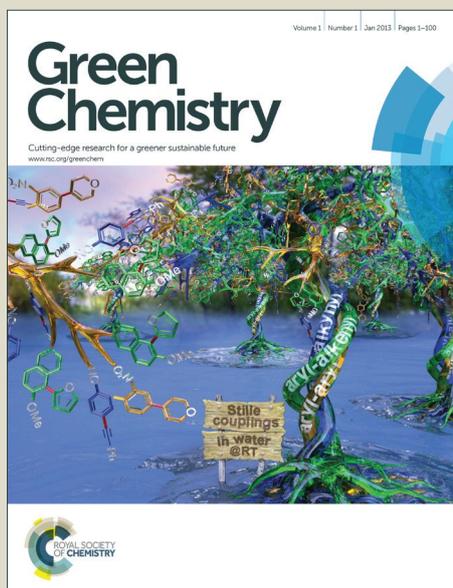


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Bigger data open innovation: Potential applications of value-added products from milk and sustainable valorization of by-products from the dairy industry.

Review

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Abstract

Milk is a natural suspension of essential nutrients and it can be exploited not only to be consumed directly as a beverage or transformed into a range of traditional dairy foods, but as a source of ingredients for food product innovation. In addition, by-products from the dairy industry, that used to constitute an economical and environmental problem, are still rich in proteins, lactose and minerals. Therefore, from a green point of view, they can be valorized and are being exploited for multiple food and non-food applications. This publication aims to give an overview of the different applications of milk ingredients and by-products from the dairy industry. Additionally, enzymatic modification and production of bioactive peptides and bioemulsifiers broaden their functionality and applicability. Finally, a critical view of the most promising aspects as well as some features that need further development is presented. The unmet needs, the cross fertilization in between protein domains, the carbon footprint requirements, the environmental necessities, the health and wellness new demand... are dominant factors in the search for an innovation approach outlining the potential that those "apparent" constrains oblige science and technology to account for. The adjacent technology analysis opportunity, that such a review generates, is in essence an open innovation step.

Keywords: Valorization, Milk Fractionation, Caseins, Whey Proteins, Non-Food Application, Enzyme Modification, Bioactive Peptides, Bioemulsifiers, Innovation - closed, open, collaborative, disruptive, inclusive, nested -, Bigger Data.

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1. Introduction

Often recognized as an outstanding source of essential nutrients, milk can be even defined as "the most nearly perfect food".

This might as well be challengeable given the increased "societal" concerns associated with the current production, ingredient processing of milk and allergies. Perception or reality, a certain orthodoxy or paradigm is perpetuated by the fact that bridges are possibly missing between the various sources of proteins, such as milk and vegetable sources.

Before getting on with a review of the milk ingredient potential and by-product valorization, keeping in mind that this review has also innovation incentivizing and educational purposes, we want to illustrate using bigger data analysis whether this bridging resistance or risk adversity is a myth or a reality?

Then let's try to depict a much larger picture of that precise aspect, from a BIGGER DATA standpoint...

Representations of liaisons between the most appearing keywords/concepts (nodes) in, for example, selected patent sections, can help understand the existing technology relationships; between various protein sources for example.

Illustration 1 provides, for educational value, an example of a representation based on 20 000 relevant patents and associated pertinent scientific papers, non-patent literature (NPL). On that representation a tetrahedral figure, delineated with the coarser and broader lines and bigger nodes, representing the largest numbers of connections, appears between the three selected sources of protein, i.e. milk, whey and vegetable, and food ingredients.

This tends to reflect that the paradigm or orthodoxy that assumes a lack of relationship in practice between food and vegetable proteins would not be supported by technology and scientific similarity/concordance/co-occurrence analysis.

The Illustration 1 below does not require more explanations on that matter and proves that the lack of bridging between the various protein domains is not confirmed from that analysis angle. This review can help alleviate the orthodoxy and promote technology disruptive innovation.

