The Pharma Innovation Journal 2021; 10(2): 387-390

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(2): 387-390

www.thepharmajournal.com
Received: 01-12-2020
Accepted: 03-01-2021

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Industrial applications of whey

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DOI: https://doi.org/10.22271/tpi.2021.v10.i2e.5695

Abstract
Almost 85% of the entire milk utilized for manufacturing cheese/paneer is dismissing as whey. Most of the milk plants do not have a decent pre-treatment practice for the jettisoning of whey and the discarding of whey. Whey deemed as the most significant pollutant of the dairy enterprise, not solely due to its high organic content but also owing to its lofty volume.
Whey protein-enriched method can develop for end groups such as infants, cardiac-risk group, and diabetics. Whey is assuredly an attractive growth medium for several kinds of microorganisms. The notable decline of BOD with the subsequent generation of valuable bio-products such as ethanol, bacteriocin and biogas has accomplished. Therefore, now whey is not a pollutant but a possible raw material for innovative products and processes.

Keywords: whey protein, lactalbumin, lactoglobulin, lactoferrin, antioxidant, antihypertensive, antidiabetic, antiviral, ethanol, bacteriocin, biogas

Introduction
Milk is a copious reservoir of protein with a crucial role in neonates and adults. Milk protein and bioactive peptides have health-enhancing traits. Almost 85% of the entire milk utilized for manufacturing cheese/paneer is dismissing as whey. Most of the milk plants do not have a decent pretreatment practice for the jettisoning of whey and the discarding of whey. It represents 85-90% of milk volume and comprise a vital loss of potential food and energy source as whey retains approximately 55% of the whole milk nutrients including, lactose, soluble proteins, lipids, and mineral salts, and so on (Siso, 1996; Smithers, 2008) [29, 30] which contributed to high biochemical oxygen demand. Whey deemed as the most significant pollutant of the dairy enterprise, not solely due to its high organic content but also owing to its lofty volume (Walzen *et al.*, 2002) [36]. Nevertheless, the discernment of whey as a pollutant has transformed with the development of its utilitarian and bioactive attributes, being viewed as a supplementary commodity of cheese manufacture (Smithers, 2008) [30].

Antioxidant activity
Oxidation of food components is a pivotal phase in food decomposition. It is quite understood that lipid peroxidation of food commodities can generate decay in food quality, reduce the shelf life and minimize the acceptability of processed foods. Lipid peroxidation can produce free radicals that can begin the fatty acid disintegration, which may diminish the nutritional aspect and food safety by producing unpleasant flavors and toxic elements (Niki *et al*., 2005) [20]. Synthetic antioxidants render potent antioxidant activity against several oxidation systems. However, their use is restricted in some countries because of their marked toxic impact on individual physiology and metabolism (Okada and Okada, 1998) [22]. On the other hand, bioactive peptides are recognized as natural antioxidants and possess a vast interest because of their safety and distribution properties. Zhidong *et al.* (2013) [38] studied the antioxidant activity of whey protein isolate (WPI) hydrolysate and found that DPPH radical scavenging activity of hydrolysate was 31.48% and reducing power was 0.612 at 700 nm for enzyme to substrate ratio (E/S, w/w) of 2.22%, hydrolysis time 3.60 h at 45.70 ^{o}C temperature. Mann *et al.* (2015) [15] hydrolyzed the whey protein concentrate (WPC) with three different protease flavourzyme, alcalase and corolase and evaluated the antioxidant activity by using 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method. The Trolox equivalent antioxidant activity of all the hydrolysates i.e. flavourzyme (0.81±0.04), alcalase (1.16±0.05) and corolase (1.42±0.12) were higher than the WPC (0.19±0.01). Among these, whey protein hydrolysates prepared using corolase showed maximum antioxidant activity.

DPPH radical scavenging activity of fermented whey from cow and buffalo was 32.73% and 25.65% respectively(Vankudre *et al.,* 2015) [35].

