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Production of whey protein as nutritional valuable foods

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ABSTRACT

Whey is a low calorie milk serum remaining after casein removal in cheese manufacturing. Composition and properties of whey depend on the type of cheese production process, type and the quality of the used milk. Whey mostly consists of lactose, proteins, vitamins, minerals, enzymes, hormones and growth factors. Functional and nutritional roles of whey components meet the requirements for dietary food.
Whey proteins are liquid fractions of 20% milk proteins, which remain in whey after casein removal. The increase in the temperature of milk processing leads to denaturation of proteins and a decrease in their content. The most common whey proteins are albumin and globulin. Separation and fractionation of whey proteins may be carried out as precipitation, combined with acid treatment, high temperature treatment or centrifugation (centry-whey process), and as a membrane process (usually ultra- or microfiltration followed by a diafiltration and spray drying). Whey proteins can also be separated based on their molecular mass, charge and hydrophobicity.
It is a well-known fact that whey proteins possess admirable nutritional and functional components, such as essential amino acids, bioactive peptides, antioxidants and immunopotentiators. Research of bioactive compounds and nutrients pushed whey protein to the forefront of the functional food sector, therefore, the implementation of these proteins in the diet and supplements has the potential to address many metabolic imbalance-caused diseases, and it seems imperative to harness their potential.

Introduction

Whey, also known as milk serum, is a by-product of the dairy industry that corresponds to the liquid fraction remaining after milk clotting and casein removal during cheese manufacturing. It has a yellow/green color, sometimes even a bluish tinge, but the color depends on the quality and type of the used milk. Whey may be sweet or acid depending upon the type of casein or cheese coagulated; it represents about 85–90% of milk volume and retains approximately 55% of milk nutrients (Smithers, 2008). Sweet whey contains higher level of fat, lactose and proteins than acid whey, which contains higher level of lactic acid and calcium (Gregurek, 2015). It has been considered an excellent source of functional proteins and offers a great number of vitamins, minerals and lactose, whose amount depends on the technological processes of cheese manufacturing and the quality of the used milk (Nagar and Nagal, 2013). Mineral content includes: potassium, sodium, calcium, magnesium, chlorides and phosphates, which are present in whey in almost the same amount as in milk, and have an important role in metabolism and osmosis regulation. Sweet whey contains higher amount of vitamins than acid whey, and includes: thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), folic acid (B9), cyanocobalamin (B12), ascorbic acid (C) and biotin (H) (Gregurek, 2015).
Whey has been used as a fertilizer for a long time, or has simply been discarded into the rivers or the sea, but further research of its properties, nutritional and biological value has led to a conclusion that it is considered a valuable component in various industries, including food, pharmaceutical and

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nutraceutical industry (Smithers, 2008). Enthusiasm for seeking benefits from the dietary and nutritional supplements that help maintain physical well being due to active lifestyles, makes people familiar with whey proteins and amino acid supplements, and has pushed whey protein (serum protein) to the forefront of the functional food sector (Patel, 2015). A whey protein constitutes 15–20% of total milk proteins and has been found to possess a plethora of healthy components such as essential amino acids, bioactive peptides, antioxidants and immunostimulators (Gregurek, 2015). It is rich in essential, branched-chain amino acids, which include leucine, isoleucine, and valine that play crucial roles in metabolism, blood glucose homeostasis and neural function. It is considered that whey proteins also have anti-inflammatory, antitumor, hypotensive, gut homeostasis, antiobesity, antidiabetic, muscle biosynthesis, osteoprotective and radioprotective roles.

