A review on whey composition and the methods used for its utilization for food and pharmaceutical products

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ABSTRACT

Whey is a by-product of the dairy industry, which for years was thought to be insignificant and was either used as an animal feed or it was disposed of as waste. In the latter case, whey is a problematic to dispose of for several reasons First, its high BOD_5 (the amount of O_2 in mg, needed for the biological oxidization of the organic load per litre of whey, in five days time), which is about 35.000 – 55.000mg O_2/litre. Considering that over 145.000.000 tons of whey is produced worldwide annually, the desire for new methods to utilise whey can be appreciated . Over the last years several studies were carried out concerning the importance of whey's nutritional value and the properties of its ingredients.. It is now accepted that it's main content, whey proteins, have antimicrobial, antiviral and anti-oxidant properties, can offer a kind of protection against cancer and heart diseases and assist at the enhancement of immune-defence. Due to the substantial difficulties encountered in the treatment of whey as a biological waste and its high potential to be valuable raw material for added value food and bioactive substances production the later tend to be the only accepted and popular trend of dealing with this dairy industry by-product. For this reason, the aims of this work is first to put together all the necessary information about whey, ingredients and their properties and potential uses and second to present and describe the nowadays known methods of processing it for either isolation of the several useful and mainly bioactive ingredients or use it to produce foodstuffs.

INTRODUCTION

Milk is the only food designed for mammals by nature through evolution. Mammals have adapted to consume all other foods. Milk provides nutrition in the form of energy from the carbohydrate present in the form of lactose, nitrogen from the protein content and a rich source of calcium to build bones to name but a few. Milk also provides other important benefits. For example, there are many biologically activities associated with certain components in milk. Almost without exception, these biologically active components are exclusively to be found in the whey or serum fraction of milk. Whey is the watery and thin liquid, which is received during cheese making by coagulating and separating casein proteins from milk. In the case of sweet whey rennet type enzymes are used at a min pH of 5,6 to induce coagulum and in the case of acid whey coagulum is created when milk is acidified by lactobacillus culture or mineral acid at a max pH of 5.1 Whey's composition and sensory characteristics vary depending on the kind of the whey (acid or sweet), the source of the milk (cow, sheep, bovine milk etc) and the feed of the animal which produced the milk, the cheese processing used, the time of the year and the stage of lactation. With this in mind, it is perhaps surprising that for many years, whey produced as a by- product of cheese production was considered a waste material and was either dumped, sprayed on fields as fertiliser or at best, dried as cheese whey powder destined for the animal feed applications. The global utilization of the whey is briefly given in Figure 1. Increasingly, over the last few decades, dairy companies have applied different technologies to process cheese whey resulting in

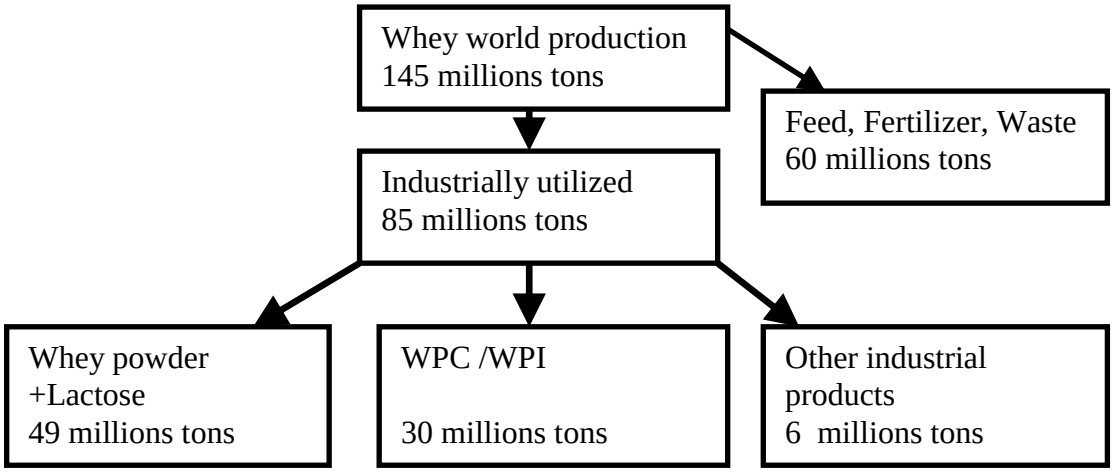

Figure 1. The present balance of Global Utilization of whey

its separation into its principle components, comprising fractions enriched in proteins, lactose and minerals. These technologies have been generally based around crystallisation, membrane and chromatographic processes. In the last decade, whey processors have somewhat been a victim of their own success. The increasing volumes of edible lactose and whey protein concentrates flooding into the market have resulted in a downward trend in relative prices. Converting these semi-commodity products into added value products is the challenge facing the industry presently. This article will give a brief overview of traditional methods for valorisation of whey and then turn towards opportunities for real value creation that are embedded in the nutritional, functional (meaning structure-building) and physiological properties that are unique to whey components.

