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REVIEW ARTICLE

BIOTECHNOLOGICAL VALORISATION OF WHEY

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Abstract
Cheese whey, the liquid remaining after the separation of milk fat and casein from whole milk, is a major problem as organic pollutant for the environmental natural quality, which demands simple and economical solutions for valorization or bioepuration. The bioconversion of lactose from whey to valuable products has been actively explored. Since whey and whey permeates contain significant quantities of carbon and nitrogen compounds, an interesting way to upgrade this effluent could be as a substrate for different fermentations. Due to the large lactose content generated, its conversion to bio-ethanol has long been considered as a possible solution for whey biovalorization. Bioactive whey proteins and peptides are gradually finding more applications not only as food or feed products but also as functional compounds to be use in the pharmaceutical field.

Keywords: whey, carbon and nitrogen compounds, biovalorization, environmental protection

Introduction

Cheese making is a process concentrating milk components, in particular fat and protein contents which are determinant factors of cheese yield. It is a linear process which involves many technological steps. Nowadays are using many types of different processing methods for cheese making.

In general, the production scheme of cheese manufacturing include the following steps: production of coagulum through the enzymatic or acid coagulation by using rennet and/or lactic acid, separation of the resulting curds from the whey and then processing of the curds to obtain the desired characteristics of the cheese (Dragone *et al*., 2009). A flowchart of cheese manufacturing consists of seven main steps and the waste flows from each manufacturing step are presented in Figure 1.

In the first step starter cultures are added to the milk to produce lactic acid. The rennet is then used to coagulate the milk protein. The curds and whey are separated and the curds washed and cut into cubes. The firmness of the cheese involves compressing and stretching the curds and can be carried out in tower systems.

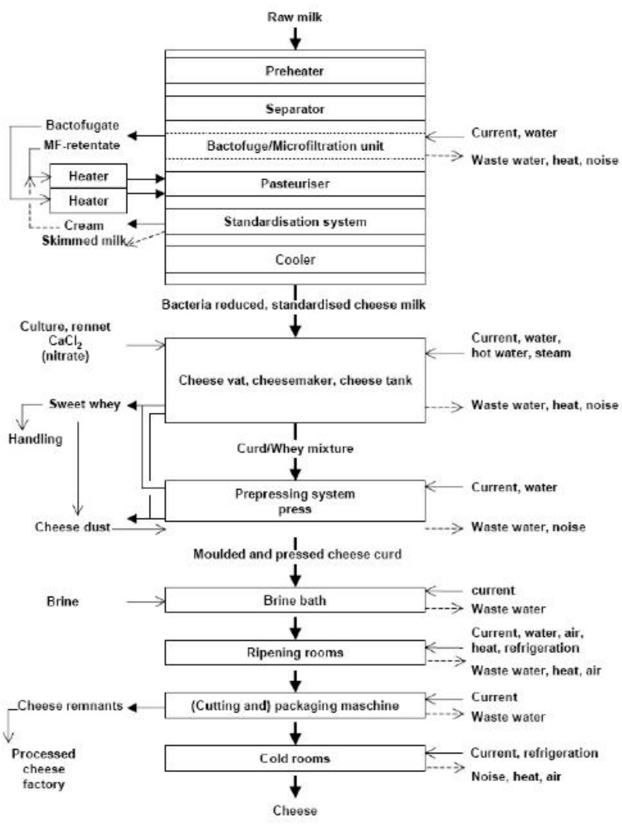

Figure 1. *Process diagram for cheese making (European IPPC Bureau, 2003 cited by Türkmenoğlu, 2006)*

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Based on its low concentration of milk constituents (6-7 % dry matter), whey has commonly been considered a waste product (Koller *et al*., 2008; Staniszewski *et al*., 2009).

As cheese production increased, the volume of whey also grew and many cheese factories were built near waterways. So that most of the whey was diverted to these streams or rivers.

The polluting problem that whey represents and the consequent regulations prohibiting its dumping into waterways and even into municipal sewage systems, whose conventional treatments are not appropriate to sufficiently reduce whey polluting load (Kosikowski, 1979; Guimarães *et al*., 2010).

Whey composition

Whey is the basic liquid by-product remaining after the precipitation and removal of milk casein during cheese manufacturing.

The chemical composition of whey is dependent upon chemical composition of the milk, which varies with stage of lactation, feeding, breeding, individual animal differences, and climate. Few studies have shown that the whey protein composition of these milks follow the general lactational pattern of the cow. In addition, whey composition varies according to slight changes in milk processing parameters (Polat, 2009).