Whey proteins

Whey proteins are a mix of globular proteins that can be isolated from whey and have excellent nutritional properties. They are smaller than casein, have simpler protein structure, higher amino acid content, and are easier to digest. Although whey proteins have been reported to possess relevant nutritional and biological properties, their activities are mainly associated with the bioactive peptides encoded within the protein sequence. Whey proteins are a rich source of bioactive peptides, which may play a role in the dietary management of chronic diseases (Fig. 1). The major whey protein fractions are:

1. β-lactoglobulin - soluble milk protein which captures hydrophobic molecules and participates in the reduction of intestinal absorption of lipids (50% of the total whey proteins);
2. α-lactalbumin - thermostable whey protein which supports lactose biosynthesis and has ability to bond metal cations (20% of the total whey proteins);
3. serum albumin (SA) - binds free fatty acids, other lipids, and flavours; SA can stabilize the molecule against thermal denaturation, but its specific function has not yet been revealed (5% of the total whey proteins);
4. immunoglobulins - specific antibodies generated in response to the presence of foreign antigen. Their primary function is to increase a passive immunity (10% of the total whey proteins) (Samaržija, 2015).

Minor, but commercially important proteins, are lactoferrin (LF) and lactoperoxidase (LP) (Doultani et al., 2004). Biological value (BV) of whey proteins stands between 104 and 124, while the percentage of their BV is under 100%, which is higher than biological value of casein (between 73 and 77) (Gregurek, 2015). They are considered as GRAS (Generally Recognized As Safe) substances for food product applications (Sinha et al., 2007). In addition to their nutritional value, whey proteins have good water-holding capacity, are effective emulsifying and foaming agents, and can improve rheological properties (Zydney, 1998).

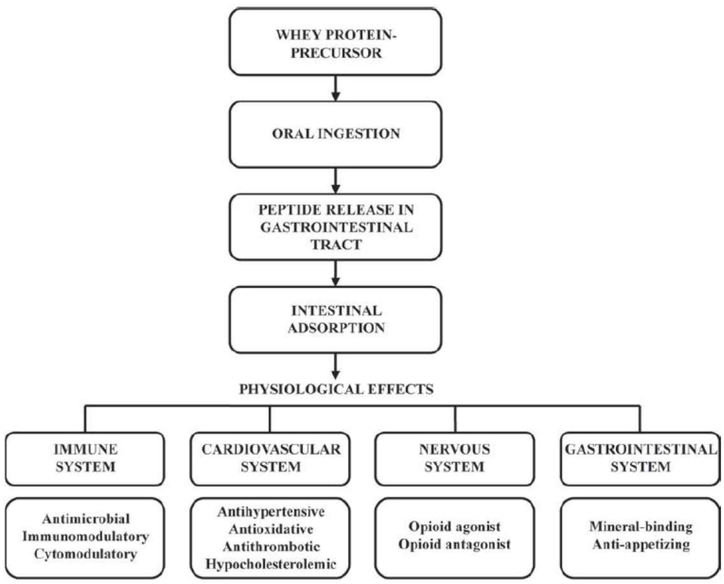

Fig. 1. Bioactivity of whey peptides (Madureira et al., 2010).

Preparations of whey proteins

Even if whey proteins are so rich in bioactive peptides, they are still in need of a fractionation or purification process to turn them into saleable products with the defined properties and characteristics. The biological properties of individual whey constituents (*α*-lactalbumin, *β*-lactoglobulin, lactoperoxidase, lactoferrin, glycomacropeptide and immunoglobulins) have become a focus of a commercial interest as functional (health-promoting) foods. Whey proteins can be fractionated based on their molecular mass, size, charge and relative hydrophobicity. Because of the very high concentration of water (93.5%) and lactose (4.5–5.0%) in whey, protein fractionation requires separation of proteins from whey, which can be conducted by various membrane processes, precipitation, or a combined acid and temperature treatment. Generally, they are marketed in three forms, such as whey *protein concentrate* (WPC), *whey protein isolate* (WPI), *whey protein hydrolysate* (WPH) (Sousa et al., 2012). These preparations of whey proteins differ in their protein content and their properties.