WHEY COMPOSITION & METHODS EMPLOYED FOR ITS UTILIZATION.

Whey is a fairly dilute product with a total solids of about 6,5%. As mentioned before the solids are basically consisted by lactose, whey protein, ash, lactic acid and fat (Table 1).

Table 1.
The Whey Composition

S.No	Constituent	Unit	Sweet whey	Acid whey
1	Water	%	93-94	94-95
2	Dry matter	%	6-6.5	5-6
3	Lactose	%	4.5-5	3.8-4.3
4	Lactic acid	%	traces	up to 0.8
5	Total protein	%	0.8-1.0	0.8-1.0
6	Whey protein	%	0.6-0.65	0.6-0.65
7	Citric acid	%	0.1	0.1
8	Minerals	%	0.5-0.7	0.5-0.7
9	pH		6.4-6.2	5.0-4.6
10	SH Value		about 4	20-25

Source : *www.dairyforall.com*

1 .Production of Whey powders

Whey drying is the simplest operation used in whey utilization. It aims and it is used just to reduce the amount of moisture in order to produce whey powders. Typical traditional whey drying operations consists of evaporation in multistage vacuum evaporators, followed by spray or roll drying. Whey is firstly concentrated in 40-70% total solids and then by the use of

a spray dryer or a roll dryer moisture is removed until the final product reaches 5% moisture content. Although it sounds as a simply process it can get pretty complicated partly because of the high lactose content in whey. Necessary stages are the pre-crystallization of lactose before the drying in order to minimize problems of hygroscopicity as well as the careful manipulation of the heat conditions to minimize problems caused by the heat sensitivity of whey proteins. In the case of the production of non-hygroscopic whey powder, a holding period is required to allow the crystallization of lactose into non-amorphous, non hygroscopic form prior to drying. But lactose may causes defects, such as lumping or caking to the final product. If drying is rapid a- lactose may not have enough time to form as monohydrate and it form as amorphous a-lactose, which is highly hygroscopic and it will absorb moisture from air resulting in a hydrate that occupies more space than the amorphous form.

2. Production Methods used for separating whey proteins

The basic idea behind the manufacture of whey protein concentrates, isolates and fractionates is the separation of the proteins from the rest of the whey components at a first stage and then the further concentrate of the lactose solution which remains by evaporation and spray drying.

There are several processes to separate the proteins from whey, which can be divided in following main categories:

a .Heat precipitation (Thermocoagulation) and/or Selective precipitation

Whey proteins are heat sensitive and can be precipitated by heat treatment under appropriate conditions of pH and ionic strength. This property is used in the manufacture of lactalbumin. Lactalbumin is the product that derives from heat precipitation of whey proteins and it is a mixture of denatured a-lactalbumin, β-lactoglobulin and other whey proteins. For its production whey is heated to denature coagulate and precipitate the whey proteins; the sediment is recovered by settling and decantation (or centrifugation), washed to remove excess salt and lactose, and the product recovered by centrifugation or filtration prior to drying, grinding and bagging. The heat treatment used results to extensive denaturation of whey proteins therefore the final product is of poor functionality. Because of that, lactalbumin finds its best applications in products where protein fortification is necessary, but it's not required to provide any functional properties. A potentially attractive whey purification process based on thermal precipitation was introduced by **Pearce (1983).** Whey concentrated is heated to 65 ^{o}C at pH 4.2, which causes α-lactalbumin aggregation and co-precipitation of BSA and immonoglobulins. The supernatant is collected and purified using diafiltration yielding a highly purified β-lactoglobulin product. Techniques for the separation of β-lactoglobulin and α-lactalbumin have been developed based on the reversible thermocoagulation of latter. Whey or a mixture of these two is heat treated at moderate temperatures (< 55^{o}C) for several minutes at low pH, produces the reversible aggregation of α-lactalbumin, which can then be separated from the mixture by microfiltration; the permeate , rich in β-lactoglobulin can be treated separately by ultrafiltration / diafiltration to concentrate the protein, while the α-lactalbumin in the retentate can be redissolved at neutral pH and then concentrated by ultrafiltration (**Bramauld et al., 1997; Gesan –Guizion et al.,1999**) Selective precipitation can be accomplished using pH, salts and temperature. In the case of β-lactoglobulin at pH 4.65 it can be selectively separated from whey. β- lactoglobulin can also purified by selective precipitation of other whey proteins at pH 2.0 using 7% NaCl (**Mailliart , 1988; Mate and Krochta, 1994**). β- lactoglobulin and BSA can be selectively precipitated with the use of 7.5mM $FeCl_3$ at pH 4.2 and 4^{o}C (**Kaneko *et al*. 1985**), yielding a supernatant concentrated in α-lactalbumin and immonoglobulins. Immonoglobulins can selectively precipitated from whey using ammonium or sodium sulfate (**Maubois and Ollivier, 1997**) Athough these processes have generated considerable commercial interest, they have not be widely implemented for large-scale whey protein purification because of their complexity, high cost, low overall yield, poor sensitivity, and/or unacceptable product degradation associated with the extremes of heat, pH, or salt used during the process.