Cheese-whey represents about 90-95% of the milk volume and retains about 55% of milk nutrients. The most abundant of these nutrients are lactose (4.5-5.0 % w/v), soluble proteins (0.6-0.8% w/v), lipids (0.4-0.5% w/v) and mineral salts (8.0 - 10.0 w/v of dried extract) (Kosikowski and Wzorek, 1977; Kosikowski, 1979).

Cheese-whey salts include NaCl and KCl (more than 50%), calcium salts (primarily phosphate) and others. Besides those, cheese-whey also contains lactic (0.05% w/v) and citric acids, non-protein nitrogen compounds like urea and uric acid, B group vitamins etc. (Anon, 1983; Marwaha and Kennedy, 1988).

According to the procedure used for casein precipitation, the cheese whey produced can be acid (pH<5.0) or sweet (pH 6.0-7.0).

Acid whey, also known as salty, has higher salt and lower protein contents than sweet whey. As a consequence of its high salinity, salty whey is more difficult to processing and has higher disposal costs than sweet whey (Blaschek *et al*., 2007; Siso, 1996).

Their use in food is more limited than that of sweet whey, because of their acidic flavor and high salts content (Kosikowski, 1979; Mawson, 1994).

Characteristics whey as pollutant

Cheese whey is a protein and lactose rich by-product of the cheese industry. It is very biodegradable substrate (~99%) with very high organic content (~ 70 g COD/l) and low alkalinity content (Mawson, 1994).

The world whey production is over 160 million tones per year (estimated as 9-fold the cheese production), showing a 1–2% annual growth rate (Guimarães *et al*., 2010).

In Romania during the 2011 year about 40.000 tones of cheese were produced. About 30 000 tons of cheese are obtained from cow's milk resulting in approximately 250 million liters of whey. In the south of the country about 100 million liters of whey were produced and represent a serious problem for the dairy industries and for environmental protection.

However, approximately half of the world cheese-whey production is not treated and is being discarded as effluent. Thus, cheese-whey represents an important environmental problem because of the high volumes produced and its high organic matter content, with lactose being largely responsible for the high BOD and COD exhibition in the natural polluted media (Marwaha and Kennedy, 1988; Mawson, 1994).

The biochemical oxygen demand (BOD) of 30–50 g/l and a chemical oxygen demand (COD) of 60–80 g/l of the whey due to a high organic pollution by discharge it in the streams or rivers. Lactose is largely responsible for the high BOD and COD. Protein recovery reduces the COD of whey only by about 10 g/l (Domingues et al., 1999a; Siso, 1996).

Cheese-whey valorization has been the subject of much research (Türkmenoğlu, 2006; Guimarães *et al*., 2010).

The BOD reductions of higher than 75%, with the concomitant production of biogas, ethanol, single cell protein or another marketable product, have been achieved (Siso, 1996). Thus, the half of the whey that was seen as a pollutant is now seen as a resource.

Possibilities of whey valorization

Approximately 50% of worldwide cheese-whey production is treated and transformed into various food and feed products.

About half of this amount is used directly in liquid form, 30% as powdered cheese-whey, 15% as lactose and its byproducts and the rest is as cheese-whey-protein concentrates.

Due to its characteristics cheese-whey can be utilized as ingredient for many products (Türkmenoğlu, 2006). Many researches are still being conducted with cheese-whey to find new whey products formulation (Marwaha and Kennedy, 1988).

Traditional, liquid cheese-whey can be supplied to farmers for either agricultural biofertilizer or for supplying proteins and lactose for feeding farm animals. However, it must be noted that the transport of liquid whey is very expensive.

Powdered cheese-whey is used in animal feeding and some smaller quantities are used in human foods as sweeteners. However, due to its excessive saline taste its utilization in human foods is not favored.

Another possible utilization method of cheese-whey is using it as protein source. It can be converted into whey protein concentrate (WPC) and used as food additive and ingredient.

Whey processing for protein recovery has been benefited by the developments in membrane technology. Nowadays whey ultrafiltration and diafiltration are standard operations in the dairy industry (Pouliot, 2008) that allow their recovery without significant loss of their functional properties and with a low salt content, making it apt for human consumption (Figure 2).

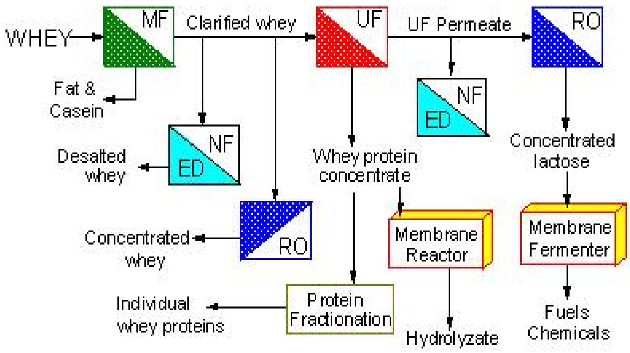

Figure 2. *Whey valorization by using membrane techniques (Cheryan, 1998)*

Whey proteins represent between 15 and 22% of the proteins in milk. There are five major protein fractions with defined molecular weights, i.e., immunoglobulins (Igs), mainly immunoglobulin-G (IgG), bovine serum albumin (BSA), α-lactalbumin (α-La), β-lactoglobulin (β-Lg) and proteose peptones (Merina *et al*., 2001).

