/L and 31,900 to 40,000 mg/L, respectively. This makes disposal of acid whey without harming the local ecosystems challenging [18]. Additionally, the excessive lactic acid content accompanied by a low pH leads to the crystallization of lactose during spraying. Similarly, the high calcium phosphate concentration of acid whey causes considerable fouling of machinery. The above difficulties complicate its valorization, necessitating deeper studies regarding its potential application. Although acid whey is most commonly used on agricultural land as fertilizer, its implementation is limited due to the risk of running off [19]. Livestock can be fed acid whey when combined with silage. Nevertheless, this utilization is also restricted because of the likelihood of animal salt toxicity. Acid whey can also be digested anaerobically to produce combustible fuel, such as methane gas, which can then be used as a source of energy [14]. Besides, purified acid whey is also used for the growth and multiplication of yeasts in vitro. The use of acid whey as a nutritional supplement for humans has also gained attention in recent times owing to the nutritional composition and functional benefits of this by-product. In fact, food producers and researchers have been attempting to maximize the utilization of acid whey by separating valuable components from the former and minimizing its disposal to save on environmental impact as well as costs [20]. Indeed, several companies, including General Mills, Danone, and Arla Foods Ingredients have purified acid whey and used it for fortification of food products, production of soluble fibres, and generation of lactose [21].

# NUTRITIONAL COMPONENTS OF ACID WHEY

 Chemically, acid whey is a mixture of water, fat, protein, lactose, carboxylic acid, and minerals [22]. The pH of acid whey ranges from 4.3 to 4.6, with pH 4.2 to 4.4 for GAW and 4.3 to 4.4 for CAW, respectively. Moreover, the total solid content spans from 3% in CAW to 6% in GAW [23].

## **Carbohydrates**

 Lactose, which makes up the majority of whey, amounts to about 5%. It accounts for approximately 70% of the total solids in the same. Due to the fermentation process, in which some lactose is transformed into lactic acid, acid whey typically has a lower lactose level. Lactose conducts a gradual chemical process during digestion that increases energy supply [24]. The content of these macromolecules affects the textural properties and impacts the utilization of acid whey in food products. The degree of non-Newtonian behavior and viscosity are both decreased by lactose hydrolysis. Perhaps as a result of the creation of heat-induced complexes, an increase in lactose concentration has been observed to decrease the denaturation of proteins. Only 20 g of lactose dissolves in 100 g of water at ambient temperature, while 60 g dissolves in 100 g of water at 60 °C. Therefore, crystallized lactose develops when whey concentration exceeds 36-38% of total solids. Interestingly, this forms the basis of lactose manufacturing by using a technique that crystallizes highly concentrated whey [23].

## **Proteins**

 β-lactoglobulin accounts for 65% of whey proteins, followed by α-lactalbumin (25%), albumin (8%), and glycomacropeptide (GMP). Other significant whey proteins include bovine albumen, lactoferrin, immunoglobulin, and phospholipoproteins. In comparison to casein, soy, beef, or gluten, the whey macromolecule has a biological value (BV) of 110. Lactoferrin (LF), is a globular milk protein that represents between 1 and 2 percent of the acid whey protein and has numerous biological functions. Additionally, lactoferricin, a tiny cationic peptide that is several times more active than LF, is produced by the enzymatic hydrolysis of LF [25]. Importantly, acid whey proteins have been indicated to be efficiently utilized by the human body because of its high biological value, thereby reflecting its scope of use.

## **Lipids**

 Despite being poor in lipids (< 0.04%), acid whey has the potential to be a significant source of the milk phospholipids (MPL) that promote health. Acid whey contains MPL in the form of lipoproteins, small fat globules, and milk fat globule membrane (MFGM) fragments. In fact, some acid whey by-products, such as acidic buttermilk, and cheese whey, have MPL levels up to five times higher than those in sweet whey, at 0.1% and up to 0.39 percent, respectively [26]. Notably, before being used in other applications, lipids are separated from acid whey to prevent unwanted turbidity. The most abundant lipid class in acid whey is triacylglycerol, which is followed by phospholipids, diacylglycerols, FFA, cholesterol esters, cholesterol, and monoacylglycerol. Sphingomyelin, phosphatidyl choline, and phosphatidyl ethanolamine are the three main phospholipids in whey protein concentrates, followed by phosphatidyl inositol, phosphatidyl serine, and cerebrosides. Furthermore, when compared to milk, the free fatty acids display a higher butyric acid content compared to oleic, caproic, and caprylic acids [27].