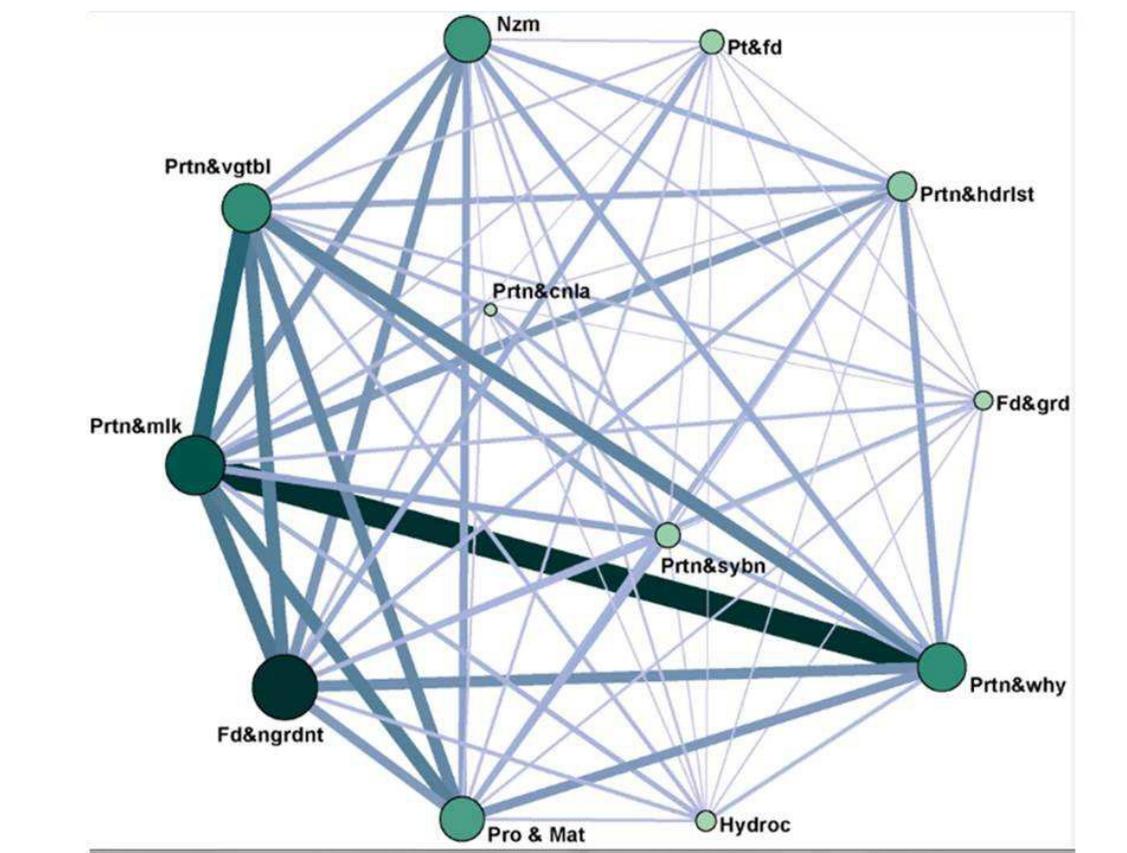


Illustration 1. The protein (Prtn) neural network (co-occurrence chart) based on 20 000 patents and NPL of the selected field. (Prtn&x), (x=mlk, why, vgtbl respectively milk, whey, vegetable), (food ingredients = Fd&ngrdnt).

Indeed reviews are “immensely” stimulating in terms of innovation, although they tend to miss the adjacent technology analysis (ATA) that innovation most often derives from. One objective of the current review is to promote innovation from a public literature analysis and to propose some interpretations with an educational illustrative mindset.

As mentioned before, as an outstanding source of essential nutrients, milk can be defined as “the most nearly perfect food”. Those nutrients can be classified in three fractions: the carbohydrate fraction, where lactose is the main component; the fat fraction, essentially composed by acylglycerols and phospholipids; and the protein fraction, with two groups of proteins: caseins and whey proteins. A general composition of cow’s milk is summarized in Table 1. Though similar, milk composition is not the same for all the species of dairy animal; some of the main differences are: goat is quite similar to cow’s milk, sheep’s milk has higher fat and protein contents, buffalo’s milk is similar to yak’s milk and has twice higher fat content than cow’s milk, etc. Being the principal constituent of milk, water content from different dairy species range from 83 percent in yaks to 91 percent in donkeys. In addition, milk composition is also affected by many factors such as stage of lactation, age, diet, breed, physical environment and season^{1, 2}. The unique composition of nutritional components contained in milk makes it perfect not only to be consumed directly as a beverage or

transformed into a range of traditional dairy foods, but to obtain ingredients or raw materials for different food and non-food applications.

Table 1 Composition of cow's milk ^{1,3}

<i>Nutrient</i>	<i>Component</i>	<i>Content (%)</i>
<i>Water</i>	--	87.4
<i>Caseins</i>	α_{s1} -casein	1.2
	α_{s2} -casein	0.3
	β -casein	1.0
	κ -casein	0.35
	γ -casein	0.12
<i>Whey proteins</i>	α -lactalbumin	0.12
	β -lactoglobulin	0.32
	Serum albumin	0.04
	Immunoglobulins	0.08
	Proteose-peptone fraction	0.1
<i>Fat</i>	Triacylglycerols	3.54
	Mono- and Diacylglycerols	0.09
	Phospholipids	0.04
	Sterols	0.02
	Free fatty acids	0.01
<i>Lactose</i>	--	4.8
<i>Ash</i>	--	0.7

When milk is used in the production of traditional dairy products, such as yoghurt, cheese or kefir, huge amounts of by-products and wastes, especially whey, are generated. Whey is an important disposal problem in the dairy industry, but at the same time it is still rich in nutrients. Therefore, from the green point of view, it can be valorised and transformed from waste to a source of valuable ingredients.