b. Membrane processes

Membrane systems are used extensively throughout the dairy industry to control protein, fat and lactose content of a variety of products. Theses membrane processes have been successful because they can be effectively and economically implemented at the large scale required for most dairy applications. These techniques are basically filtration processes in which tiny – diameter porous membranes are used as filtration media in order to separate solid components from liquid phase. These processes have been used for whey treatment since the late 1970s and early 1980s, using cellulose acetate membranes in the early stages of development , which were later replaced by more resistant and durable membranes made of poly-sulphones or poly-ethero-sulphones. Pressure driven membrane techniques used to produce whey protein concentrates and isolates are reverse osmosis, ultrafiltration, microfiltration and nanonofiltration. Electrically driven membrane processes used are electrodialysis and electroelectrodeionization.

WPCs

The most commonly method used in the manufacture of WPCs is certainly Ultrafiltration (Picture 1). It has been alone or in a combination with another membrane processes such microfiltration or nanofiltration. The principal aim of ultrafiltration of whey is to concentrate the native or pre-denatured whey proteins in order to obtain a whey protein powder with varying protein content and reduced lactose and ash content (**Da Costa et al, 1993; Huffman, 1996; Marshall, 1982**) Ultrafiltration (UF) uses polymeric or ceramic membranes, which are fully retentive to the whey proteins, to remove lactose and minerals, yielding a retentate stream that can be further processed by evaporation and spray drying. The net result is a whey protein concentrate that is around 60% protein by weight. The lactose and mineral content in whey can be further reduced using a subsequent diafiltration (DF) in which deionised water is continually added to the retentate while lactose and minerals are simultaneously removed in the filtrate. This combined UF-DF yields a high value retentate of about 85% protein. Nanofiltration can also be used for concentration of the whey up to 20-24 % w/w solids or alternatively for concentration of the permeate which penetrates the membrane during

Picture 1. The overview of a UF unit for whey

ultrafiltration processing of whey and it which contains lactose in the same concentration as in the water phase of the original fluid. Finally, microfiltration has been examined for the removal of residual lipids from whey, prior to UF and sterilize the whey **(Lee & Merson, 1976; Merin et al. 1983).** This often involves heat treatment and/or pH adjustment to aggregate lipids and calcium phosphates **(Fauquant et al., 1985; Gesan et al., 1995).** (**Maubois and Ollivier, 1997**). Microfiltration can also be used to remove microorganisms from whey thus reducing the bioburden without need for high temperature pasteurisation

WPIs

Commercial WPIs contain between 88-95% protein. For the production of these , whey has to be skimmed by microfiltration and demineralized by ionic exchange, electrodialysis or nanofiltration. Further purification of the proteins can be carried out by diafiltration. Finally the purified retentate is concentrated and spray dried. Alternatively, WPI can be obtained by a combination of ion exchange chromatography and ultrafiltration (**Morr, 1989**). Protein fractionates, like protein isolates, are high protein products with a higher ratio of a particular protein than that present in whey. Such protein concentrates are generally manufactured by the use of a non-specific absorbent to bind the proteins in whey, followed by elution of the proteins treatment of the absorbent with a specific eluent. Absorbents that have commercially used include carboxy-methy-cellulose and a range of mineral oxides. Although these absorbents are comparatively non specific, they can show a preference for binding particular proteins under set conditions of pH, temperature and ionic strength.

c. Chromatographic fractionation of whey proteins

Adsorption chromatography is a separation technique that has great potential in the isolation of functional dairy protein ingredients with retention of bioactivity, as well as meeting essential criteria such as cost effectiveness and consistency of operation (**Nielsen *et al.* 2002**). Chromatography has been successfully used at large scale at biotechnology industry to separate components of high value, while maintaining their biological activity. By contrast, the dairy industry has not exploited this technology to its maximum potential.