Whey protein concentrate (WPC)

At first, isolation of WPC resulted in denatured proteins with high nutritive and low functional value. Over the years, progress in membrane technology led to the production of un-denatured WPC, which can be fractionated on various peptides for the production of functional foods and nutraceuticals. Nowadays, WPC is prepared using different types of membrane processes such as ultrafiltration (UF), diafiltration (DF), microfiltration (MF) and reverse osmosis (RO). In addition to membrane processes, ion-exchange and electro dialysis are sometimes used for demineralization (Samaržija, 2015). It is a low cost technology, and mild operation conditions are used, so substrate nutritional properties remain almost intact (Tavares et al., 2012). WPC has lowest levels of fats and cholesterol when compared to other forms of commercially available whey, and contains carbohydrates in the form of lactose, and a high level of bioactive compounds (Ganju and Gogate, 2017).
According to protein concentration, WPC is divided into three categories:

1. WPC with low content of protein: 25–40% of the total solids;
2. WPC with medium content of protein: 45–60% of the total solids;
3. WPC with high content of protein: 60–80% of the total solids (Gregurek, 2015).

WPC with protein content below 60% of the total solids, can be prepared using only ultrafiltration, while for those between 60-80% of the total solids, diafiltration is required. Technological process starts with the separation of fat and cheese particles on a centrifugal separator, followed by pasteurization, cooling and ends with ultrafiltration/diafiltration and drying (Herceg and Režek, 2006). Because of its great solubility, WTC is extensively used as a food additive in meat, beverages, dairy products, baked goods, and infant formula.

Whey protein isolate (WPI)

Concentration of protein in dry matter is the basis for distinguishing WPC from WPI. Whey protein isolates have 90% of the total solid proteins and are mainly prepared by ion-exchange (IE) (Herceg and Režek, 2006). High levels of proteins and minimal amounts of lactose, fat and ashes (gram of the total solids) are main advantages of WPI over WPC. To maintain bioactivity of proteins, it is necessary to ensure a natural and un-denatured protein state, so temperature and acid usage during process needs to be maintained low, therefore microfiltration (MF) finds its application as a less intense process, followed by ultrafiltration (UF) and spray drying (Herceg and Režek, 2006). Membrane fouling is the main problem of protein filtration, so cross-flow microfiltration (CFMF) is usually used to increase WPI production efficiency. The addition of WPI in food production improves the taste and texture of a final product. They are mostly added to frozen desserts, drinks, ice creams and sport supplements.

Whey protein hydrolisate (WPH)

WPH is a pre-digested and partially hydrolyzed whey protein manufactured with the aim to achieve easier metabolism. WPHs have anti-oxidative properties and a protein content of 70-80% (Sinha et. al., 2007). During hydrolysis, proteins are broken down into peptides of different sizes and free amino acids, as a result of cleavage of peptide bonds. Degradation of proteins can be carried out by enzymes, acids, alkali or by a high temperature treatment (Samaržija, 2015). Acid and alkaline hydrolysis yield products with reduced nutritional qualities and tend to be a difficult process to control. Enzymatic hydrolysis developed under mild conditions of pH (6–8) and temperature (40–60 °C) may also lead to the development of the biologically active nutritional components which can be used as dairy ingredients with higher nutritional value (Sinha et al., 2007). Hydrolyzed whey protein-based formulas are beneficial for infants intolerant to cow's milk protein.

Production of whey protein constituents

Because of a substantial amount of nutritional value associated with whey, there have been several methods which have been used to separate whey into its various economically-useful components. For the commercial scale production of whey protein fractions, three main processes have been proposed:

1. selective precipitation induced by the adjustment of the physical properties of solution;
2. membrane filtration based primarily on differences in size and charge;
3. selective adsorption (Doultani et al., 2004).

Precipitation and membrane filtration are volume-dependent methods, wherein capacity and cost of a manufacture is proportional to the volume of whey and not the mass of the proteins produced, while selective adsorption depends mostly on the mass of the protein recovered. The individual whey protein can be concentrated or fractionated by the application of a membrane technology.