The product whey protein concentrate (WPC) contains between 50 - 85% protein on a dry basis, while a more refined product, known as whey protein isolate (WPI) contains between 90 - 98% protein and very small amounts of lactose and fat. Whey proteins are high quality proteins with a protein efficiency ratio (PER) of 3.4, higher than casein (2.8) and similar to egg albumin (Siso, 1996).

In Table 1 are presented the profile of whey protein and their properties.

The enzymatic hydrolysis of whey proteins increases their solubility in water and modifies their functional properties (Gauthier *et al.,* 1993). These hydrolyzates are being used as protein supplements for infant formula, athletes and bodybuilders. Resulting peptides are more easily absorbed but the hydrolysis level has to be carefully controlled to avoid formation of the bitter peptides (Mann, 2000).

The knowledge of the physico-chemical and functional properties of whey protein components and the advances in protein fractionation, mostly molecular membrane fractionation, are on the basis for the development of new applications (Etzel, 2004).

Table 1. *Profile of whey protein and their properties (Madureira et al., 2007)*

Proteins	Concentrations, g/l	MW, Da
β-lactoglobulin (β-Lg)	3.2	18277
α-lactalbumin (α -La)	1.2	14175
Bovine serum albumin (BSA)	0.4	66267
Immunoglobulin (A,M and C)	0.7	25000-70000
Lactoferrin	0.1	80000
Lactoperoxidase	0.03	70000
Glycomacropeptides	1.2	67000

Pure fractions of β-lactoglobulin and α-lactalbumin are now produced at industrial scale (Smithers, 2008). The fractionation of whey protein hydrolyzates initiate interesting options for the bioactive peptides production (Fitzgerald and Murray, 2006) (e.g. angiotensin converting enzyme and other antihypertensive peptides) (Abubakar *et al.*, 1998; Pihlanto-Leppälä *et al.,* 1998; Murakami *et al*., 2004; Welderufael and Jauregui, 2010 cited by Illanes, 2011).

Whey proteins are also been recently used in the production of iron propionate, an antianaemic preparation. During the manufacture of whey-protein concentrates, permeate with a high lactose content is formed as a byproduct. It is very important to take into account that protein recovery does not solve the environmental pollution problems (Mawson, 1994).

Moreover, cheese-whey can be converted to lactose. Lactose is used as supplement in baby formulas, since cow's milk contains 30% less lactose than human milk. It is also used as a building agent in confectionary due to its low sweetness as compared to sucrose or glucose.

These two applications represent more than 80% of lactose consumption in the USA, but only 35% in the European Union, where other significant applications exist in bakery and meat products.

However, the use of lactose in foods is restricted by its low solubility and intolerance. In the pharmaceutical industry, lactose is used as excipient for most tablet drugs because it is inert, non-hygroscopic, and available with high purity and having good binding properties (Fox, 2009).

Beyond its direct applications in the food and pharmacological sector, lactose is a valuable raw material for upgrading by fermentation or chemical transformation. Some of these processes employ purified lactose while others use whey or whey permeates as source of lactose. The decision of using already purified lactose, whey permeates or whey as raw material will depend on the desired final product and also on economic considerations.

Use of lactose as culture medium ingredient for fermentation was already mentioned by different authors. In some cases, lactose is previously hydrolyzed, chemically or enzymatically (Tin and Mawson, 1993; Roukas, 1999), to improve cell metabolism or making it possible for those microorganisms that do not synthesize β-galactosidase (Domingues *et al.,* 1999).

Different products derived from lactose hydrolysis can be their products (glucose and galactose) or products synthesized from it by different chemical reactions like oxidation, reduction, isomerization and esterification.

The enzymatic hydrolysis of lactose is technologically important and applies to milk and dairy products, as well as to whey and whey permeate. The biological conversion of lactose content into lactic acid using appropriate species of *Lactobacillus* has the double advantage of alleviating a pollution problem and at the same time, producing a marketable product (Polat, 2009).

Different commercial products derived by chemical transformation of lactose are made (e.g. galacto-oligosaccharides, lactulose lactitol lacto-sucrose, lactobionic acid, gluconic acid, lactosyl urea, lactosyl monolaurate and tagatose).