The fractionation of milk to obtain products with different composition and properties opens up a wide range of applications for components obtained from milk or the dairy industry. A compound annual growth rate of 4.9% from 2013 to 2018 has been projected for the overall industry for dairy ingredients, reaching more than \$53.6 billion by 2018 ⁴. The increasing number of applications they can be used for and the growing health-conscious consumers are the major factors fueling this growth. Additionally, those substances can be processed in various ways to create numerous ingredients with diverse functional and nutritional characteristics. In 2013, a volume of approximately 2200 KT was estimated for the industrial market of proteins (whey ingredients, MPC & MPI and caseins). While nearly 16800 KT are projected for the dairy ingredients in general to reach by 2018, around 5100 KT corresponding to proteins ⁴.

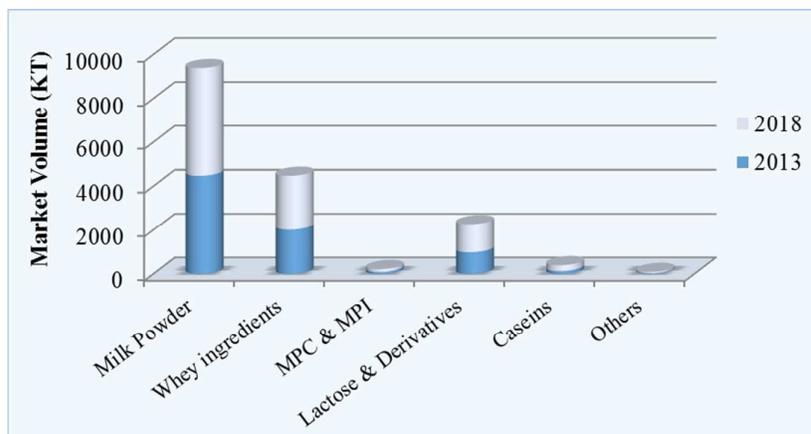


Figure 1 Trend of the industry in terms of volume by type of dairy industry ⁴.

Of the different components, protein can be considered to be the most valuable with an expanding demand for dairy proteins, including those obtained from the valorization of dairy by-products, and its derivatives. It has been reported that the fastest growing segments are milk protein concentrates and milk protein isolates, followed by whey ingredients ⁴. According to U.S. Dairy Export Council, dairy commodity prices have been decreasing during the last year. The reported values in Europe for August 2015 were: 1770 \$/MT for skim milk powder and non-fat dry milk, 700 \$/MT for whey and 1500 \$/MT for whey protein concentrates (34%) ⁵.

This review highlights the great potential of value-added products manufactured from the protein fraction to be used not only as food ingredients or nutritional products, but also in the non-food area. A new concept defined as “Bio-inspired Technology Innovation” ⁶, indicates that the future of the innovation should rely on the observation of nature and application of its resources in different domains, using multi-disciplinary research translation. In this review this concept is clearly exemplified, as multiple potentials of transferring natural properties of milk proteins to different areas are presented.

2. Fractionation of milk and production of milk proteins

To be used as ingredients, milk components need to be fractionated and purified. These substances differ in size, structure and physical properties and can be isolated by different processing techniques, usually involving the use of filtration methods based on molecular weight. Membrane separation technology is being applied both in traditional dairy processing and for new and innovative applications, to produce value-added dairy products.