## **Minerals and vitamins**

 Acid whey contains significant concentrations of calcium (1.12%), phosphorous (1.78%), copper, and zinc. Moreover, it also inhabits the presence of potassium (0.16%), magnesium, manganese, sodium, chloride (0.11%), and boron [18]. Several novel methods have indeed been employed for the treatment of acid whey because of their rich mineral profile. This by-product also harbors certain types of water-soluble vitamins, particularly those belonging to the B vitamin group, including Thiamin, Riboflavin, Niacin, Panthothenic acid, B6, and B12, hence indicating their use for micronutrient deficiency disorders.

# HEALTH BENEFITS OF ACID WHEY

 In recent years, due to their advantages in terms of health benefits and various applications, acid whey fractions have gained considerable attention. Whey-based drinks target a wide variety of customers, from elderly people to small children. It has been stated that proteins, lipids, lactose, and bioactive molecules derived from whey carry potential biotechnological ability and promise to be used in the health sector (Figure 1). The effect of acid whey on human health and potential disease-ameliorating applications are highlighted in this section.

## **Energy and Mucoskeletal Health**

 Acid whey based foods and beverages are attractive because of their energy and nutrient content. Notably, these are desired especially by athletes in the form of sports-drinks because of their amino acid and protein content [28]. These by-products are one of the significant reservoirs of branched-chain amino acids (BCAA) such as isoleucine, leucine, and valine that are essential for muscle mass development as well as metabolism in the muscle. Interestingly, acid whey consumption has been reported to delay sarcopenia in elderly individuals and prove beneficial for instances of osteoporosis and phenylketonuria. Lactic acid derived from acid whey has been linked to improving the bioavailability of calcium through the solubilization of the insoluble forms of this mineral [23]. Moreover, the lactose, casein, vitamin, and mineral compositions discussed in section 3, help support physical activity and wellbeing. Notably, components such as β-lactoglobulin, α-lactalbumin, and vitamin B12 are used as supplements for medicinal purposes. Lactoferrin in acid whey has been noticed to elevate the absorption of iron from food. Nonetheless, the protein content of acid whey is lower compared to sweet whey, with the former possessing a higher percentage of lactose, thereby making it a valuable energy source. Unfortunately, acid whey has been observed to develop a granular consistency upon heating, necessitating the requirement of technological interventions and innovations for efficient valorization of nutrients and health promoting ingredients [29].

## **Gut Health**

 Acid whey consumption has been associated with improved gut health and amelioration of digestive disorders. The electrolytes present in this by-product, including magnesium and phosphorus, help in the management of diarrhea. Moreover, the α-lactalbumin boosts intestinal health and increases nutritional absorption, especially of iron and zinc, apart from portraying prebiotic effects [30]. In adults, α-lactalbumin proteolysis produces bioactive peptides with antibacterial and prebiotic properties. Phospholipids derived from acid whey have been reported to increase intestinal crypt depth in mammalian models and promote mucosal immunity. Besides, these phospholipids also elevate the number of viable probiotics, mainly *Bifidobacterium sp,* hence contributing to gut homeostasis [31]. The antimicrobial agents present in acid whey also protect the gut against pathogenic infiltration, thus maintaining stability. Additionally, lactoferrin and its derivative lactoferricin have been documented to inactivate gram positive and negative bacteria through interference with their cell wall teichoic acids and lipopolysaccharides, respectively. Also, these bioactive components prevent the adherence of pathogens to the intestinal walls of the host. Even though acid whey contains a substantial amount of lactose, most of it is converted to lactic acid through fermentation by starter cultures during acidification processes, thereby making it suitable for lactose-intolerant individuals. Notably, acid whey holds approximately 16 times more lactic acid compared to sweet whey [32].