Membrane technology (MT) is an environmentally friendly green technology that minimizes the adverse effect of temperature rise such as a change in phase, denaturation of proteins and a change in sensory attributes of the product. The membrane configurations are suitable for industrial applications due to their compact design and low maintenance, and the fact that existing capacity can be increased by adding membrane modules parallel to the existing ones. MT represents the separation processes by specific semi-permeable membrane filters to concentrate or fractionate a liquid into two liquids of different compositions by selectively allowing some compounds to pass while encumbering the others (Kumar et al., 2013). In membrane separation, particles are separated based on their molecular size and shape, using pressure and specially-designed semi-permeable membranes. Most common membrane processes are ultrafiltration (UF), microfiltration (MF), reverse osmosis (RO), pervaporation, nanofiltration (NF), electrodialysis (ED). By applying different membrane technology in whey processing (Fig. 2), nutrients are concentrated, fractionalized or purified into whey protein concentrate/isolate, α-lactalbumin, β-lactoglobulin, lactose and salts (Kumar et al., 2013). Membrane technology enables concentration and separation of whey proteins in their un-denatured form with high functional property in native whey.

Ultrafiltration (UF) of whey was the first application of a membrane fractionation to reach full commercial scale (Akpinar-Bayizit et al., 2009). UF represents membrane filtration driven by a hydrostatic pressure gradient which forces liquid against a semi-permeable membrane, in which suspended solids and solutes of high molecular weight (molecular weight between 103 and 106 Da) are retained, while water and low molecular weight solutes pass through the membrane (Kumar et al., 2013). Protein and fat in whey are recovered commercially by ultrafiltration (UF), because these molecules, by virtue of their size, are retained, while lactose and ash are able to pass through the membrane into the permeate (Yee et al., 2007). UF offers a unique method for the recovery of whey proteins in their native form.

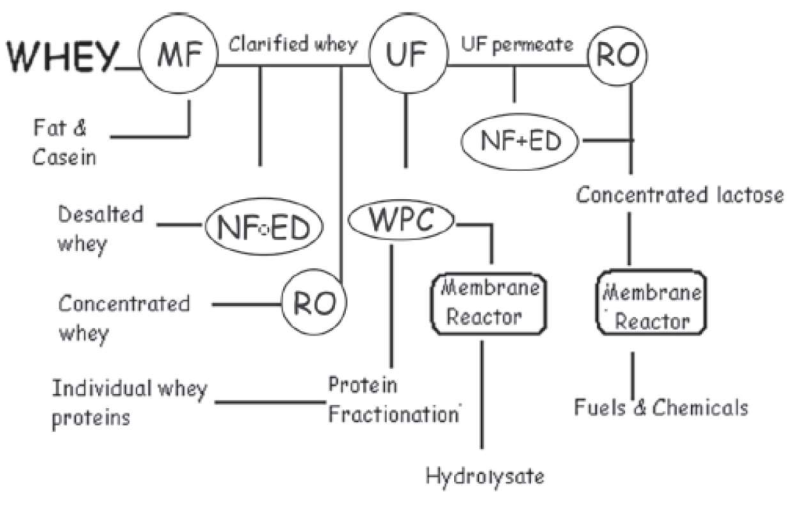

Fig. 2. Membrane applications used in whey processing (Akpinar-Bayizit et al., 2009)

The retentate stream is then fed into spray dryers to produce powdered whey protein concentrate (WPC). UF combined with diafiltration (DF) increases protein content in WPC by 35% to 85% of the total solids. The addition of DF water increases the permeation of non-protein species across the membrane, thereby increasing the protein concentration of the total solids in the retentate stream. *Diafiltration* (DF) is used for the production of WPC and WPI with high protein content, and for protein purification to eliminate problems associated with high concentrations in the retained product, generating high purification, while retaining good process performance (Baldasso et al., 2011).