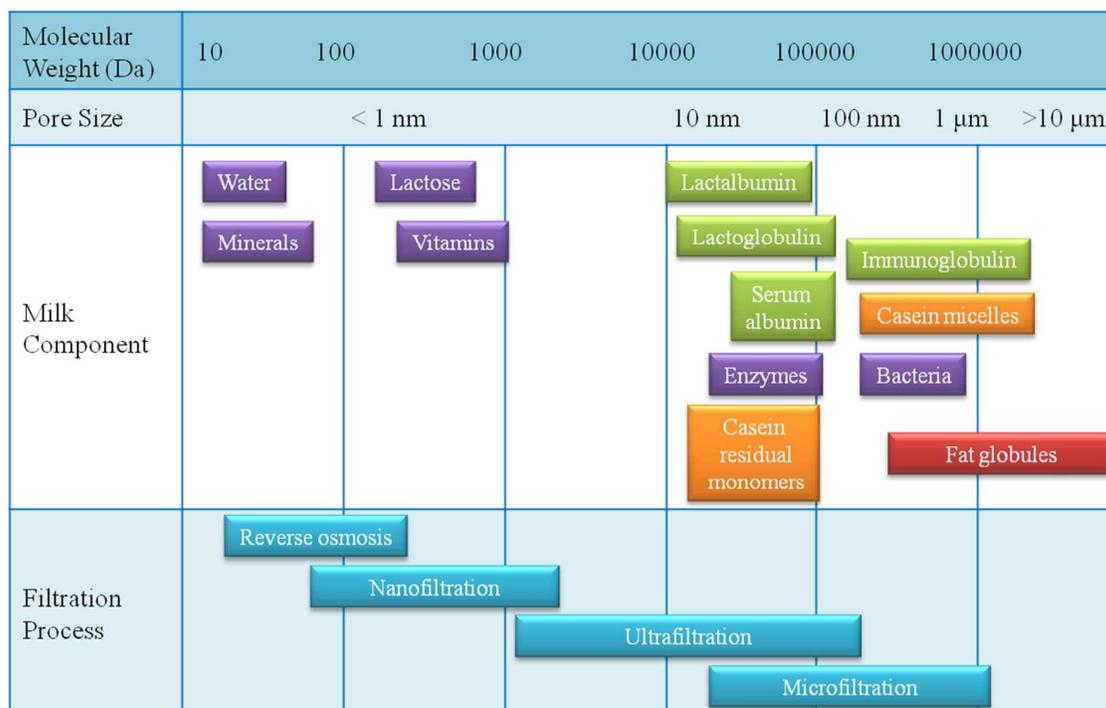


Figure 2 Relative milk component sizes in comparison with membrane pore size ranges (Adapted from ⁷)

Ultrafiltration (UF) was initially used in the dairy industry to pre-concentrate milk before cheese making. It has also been successfully used to recover proteins from whey and concentrate milk proteins from smaller compounds such as lactose, vitamins and minerals. Microfiltration (MF) emerged as an industrial separation technology in the dairy industry for bacteria removal, defatting of whey and micellar casein enrichment for cheese making ⁸. Nowadays, it has been investigated for its capability to directly isolate caseins and whey proteins from milk ⁹⁻¹¹. A wide variety of microfiltration-membrane materials, geometric designs, system configurations and operating approaches are available. Two major types of membranes exist: polymeric and ceramic. Their differences lie in cost, membrane life, flux, efficiency, purity of the fractions, cleaning and energy consumption ¹².

Fouling is considered the limiting factor in milk filtration, which has led to the development of different strategies to minimize this problem, e.g., uniform transmembrane pressure (UTP), backpulsing, air slugs, rotating or vibrating modules and ion-exchange membranes ^{8, 13}. Solutions based on the porosity gradient of the membrane support (Membralox GP[®], Pall Corporation) or a variable thickness active membrane layer (Isoflux[®], Tami Industries), have also been developed ^{14, 15}.

One of the indicators of the great potential of the fractionation of milk is the number of patents that have been published over the last decade regarding the purification and concentration of its different ingredients. Some examples are: protein fractionation of skim milk by means of microfiltration ¹⁶, purification of β -casein using cross-flow polymeric microfiltration membranes ¹⁷, fractionation of α_s -casein, κ -casein and β -casein ¹⁸, concentration of milk proteins using negatively-charged ultrafiltration membranes ¹⁹ and supercritical carbon dioxide to effectively fractionate whey proteins (α -lactalbumin and β -

lactoglobulin), as well as to isolate casein glycomacropeptide (an amino acid fragment of κ -casein)^{20,21}.

Milk proteins can be obtained directly from skim milk or from wastes and by-products of cheese making; Figure 3 summarizes the main steps to obtain milk protein products through different routes. Direct ultrafiltration of skim milk produces milk protein concentrate (MPC) that contains whey proteins and caseins in the same ratio as milk, while ultrafiltration of cheese whey produces whey protein concentrate (WPC)¹⁵. With a combination of micro- and ultrafiltration, separated fractions of micellar casein and whey protein concentrates are obtained. This is also possible with the coagulation of casein.