Another solution to solve the cheese whey management problem and to reduce high disposal cost would be to use cheese whey as an alternative substrate for cultivating mycelium of edible mushrooms as *Ganoderma lucidum*, *Lentinus edodes, Pleurotus sajor-caju* (Bhak *et al.,* 2005; Mukhopadhyay *et al.,* 2002, 2005; Inglet *et al.,* 2006).

Another major application for the lactose in whey or permeate involves its use as a substrate for the production of valuable compounds by fermentation (Audic *et al.,* 2003). The classical examples are ethanol and single cell protein (SCP) production in yeast-based bioprocesses, although biotech-nologists have proposed a multitude of alternative bioproducts (Pesta *et al.,* 2007; Siso, 1996; Yang and Silva, 1995).

The fermentation of whey lactose to ethanol, using selected yeasts, has been frequently referred in the literature, since at least the 1940s (Guimaraes *et al.,* 2010).

Although the yeasts that assimilate lactose aerobically are widespread, those that ferment lactose are rather rare (Fukuhara, 2006), including *Kluyveromyces lactis, Kluyveromyces marxianus* and *Candida pseudotropicalis* selected strains.

The conversion of the lactose in cheese whey or whey permeate into fuel ethanol is hardly economically competitive with the currently established processes, using cane sugar and cornstarch as substrates, or with emerging second generation technologies using lignocellulosic biomass as raw material (Guimaraes *et al.,* 2010).

Being a waste product represents an advantage of whey over food-related fermentation feedstocks, such as corn, for ethanol production.

Moreover, the availability of diverse solutions for whey bioremediation is valuable, so that each dairy company can evaluate, according to its own specificities, the best way to deal with the environmental problem created by whey surplus.

Finally, whey ethanol is potable, and therefore can find proper markets, e.g. in food and beverages, pharmaceutical and cosmetic industries.

Anaerobic bioconversion producing methane to use as a direct energy source has been employed in industrial waste treatment.

Several kinds of fermenters and several large scale plants have been established achieving more than 95% COD removal efficiencies.

However, the effluents from the anaerobic reactors are generally not suitable for stream discharges. Therefore, some secondary aerobic polishing steps are usually required (Kosikowski, 1979; Anon, 1983; Marwaha and Kennedy, 1988; Mawson, 1994).

Other whey fermentation pathways provide for the production of some additives used in food and textile industries and medical sectors.

Volatile flavoring compounds, plant hormones, particularly gibberelic acid, as well as polygalacturonase and other enzymes can be produced by using whey as fermentative substrates.

Deproteinized whey permeate has also been studied as a substrate to produce with a selected strain of *Phaffia rhodozyma* large amounts of the β-carotene and astaxanthin, colorants used in the coloring of eggs on farms or of salmonids in aquaculture (Siso, 1996).

The production of fructose-diphosphate, the salts of which are used in pharmacology, through the bioconversion of whey with genetically engineered *Saccharomyces cerevisiae* cells has been described (Siso, 1996).

In addition, some other bioproducts can be produced from cheese-whey, such as several organic acids with food uses (HAc, HPr, lactic, lactobionic, citric, gluconic, and itaconic) (Blanc and Goma, 1989; Roukas and Kotzekidou, 1991; Zayed and Zahran, 1991; Colomban *et al.,* 1993; Norton et al., 1994), vitamins from B group, few amino acids (glutamic, lysine, threonine) (Nielsen *et al.,* 1990; Fournier *et al.,* 1993) or starter cultures (baker's yeast or probiotic starter cultures for fermented milk products and for cheese ripening)(Koutinas *et al.,* 2009).

Whey can be used as substrate for xanthan gum production with application in oil drilling and in the textile and food industries as a thickener, emulsifier and stabilizer, to produce calcium magnesium acetate from cheese whey, which can be used as a road deicer with advantages over traditional products (Siso, 1996).

Conclusions

Whey is a byproduct that has been traditionally considered as a residue of cheese production. The magnitude of the dairy industry makes whey production volumes enormous and it became an very large organic pollutant for environmental.

A significant portion of that volume is underutilized or discarded, increasing the production costs as a consequence of waste management and treatment to comply with environmental regulations or, even worse, disposing it without treatment when such regulations are soft, not enforced, or inexistent.

Few big companies have the capacity to implement different technologies for the efficient recovery and upgrading of whey.

A challenge exists in incorporating medium and small size whey producers to strategies for its management that include recollection, processing and upgrading.

Recovery, fractionation and transformation of whey may acquire a significant economic value for their producers, conciliating the interest of the productive sector with the social demands of environmental protection, within the framework of sustainable development.

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