Microfiltration (MF) is a membrane filtration similar to UF, but with a larger membrane pore size allowing particles in the range of 0.2 to 2µm to pass through the membrane, and lower pressure used than that of a UF process (Kumar et al., 2013). MF is often used as a pretreatment for UF in the production of WPI, wherein bacteria and fat are removed, and protein content is increased to 90% of the total solid content.

Reverse osmosis (RO) is a high pressure-driven membrane filtration process, which is based on a very dense membrane with a very small membrane pore size, allowing only small amounts of very low molecular weight solutes to pass through the membranes. In whey processing, RO is mainly used for whey concentration. Osmotic pressure, viscosity and solubility of the lactose and calcium salts present in whey are limiting factors in RO (Akpinar-Bayizit et al., 2009).

Nanofiltration (NF) is a type of RO where a membrane has a slightly more open structure allowing monovalent ions to pass trough the membrane and rejecting the divalent ions to a great extent, and is usually used for desalting (demineralization) of whey and the recovery of lactose from a whey permeate.

The major problem faced in the separation of whey proteins is the fouling of membranes. Membrane fouling is caused due to a combination of several phenomena such as a concentration polarization, pore blocking or a cake formation. This leads to a reduction in flux and efficiency of whey protein separation and often requires the use of pre-treatment techniques (chemical, temperature, ultrasound treatment or use of turbulence promoters) to negate the effects of membrane fouling (Ganju and Gogate, 2017). Whey protein separation using membrane-aided methods will greatly depend on the discovery of new methods to combat membrane fouling.

Drying of whey proteins

For the commercial scale production, whey proteins need to be dried in order to get rid of the moisture content and yield a commercially required powder form. Drying also increases the physical and microbiological stability of whey proteins, reduces transportation costs and increases their shelf life. A major challenge in the drying of whey proteins is to optimize the application of heat to the separated whey proteins in order to minimize the possibility of denaturation of the proteins. Drying operations need to be precisely controlled and optimized in order to produce a good-quality product with the highest level of nutrient retention. Whey protein drying is achieved using two major approaches of spray drying and freeze drying.

Spray drying (SD) is based on a generation of very fine droplets using a nozzle or a rotary atomizer into a hot convective medium (180-220 °C) converting the droplets into fine solid particles (Gharsallaoui et al., 2007). Solution of soluble or suspended slurry of materials is sprayed into a drying chamber using an atomizer (e.g., a nozzle), and hot gas flows concurrently or counter currently with the dispersed liquid droplets, removing moisture from the particles and resulting in dry powder particles. While this method has several advantages, including rapid drying, large throughput, and continuous operation, spray drying is a very expensive technique, because of its low energy efficiency, and losses of certain quality and sensory attributes, due to the relatively high temperatures involved in spray drying processes (Sabarez, 2016).

Freeze drying (FD) (also known as lyophilization) is a drying process in which the food is first frozen, then exposed to heat under vacuum which causes sublimation of the frozen liquid under the reduced pressure (Sabarez, 2016). Generally, it is used to produce high-quality dried products which are very sensitive to heat. As freeze drying is performed at low temperatures and under vacuum (absence of oxygen), this process leads to great retention of properties in the components to be dried. High production costs, high energy consumption, and low throughputs, are main disadvantages of FD.

Conclusion

Whey is a major by-product of the dairy industry, which has severe biological and environmental implications. It also contains a significant nutritional and functional value which can be harnessed in a positive manner. Whey proteins have been reported to possess relevant nutritional and biological properties. Integration of these proteins in the diet and supplements has the potential to address many metabolic imbalance-caused diseases. Further investigations are likely to unveil many more physiological benefits. It has been established, based on the overview presented, that the most economic and feasible method for the separation of whey proteins is ultrafiltration. The efforts made for the development of superior membranes will further expand the role of membranes in whey processing. The future development of the whey processing industry and whey protein separation using membrane-aided methods will greatly depend on the discovery of new methods to combat membrane fouling, and novel materials (with high affinity for whey proteins) for the construction of membranes.

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