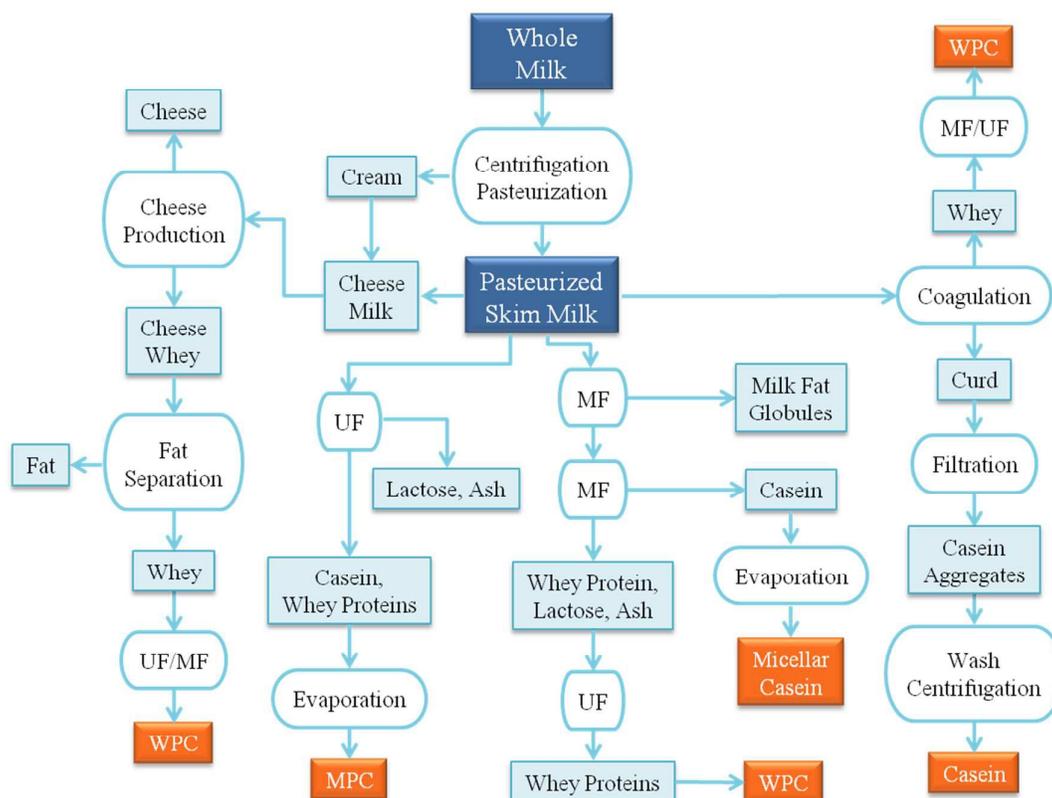


Figure 3 Different processes of manufacture of milk protein products (Adapted from ⁷)

It is estimated that more than 90% of whey protein concentrates originates from separating the coagulum from milk, cream, or skim milk in cheese making and also from the production of yogurt. Less than 10% is obtained from casein production, from the curd formation by direct acidification of milk. Depending on their source, WPC have different flavor and appearance, WPC from the production of cheese is more colored than the one obtained from milk. A solution of milk-derived WPC has a clear appearance, while using cheese-derived WPC is very milky and cloudy. This is due to the higher fat content of the second. The presence of glycomacropeptide only in cheese-derived WPC is another difference.

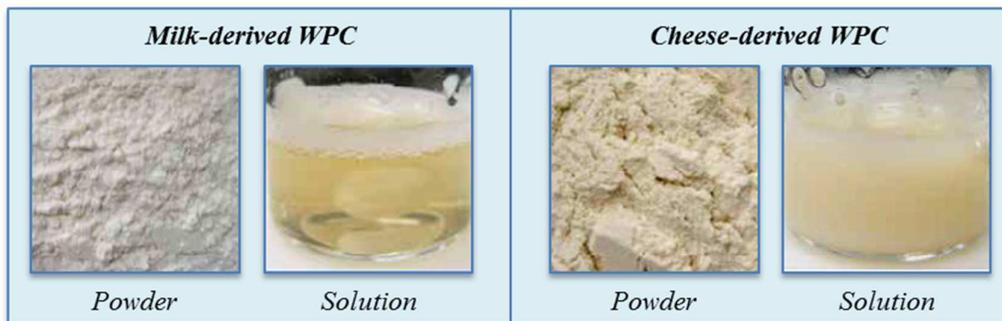


Figure 4 Appearance of WPC from different sources, powdered and in solution

Whey as the largest by-product of the dairy industry constitutes a challenging disposal problem; this is due to the large amounts that are generated and the high concentrations of dissolved organic substances. In general, approximately 8-9 kg of whey is generated from the production of 1 kg of cheese. The world whey production is over 160×10^6 tons/year, showing a 1–2% annual growth rate²². The biochemical oxygen demand (BOD) of whey varies from 30000 to 50000 ppm²³ and its chemical oxygen demand (COD) from 50000 to 80000 ppm²⁴. These high values are mainly attributable to lactose and depend upon the source of milk and the variety of product being made. A reduction of 13% of the whey BOD by deproteinization by heat or chitosan treatment has been reported. On the other hand, after isolation of lactose, BOD was reduced by 87%²³.

3. Milk proteins

As commented previously, milk proteins consist of two major groups of proteins called caseins and whey proteins. Of the approximately 3.6% protein in milk, roughly 80% is casein and 20% is whey protein³.

3.1. Milk proteins as food ingredients

Dairy proteins are isolated from skim milk using membrane filtration and obtaining milk protein concentrates (MPCs). MPCs have concentrations ranging from 40 to 80%, with higher concentrations (>90%), they are referred to as milk protein isolates (MPIs). Those milk fractions are rich in bound calcium and contain both whey protein and casein in the same ratio as milk, having good heat stability and clean flavor profile.

The excellent surface-active and colloid-stabilizing characteristics of milk proteins make them highly valued food ingredients, both in soluble and dispersed form²⁵. Some of their main functional characteristics are solubility, water binding, gelling, foaming, emulsification and heat stability. Their hydrophilic and hydrophobic polar nature makes them desirable emulsifiers used as fat replacers, and to improve texture and increase shelf-life in diverse products such as breads, meats and frozen desserts. Thanks to their foaming properties, milk proteins can also be used in whipped products, function that has traditionally relied on eggs. MPCs are currently used as an ingredient for manufacturing products including: cheese and yogurt, ice cream, dietetic formulations, cereal and energy bars, infant formulas, desserts, baked goods, toppings, low-fat spreads, sports beverages/foods and geriatric nutritional products.