Antihypertensive activity

ACE is a peptidyl di-peptidase enzyme, cleaves the carboxyl-terminal end of the substrate that may control a rise in blood pressure by switching angiotensin I to an active peptide hormone angiotensin II. This stimulates the release of aldosterone on one hand and inactivation of vasodilator, bradykinin on other hand, as a result of which sodium concentration becomes high and blood pressure goes up. ACE inhibitory peptides have gained specific recognition due to their inherent useful results in the treatment of hypertension. α-LA and β-LG hydrolysate reported possessing ACE-inhibitory properties (Tavares *et al*., 2011) [32]. Whey peptides obtained through fermentation with lactic acid bacteria like *L. helveticus, L. lactis* are immune to the digestive tract endo-peptidases, accordingly, can be easily absorbed into the bloodstream (Saito *et al*., 2000) [28]. Heptapeptide fraction (142-148) obtained from β-lactoglobulin was the most potent ACE inhibitor (Mullally *et al*., 1997) [18]. Digestion of α-lactalbumin and β-lactoglobulin with trypsin showed potential to produce peptides with high ACE inhibitory activity (Pihlanto-Leppala *et al*., 2000) [25]. Ibrahim *et al*. (2017) [9] studied the ACE inhibitory activity of goat milk. Pepsin-digested goat casein and whey protein showed 95% inhibition at 40 µg/ml. Ibrahim *et al.* (2017) [9] identified PEQSLACQCL from β-lactoglobulin (residues 113-122), QSLVYPFTGPI from β-casein (residues 56-66) and ARHPHPHLSFM from κ-casein (residues 96-106), possessing ACE inhibitory property. Several ACE inhibitory peptides of α-lactalbumin fractions (50-52), (99-108), (104-108) and β-lactoglobulin fractions (22-25), (32-40), (81-83), (94-100), (106-111), (142-146) were identified (Pihlanto-Leppala *et al.* 2000) [25].

Antidiabetic activity

Diabetes mellitus is a metabolic ailment distinguished by reduced insulin secretion by β-cells and insulin resistance in tissues, a state that is connected with the development of various convolution, including hypertension and cardiovascular disease. According to the WHO global report on diabetes, the number of people affecting by diabetes has been quadrupled since 1980 to 422 million adults. The dramatic rise is only due to the rise in type 2 DM. It encourages all individuals to eat healthily, be physically active and avoid excessive weight gain (WHO, 2016) [37]. It is published that oral administration of whey proteins and their hydrolysates have insulinotropic responses in humans (Jakubowicz & Froy, 2013) [10]. Incretin is a combination of gastrointestinal hormones that comprise glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). GIP and GLP-1 are discharged into the plasma from K cells in the duodenum and L cells in the intestinal mucosa, respectively, after nutrient-induced stimulation. The incretin concentration in the plasma rises quickly after food intake and is subjected to rapid degradation by dipeptidyl-peptidase 4 (DPP-4). DPP IV inhibitors could enhance the half-life of active GLP-1, potentiating the insulinotropic effect and glycemic control (Power *et al*., 2014) [26]. Bovine, ovine and caprine whey proteins, and peptides could act as DPP IV inhibitors (Tulipano *et al*., 2012) [34]. Lacroix and Li Chan (2013) [12] studied the α-glucosidase inhibitory activity of different fractions of whey protein. β-lactoglobulin (33% inhibition) and WPI hydrolysates (36% inhibition) showed maximum α-glucosidase inhibitory activity as compared to α-lactalbumin (24% inhibition), hydrolyzed lactoferrin (5% inhibition), and BSA (6% inhibition). Vankudre *et al.* (2015) [35] studied the α-amylase inhibitory activity of whey separated from cow and buffalo milk. Cow milk whey and buffalo milk whey on fermentation with *Lactobacillus lactis*, *Lactobacillus delbrueckii* showed 39.18%, 46.59% and 25.09%, 32.29% inhibition respectively. Such an approach describes a relevant strategy from both basic and applied perspectives, also suggesting that hydrolysates and peptides might be useful in the management of type 2 diabetes.

Antiviral activity

Bovine lactoferrin strongly repressed HIV-1 replication and syncytium formation and decreased HIV-1 DNA in the C8166 T cell line when added before the HIV infection or during the viral adsorption step. The mechanism of action may include inhibition of HIV binding to or entry into C8166 cells although a postadsorption effect cannot be eliminated (Ng *et al*., 2015; Puddu *et al*., 1998) [19, 27]. Methylated alpha-lactalbumin and methylated beta-lactoglobulin, and their hydrolysates weakened the infectivity of cytomegalovirus in MRC-5 fibroblasts (Chobert *et al*., 2007) [3] The reaction of alpha-lactalbumin, beta-lactoglobulin, and lysozyme with the chemical modification agent 3-hydroxyphthalic anhydride yielded aggregates with antiviral activity toward HSV-1 before, during, or after infection (Oevermann *et al*., 2003) [21]. Lactoferrin and lactoferricin prevented the entrance of HSV into host cells (Jenssen 2005) [11]. Lactoferrin exercised its antiviral activity at an initial phase of HBV infection seemingly by communicating with host cell surface molecules. Lactoferrin exhibited antiviral action toward respiratory syncytial virus *in vitro* but not in an *in vivo* mouse model (Gualdi *et al*. 2013) [6]. Lu *et al*. (2013) [14] reported inhibitory activity of 3-hydroxyphthalic anhydride-modified bovine beta-lactoglobulin, against human papilloma viruses, including HPV6, HPV16, and HPV18.