C. **Respiratory health**

 Acid whey has been noticed to improve prognosis in patients suffering from chronic obstructive pulmonary disorder (COPD) through reduction of inflammation and muscle wasting. By supplying necessary amino acids and boosting protein anabolism, this protein may aid in promoting the synthesis of new muscle, hence ameliorating pulmonary disorders. The anti-inflammatory and antioxidant characteristics of proteins present in acid whey have been shown to be useful in lowering levels of pro-inflammatory cytokines, including tumour necrosis factor (TNF) and interlukin-6 (IL-6) in the body [33]. Moreover, the magnesium content of acid whey may not only help in COPD management through maintaining muscle mass but also by directly restricting COPD [34]. Moreover, antioxidants present in acid whey minimize the inflammatory effects of pulmonary distress, thereby improving the disease status.

D. **Immunity**

 The majority of the bioactive proteins found in acid whey are immunoglobulins. In fact, they have been employed for the manufacture of immunoglobulin-fortified milk products. These immunoglobulin-rich isolates provide the user with passive immunity, and research suggests that they can improve intestinal health, suppress illnesses, increase athletic efficiency and recuperation times, and assist people who may have weakened immune systems. Furthermore, lysozyme in whey has also been correlated with improved immunity. Additionally, the amino acid precursors in whey that turn into glutathione can boost immunity and eliminate possible carcinogens. Additionally, the α-lactalbumin present in acid whey has also been associated with enhanced immune responses [35].

E. **Cancer**

 Acid whey proteins have gained popularity as a supplement in recent years because of their numerous health advantages. α-lactalbumin and β-lactoglobulin has been noted to portray cancer-preventive actions [36]. In fact, α-lactalbumin in combination with human proteins, forms human alpha-lactalbumin made lethal to tumor cells (HAMLET) complexes that have been connected with anticancer capabilities via destroying tumour cells, triggering liposomal destabilization, and enhancing cell death pathways. These complexes have also been proposed as a treatment strategy for aggressive tumours [37]. Moreover, the lactic acid content of acid whey has been linked with improving cancer prognosis through anti-inflammatory effects mediated by lipopolysaccharide-induced impairment in inflammatory signaling pathways by affecting extracellular signal-regulated kinase and inhibitor of nuclear factor κB-α (I-κB-α), along with up-regulation of nitric oxide, IL-6, and IL-10 [38].

F. **Obesity**

 The functionality of whey proteins in the diet of obese individuals is related to the stimulation of cholecystokinin (CCK) by oral intake of glycomacropeptide (GMP). When cheese is being made, chymosin causes the C-terminal portion of kappa-casein, termed GMP, to be released into the whey. CCK is known to be an important satiety hormone that can make whey protein a useful component of a weight-loss diet. It decelerates gastric emptying, which in turn promotes satiety [39]. Moreover, stimulation of lipase activity via β- lactoglobulin also helps in instances of obesity [40]. Besides, whey can be a desired nutrient source for obese individuals owing to its low fat percentage. There is a significant commercial impact of whey on the weight-loss diet because of its protein content. Indeed, the essential and non-essential amino acids in whey have been documented to act as protein synthesis substrates and improve body mass index. Additionally, lactic acid from acid whey has been reported to minimize lipolysis in the adipose tissues of mammals, including humans [23].

G. **Cardiovascular health**

 Acid whey has also been known to promote cardiovascular health through its protein and other bioactive components. Several proteins, including β-lactoglobulin have been noted to reduce blood pressure in mammalian models. Moreover, mineral components of acid whey such as calcium and magnesium have also been noted to reduce the systolic blood pressure [41]. Moreover, the anti-obesity effects of this by-product also help protect cardiovascular health. Furthermore, acid whey components have been observed to impact vascular stiffness, hence influencing hypertension. The anti-oxidant and anti-inflammatory actions of acid whey have also been acknowledged to promote cardiovascular well-being. Importantly, the anti-hypertensive effects mediated by acid whey have been mainly attributed to its influence on the renin-angiotensin system (RAS) [42].

H. **Antimicrobial effects**

 Lactoferrin (LF) is a globular protein, representing between 1 and 2 percent of acid whey proteins, and is one of the most important milk proteins with multifunctional activities. A tiny cationic peptide called lactoferricin is created by the enzymatic hydrolysis of LF and is significantly more active than LF itself. While it is not a naturally occurring peptide, a large spectrum of antimicrobial activity against yeast, fungi, viruses, protozoa, and gram-positive and gram-negative bacteria is shown by the artificial development of the small peptide lactoferrampin from LF [43]. In particular, dietary LF supplementation improves energy balance and metabolism and decreases adiposity [44]. Moreover, organic acids produced during the acidification process, including lactic, formic, acetic, citric, and pyruvic acids, portray antimicrobial effects through pH induced mechanisms. Owing to the above properties, acid whey has been traditionally used for the treatment of diarrhea, skin disorders, and urinary tract infections.