Not only milk proteins, but proteins in general are important ingredients in the food industry and the demand for protein-enriched food formulations is increasingly growing. Proteins provide amino acids that are required for growth, functioning and cellular maintenance of the human body. However, their composition is different depending on their source, hence their potential to meet the needs of the human body is also different.

The United States is the only producing country with official production data of MPC and MPI. The U.S. market size is estimated at 50000 - 55000 MT for MPC42-56, and 17000 - 18000 MT for MPC70-85 and MPI. It is projected a growing of more than 40000 MT by 2020²⁶.

The quality of proteins can be determined by various methods. Protein efficiency ratio (PER), which “measures the weight gain of growing rats when being fed the test protein”. This method only reflects the amino acid requirements for the rat rather than the actual human amino acid requirements. Another method to compare the quality of various proteins is known as protein digestibility corrected amino acid score (PDCAAS) and based on the amino acid requirements of humans. According to this method, “an ideal protein that meets all the essential amino acid requirements of human body will have a value of 1.0”. Moreover, the biological value (BV) provides “a measurement of how efficient the body utilizes protein consumed in the diet”. Table 2 summarizes the quality of proteins from different sources²⁷.

Table 2 Comparisons of quality of proteins from different sources

<i>Protein type</i>	<i>PER</i>	<i>PDCAAS</i>	<i>BV</i>
<i>Whey protein</i>	3.2	1.00	104
<i>Casein</i>	2.5	1.00	77
<i>Milk</i>	2.5	1.00	91
<i>Soy protein</i>	2.2	1.00	74
<i>Wheat gluten</i>	0.8	0.25	64
<i>Beef</i>	2.9	0.92	80
<i>Egg</i>	3.9	1.00	100

Vegetables proteins have a lower biological value, this is due to the presence of trypsin inhibitors, lectins and tannins, which hamper the hydrolysis of proteins and hence the obtaining and final absorption of amino acids. Besides, these proteins lack of one or more of the essential amino acids. However, vegetable proteins, may be combined to provide for all of the essential amino acids, avoiding animal sources and with a lower content of saturated fat. These proteins also include other nutrients such as phytochemicals and fiber. For instance, soy proteins provide protease inhibitors, phytosterols, saponins, and isoflavones that are beneficial for cardiovascular health. Milk proteins, however, provide high levels of the essential and branched chain amino acids. These proteins also possess many beneficial bioactivity properties and are a rich source of minerals and vitamins²⁷. Another advantage of milk proteins is that they are suitable to people that are allergic to soy products and to those that reject GMO products. From the opposite position, they are inapt to people allergic or intolerant to milk products or vegans.

3.2. Types of milk proteins and their applications

Caseins and whey proteins have a different molecular structure; in contrast to the disordered flexible structure of caseins, whey proteins have a compact globular structure. For that reason, the protein adsorbed layer at the surface of the dispersed droplets for each of them has a specific structure, and consequently the mechanical properties of the stabilized emulsion are different^{28, 29}. The relationship between the properties of milk protein adsorbed layers and stability of corresponding emulsion systems for pure milk proteins, including interactions between them and with surfactants, has been extensively studied³⁰.

3.2.1. Caseins

The caseins in milk form complexes called micelles that are dispersed in the water phase of milk. There are diverse models to describe the casein-micelle structure³¹. In one of the most commonly accepted model, the casein micelles consist of spherical subunits or submicelles of the different caseins (α_{s1} , α_{s2} , β and γ) held together by calcium phosphate bridges and hydrophobic interactions between proteins on the inside, surrounded by a layer of κ -casein which helps to stabilize the micelle in solution. There are two main types of submicelles; one consisting of α_s - and β -caseins, that constitutes the hydrophobic center of the submicelle, and another type consisting of α_s - and κ -caseins, which is distributed outside of the micelle with the hydrophilic part (the sugar residues of κ -caseins) forming an outer “hairy layer”³¹. Owing to the importance of casein micelles for many of the physico-chemical properties of milk and dairy products, their structure, properties and the effects of their composition and processing conditions have been extensively studied³².