Ethanol production

Whey is an exemplary material for ethanol production, because of its primary lactose content (Marwaha & Kennedy, 1984) [16]. Several approaches have been applied for the fermentation of whey to ethanol. In one method, the lactose is hydrolyzed with β-D-galactosidase and then fermented with non-lactose yeasts such as *Saccharomyces cerevisiae* (Bailey *et al.,* 1982; O'Leary *et al.,* 1977) [2, 23]. A modern alternative is to use recombinant yeast produced directly on cheese whey, allowing a high yield of ethanol. A mixture of acetone, butanol and ethanol can be obtained by fermentation of whey. *K. fragilis* is the yeast of choice for most commercial plants. *K. fragilis* converts 95% of the lactose of non-concentrated whey into ethanol with a conversion efficiency of 80%-85% of the theoretical value (Mawson, 1994) [17]. The effects of high concentrations of whey permeate on a lactose fermenting yeast, K. marxianus designated that the immobilized cells were more tolerant to high osmolalities if the osmolality was increased slowly over time (Dale, 1994) [5].

Bacteriocin production

Cladera-Olivera *et al.* (2004) [4] explained the process for the exploitation of whey for the generation of antibacterial substances. P. acidilactici NRRL B-5627 on whey produces

pediocin (Guerra & Castro, 2003; Pérez Guerra *et al.*, 2005) [7, 24]. The recombinant strains of Lc. lactis ssp. lactis, *S. thermophilus* and *Enterococcus faecalis* have been successfully used for the pediocin generation in skim milk and cheese whey (Somkuti & Steinberg, 2003) [31]. The results demonstrated the capacity of recombinant strains of LAB to produce pediocin in a variety of growth media including skim milk and inexpensive cheese whey-based media, requiring minimum nutritional supplementation. The immobilized Lc. lactis subsp. lactis ATCC 11454 has been exercised in a packed-bed bioreactor for the constant generation of nisin in laboratory media and whey permeate (Liu *et al.*, 2005) [13].

Biogas production

Anaerobic digestion is widely used for the treatment of wastes with a high organic load can bring numerous environmental and economic gains, including the generation of energy in the form of electricity and heat (Amon *et al.*, 2007) [1]. Methane is produced in an environment where oxygen, nitrate, and sulfate are not readily available, then CO2 is used as an electron acceptor. Anaerobic digestion happens in two steps.

Firstly, the complex organic compound is converted into volatile acids by facultative and anaerobic bacteria, in the next step, the by products formed by the former, converted into methane and carbon dioxide by strictly anaerobic bacteria. Brazil uses livestock and agricultural wastes as raw materials for biogas production. Biogas can be employed in many different activities such as home heating, heating of animals, production of electricity, lighting, or sale of purified biogas as a vehicle fuel (Teghammar *et al.*,2014) [33]. Europe adopts anaerobic digestion of waste in biogas production and for the production of thermal energy. The principal substrates utilized are manure, grass or corn silage, and often organic industrial waste (Hjort-Gregersen *et al.*, 2011) [8].

Conclusion

Whey is no longer an insignificant by product of the dairy plant because an escalating number of researchers are validating whey protein as an excellent source for various industrial applications. Whey protein-enriched method can develop for end groups such as infants, cardiac-risk group, and diabetics. Distinct tactics necessarily to be devised to augment the benefit of this underutilized resource. Its usage in the advancement of nutritional situation and alleviation of metabolic complexes ought to strengthen. Whey is assuredly an attractive growth medium for several kinds of microorganisms. The notable decline of BOD with the subsequent generation of valuable bio-products such as ethanol, bacteriocin and biogas has accomplished. Therefore, now whey is not a pollutant but a possible raw material for innovative products and processes. Despite this, economic problems in the transportation of whey created a hindrance in adopting any process of utilization of whey.

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