I. **Nerve and brain health**

 Acid whey has been known to promote nerve and brain health and assist cognitive development. Due to the large concentrations of spingomyelin (SM) and its metabolites in the myelin sheath that surrounds and insulates neurons, acid whey derived phospholipids, mainly SMs, are noted for their function in neuronal signaling and cognitive development. According to previous studies, supplementation with SM has been observed to elevate motor, behavioral, and cognitive abilities in preterm and low-weight infants [45]. Due to changed brain phospholipid metabolism, age-related disorders like Alzheimer's, Parkinson's, and Schizophrenia may also benefit from MPL's neurological effects [46]. Lactoferrin from acid whey has been observed to support neuronal development in infants diagnosed with intrauterine growth restriction through modulation of brain-derived neurotrophic factor (BDNF) and neuronal density [47]. Furthermore, acid whey minerals such as potassium, magnesium, and calcium are involved in the transmission of nerve impulses, muscle contractions, relaxation of nerves through depolarization, and maintaining the integrity of nerve impulses. Nevertheless, lactic acid in acid whey has been associated with improvement of cerebral energy metabolism in people with traumatic brain injury as well as amelioration of cognitive impairment [48].

J. **Skin health and anti-ageing effects**

 Acid whey components, especially milk phospholipids have been found to enhance skin health via several mechanisms including elevating the water holding capacity and accelerating repair processes, hence improving the quality and barrier function of the same [49]. The anti-ageing effects of acid whey are mainly mediated by their antioxidant effects. Cysteine containing proteins from whey help in the production of glutathione (GSH), a potent intracellular antioxidant that has been identified as an anti-ageing agent. Due to its ability to chelate iron and reduce oxidative stress, glutathione peroxidase (GSHPx) aids in the detoxification process. Moreover, α-lactalbumin and β-lactoglobulin, have been noted to increase the concentration of glutathione [50]. Acid whey derived lactic acid can further benefit skin health through its proven abilities to reduce the severity of photodamage, patchy hyperpigmentation, and sallowness.

K. **Oral health**

 The natural antimicrobial activity of lactoperoxidase from acid whey is exploited in a number of oral healthcare products and is used for the prevention and treatment of xerostomia [51]. The products containing lactoperoxidase have been clinically proven to prevent gingivitis-related harmful microorganisms and oral irritation, discourage gum bleeding, minimize inflammation, and tackle both the causes and symptoms of halitosis [52].

# CONCLUSION

 An increasing population is expected to elevate food demand, placing a strain on all food sectors, including the dairy industry, thereby indicating the need for waste management and its valorization to cater to these issues. Reducing food loss and waste is important in terms of minimizing production costs, improving food protection and nutrition, and contributing to the sustainability of the environment. Acid whey is a significant by-product of the dairy industry that is produced through *Lactobacillus* or organic acid mediated acidification of whey, resulting in a net pH between 4.3 and 4.6. Unless utilized, their disposals incur huge costs and may lead to environmental concerns. Nevertheless, acid whey has been noted to encompass a wide range of nutrients and health promoting components, such as macronutrients, vitamins, minerals, immunoglobulins, β-lactoglobulin, α-lactalbumin, phospholipoproteins, sphingomyelin, lactoferrin, and glycomacropeptide, that have been documented to aid in maintaining gut, respiratory, cardiovascular, mucosleletal, nerve, brain, skin, and oral health through their antioxidant, anti-inflammatory, anti-hypertensive, immunomodulatory, anti-obesity, anti-cancer, and antimicrobial properties. However, the physicochemical properties of acid whey have been observed to vary marginally depending on the source of origin, type of cheese or yoghurt being produced, type of thermal treatment being performed on the milk, and the season. Notwithstanding, acid whey has been recognized as an economical reservoir of nutrients and functional ingredients that can be employed for food product development, food fortification, and even the formulation of nutraceutical and pharmaceutical products. Therefore, acid whey valorization and usage may not only help with difficulties related to its management and disposal but also with concerns linked to the security of food, health, and nutrition.

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