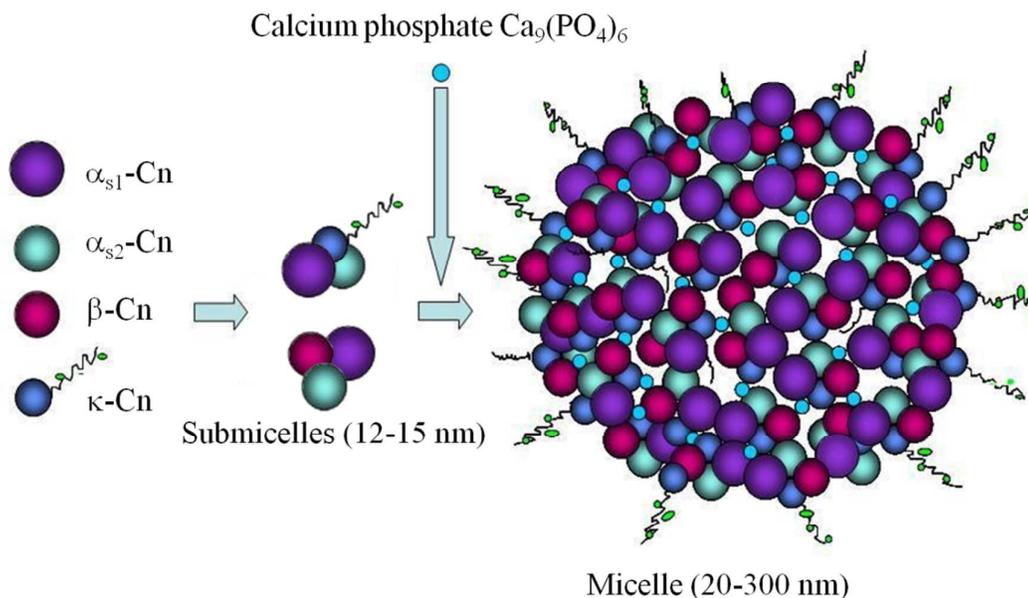


Figure 5 Schematic representation of casein submicelles and casein micelle composed of submicelles held together by calcium phosphate.

Being phosphoproteins, each of the caseins has variable degree of phosphorylation. While α_s -caseins and β -caseins are highly concentrated in phosphoserine residues, κ -casein contains only one or two residue, besides of being glycosylated. Caseins, especially β -casein have a large amount of proline residues, which disrupt the formation of α -helical and β -sheet³³. These proteins are stable at high temperatures but unstable at pH below 5.0, for that reason they are usually prepared by isoelectric precipitation at pH 4.6. Coagulation through the action of

rennet enzymes and isolation by membrane filtration are other processes to obtain caseins from skim milk, as shown in Figure 3.

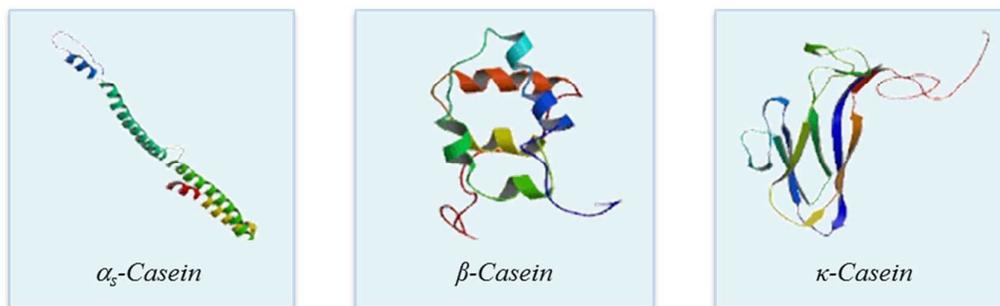


Figure 6 Protein structure of caseins³⁴

The most important applications of caseins in fabricated foods are cheese analogues, synthetic whipping creams, cream liqueurs, fabricated meats, some cereal products, various dietetic foods and as an emulsifier in coffee whiteners³⁵. The amphipathic structure and the lack of stable secondary and tertiary structures of caseins contribute to their high surface activity, which gives them good foaming and emulsifying properties. Casein functional properties are significantly affected by changes in pH, method of preparation, ionic strength and nature of the salt ion³⁶.

Regarding the isolate proteins from casein, β -casein has very high surface activity and may find applications as a high-quality emulsifier or foaming agent. The fortification of milk with β -casein improves its cheesemaking properties. This protein is also an attractive ingredient for the manufacture of bovine milk-based infant formulae that more closely mimic human breast milk, which has a higher ratio of β -casein. κ -Casein might be a useful additive for certain milk products³⁵. Nevertheless, the casein proteins find their major application in the production of biologically active peptides, which will be described in subsequent sections.

Currently, the Codex Alimentarius includes a Codex standard covering the identity, composition, labelling and quality of edible casein products intended as ingredients in food (CODEX STAN 290-1995)³⁷. Casein production is estimated at 274000 MT, the production and trade of casein products is declining in many developed markets (was 350000 MT in 2004). This is due to the replacement by MPC and MPI, which are easier to produce and generally have better organoleptic characteristics. In addition, MPC production yields milk permeate and is perceived as more environmentally friendly than casein production²⁶.