Ion exchange chromatography (IEC) is one of the methods used in the manufacture of WPIs . The principle behind ion exchange chromatography used in WPI's and more particularly, ion exchange membranes separation is the reversible interaction between target protein and membrane functional groups. It provides an additional level of selectivity above membrane processing because factors other than molecular size determine protein absorption. Separating whey protein on the basis of their iso-electric points gives two district groups: the major whey proteins β-lactoglobulin (β-lg), BSA and α-lactalbumin (α-la), which are negatively charged at the pH of rennet whey (pH 6.2-6.4); and minor whey protein lactoferrin and lactoperoxidase that hold a positive net charge at the pH of whey. In IEC cationic resins (negatively charged) are used to retain the positively charged proteins at the pH of whey (LF and LP); at the same pH, the other major proteins, β-lg, α-la and BSA, are negatively charged; thus, they are not retained by the resin, obtaining a fraction rich in this proteins. LF and LP are later released by elution with alkaline solutions. The fractions are finally washed and spray dried. (**Pearce, 1992; Chiu and Etzel, 1997**).

d. Other methods of whey fractionation

Experimentally, several techniques are currently been explored, two interesting cases in point are colloidal gas aphrons (CGAs) and molecular imprinting. CGSs are microbubbles created by intense stirring of surfactant solutions. Their large interfacial area per volume, short separation time from bulk phase, and low viscosity make them particularly attractive for protein separation. **Fuda *et. al.*, 2004 ;2005** investigated the fractionation of whey proteins using CGAs generated with either the anionic surfactant sodium bis-2-ethylhelix sulphosuccinate or cetyl trimethyl ammonium bromide and proved that CGAs can be analogous to ion exchangers when applid to whey; the selectivity of the process can be manipulated by changing the type of surfactant, pH and ionic strength. These authors demonstrated that the recovery and separation of LF,LP or β-lg from whey can be done using CGAs. Molecular imprinting is a method for preparing synthetic materials able to mimic the molecular recognition phenomena present in living systems. It consists of the selectionof a template molecule, which later associated with some functional monomers through non-covalent binds; then a polymerisation around template-monomer complex is conducted, resulting in a molecularly imprinted polymer (MIP) that has a cavity that recognises the template molecule, allowing its capture, specifically sparating itfrom a complex mixture. This method was used to recover lactoferrin (LF) (**Mendez-Palacios et al., 2006**). Using

vinylpiridin as a functional monomer and etylenglycol dimetacrilate as a crosslinker, it was possible to create a specific cavity for LF. The polymers obtained, tested against a protein mixture containing LF, had an efficiency of 27%, while the control polymer retained only 1.6%, demonstrating that the retention of the protein is not due to an unspecific adsorption in the polymer, but rather to a selective retention in the cavity formed by the template.

3. Other methods used in whey utilization

a. Lactose processing options

The options for treatment of whey involving lactose can be divide in three categories :

- those involving a fermentation step, there are many options that have been investigated such as production of biogas, biomass, ethanol, lactic acid and citric acid
- those involving separation of the lactose and its utilization, these are probably the most attractive option. The manufacture of lactose (normally α-lactose hydrate) generally involves removal of protein, concentration, refiltration, further concentration, induction of crystallization, and separation of crystal with a basket certifuge.The highest value added pharmaceutical lactose preparations are those for application in dry powder inhalation (DPI). These generally comprise subfractions of preparations that are tailored to optimise delivery of drug from the dry powder inhaler device. DPI is a fast growing market (>10% CAGR) currently focused mainly in the chronic obstructive pulmonary disease sector, which includes diseases such as asthma. This means of drug deliver has several advantages over aerosol-based devices, so called metered dose inhalers (MDIs)). Whilst the latter have largely been reformulated away from ozone damaging CFCs towards hydrofluorocarbon alternatives, there are still issues relating to poor patient compliance (cold jet of expanding aerosol propellant stimulates the user to breathe out rather than in when it hits the back of the throat). Also, DPIs are more applicable to a wide range of drug classes. Proteins such as insulin and monoclonal antibodies may in future be delivered in this manner. Here inhalation obviates the requirement to inject protein-based drugs, which would be digested if taken orally.
- those involving enzymatic hydrolysis of the lactose to produce galactose and glucose. The hydrolysis of lactose yields the sweet soluble sugars, glucose and galactose, thus increasing the applications of the product . Such hydrolysis can be carried out by treatment of whey with lactase (β-galactosidase) or by treatment of de-proteinized whey at an elevated temperature and low pH. It should be note that it is difficult to dry hydrolyzed whey, because of the tendency of the monosaccharides formed by the hydrolysis to produce glasses on the surface of the drier. Hydrolysis of lactose can be carried out by a number of processes including heat/ acid treatment (for permeate only) and enzymatic hydrolysis (for whey , permeate and WPC).

b. CreamoProt® method

According this patented technology the whey is first introduced in a UF unit where protein is concentrated and the retentate which is enriched in protein compared to the input whey (whey concentration ratio approx =27) is then fed in the CreamProt unit where is maintained at high temperature in order to happen microparticulation of the protein molecules and to be formed globules of size 0,5-15 μm with diameter similar of the fat contained in the milk. This globules can be associated easily with casein micelles and for this reason the creamy product which comes out of the CreamProt® process can be recycled and incorporated in cheese production. An overview of the process equipment is presented in Picture 2 and the principle of the full process is presented in Figure 2. where it is seen that protein molecules from an initial size of 3-5 nm become through CreamProt® of similar size with fat globules allowing the use of low calories cream in many applications that require and demand a low calories substitute of fat like in the production of sweets or in bakery applications.

Picture 2. The patented CreamProt® Processing Unit

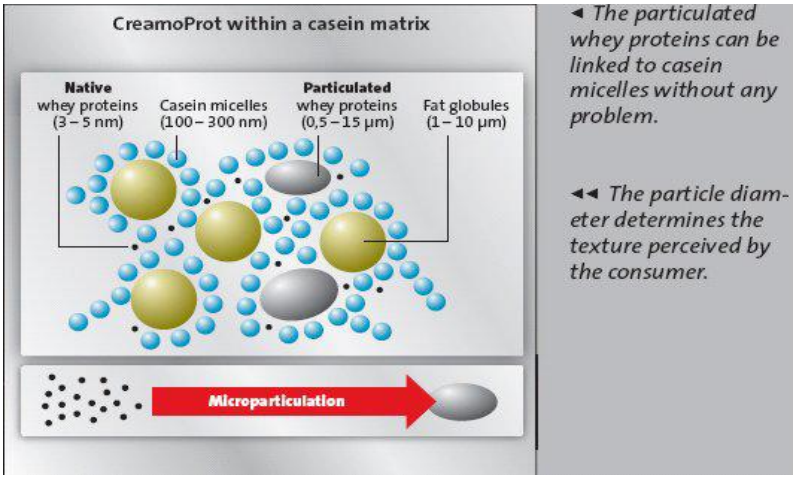

Figure 2. The operational principle of CreamProt® Process

CONCLUSIONS

Whey is a very interesting product thanks to its components. Their properties, functions and chemistry structure make whey a great base for the creation of a series of new products or an ideal alternative compound to more traditional ones. During the last decades many studies have been cared out about whey's components and especially lactose and the main proteins (α-Lactalbumin, β-lactoglobulin and bovine serum albumin). These compounds have been examined for their structure, their physical-chemical and biological properties, they have been compared to similar compounds along with ways of separating or isolating them. But still there is room for further research, mostly in the cases of the proteins that are included in a smaller percentage but they have great biological value. The first studies are very encouraging for the properties and their potential applications, of these proteins. The progress that has taken place in science and technology over the last decades, now allow scientists to have multiply ways of separating and process them, some of them in a rather low cost. The technological innovations along with the increasing demand for these compounds give optimistic aspects for their further study.

In terms of whey utilization there are so many things that can be done instead of treating whey as a waste. Valuable components can now been taken advantaged in very phosphorous ways. Apart from the obvious economical advantage of whey utilization there is the ecological concern as well. An easy to describe but complicated to apply, approach to total

utilliazation of whey would be to first purify whey, then separate it to its main components lactose, whey protein and delactosed permeate (DLP). Using the already exciting methods lactose and whey proteins can be further processed for the manufacture of various products, while in the case of DLP utilization there is still a lot of room for investigation of the applications (a first approach is to used at the production of biogas, as renewable energy source) and the economical aspects of it.

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