3.2.2. Whey proteins

The whey protein fraction consists of approximately 50% β -lactoglobulin, 20% α -lactalbumin, serum albumin, immunoglobulins, lactoferrin, transferrin, and the proteoseptone fraction. Regarding their structure, whey proteins are compact globular proteins whose intramolecular folded structure is the result of disulfide bonds between cysteine residues, which buries the hydrophobic residues into the molecule. For that reason, whey proteins do not interact with other proteins. These proteins exist as individual units dissolved in the water phase of milk and stay in solution (unless denatured) over a wide range of pH.

They can be denatured by heat, what produces breaking and randomization of the stabilizing disulfide bonds, and exhibit heat-induced gelation³⁸.

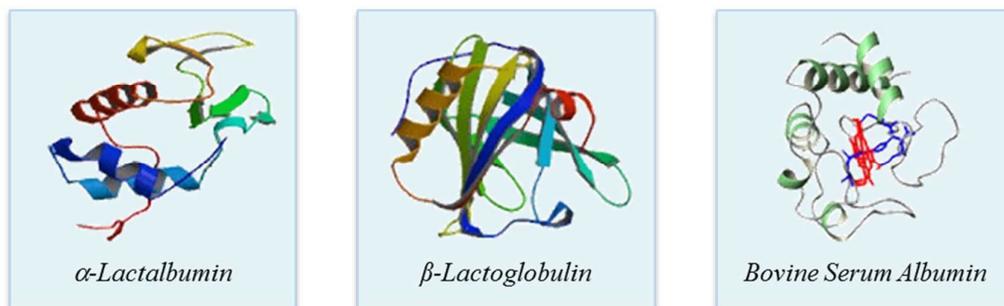


Figure 7 Protein structure of the main whey proteins³⁴

Physical, chemical and structural properties of whey proteins determine their functional properties. As the primary food application of whey proteins is as emulsifiers, the optimum conditions under which they act have been extensively studied. Some influential factors are processing conditions, the method of isolation, environmental conditions (e.g., pH, temperature, ionic strength, etc.), and interaction with other food components. Furthermore, its ability to form gels capable of holding water, lipids, and other components while providing textural properties makes them perfect to be used in processed meat, dairy and bakery products. Regarding its foaming properties, they mainly depend on the degree of the protein denaturation³⁸.

Whey protein products are produced for food, cosmetic and pharmaceutical sectors. There are diverse types of whey protein products: whey protein concentrate (WPC) (which contains 25-80% protein), whey protein isolates (WPI) (containing $\geq 90\%$ protein), whey protein hydrolysate (which will be commented in the following section), β -lactoglobulin, α -lactalbumin and protein-peptone fraction^{38,39}.

As well as for caseins, the Codex Alimentarius includes a Codex standard of whey powder for direct consumption or further processing (CODEX STAN 289-1995)³⁷. It was also affirmed as “generally recognized as safe” (GRAS) (21CFR184.1979). Production volumes of the various types of whey products available publicly from the United States are: 117669 MT for WPC25-50, 108060 MT for WPC50-90 and 39501 MT for WPI. Globally, it is likely that demand of WPC/WPI will be fueled by the nutritional benefits of whey protein along with more cost-efficient, sustainable production technologies²⁶.

WPC containing 34–35% protein (WPC35) is also rich in lactose and minerals. It has good emulsification properties, is highly soluble and has a mild dairy flavor. This product is used in the manufacture of yogurt, processed cheese, infant formulae, and in various bakery applications. WPC is also marketed for use in stews and sauces because of their thickening properties as well as nutritional benefit. WPC with $\sim 80\%$ protein (WPC80) and WPI have low carbohydrate content and are characterized by good gelation, water-binding, emulsification and foaming properties. They are extensively used in nutritional supplements, sports and health drinks, weight management products as well as for meat products³⁹.

α -Lactalbumin has a molecular weight of 14.2 kDa and an isoelectric point between 4.2 and 4.5. This protein has significant nutritional properties and is associated with some positive health benefits. Its good protein digestibility and amino acid composition are considered to be optimal to be used in infant formulas. It is relatively rich in tryptophan, an essential amino acid which is known to have a positive effect on satiety, mood, sleep and cognitive performance. α -Lactalbumin also possesses bactericidal or antitumor activity. In addition, synthesis of α -lactalbumin nanoparticles and nanotubes as drug and food delivery systems has been studied⁴⁰⁻⁴³. Application of concentrated α -lactalbumin powder in preparing health-care anti-anorexia beverage has been proposed⁴⁴.

Native β -lactoglobulin is a globular protein (18.4 kDa) with defined secondary and tertiary structure. This protein possesses a high nutritional value and interesting technological properties, but at the same time it is considered one of the major allergens in milk whey. Therefore, eliminating β -lactoglobulin from milk whey is very important in the dairy product industry⁴⁵. Despite its allergenic potential, food applications and studies concerning emulsifying and foaming properties of β -lactoglobulin have been reported⁴⁶⁻⁴⁸.

Proteose-peptone (PP) is a heat-stable and acid-soluble protein fraction, a mixture of heterogeneous proteins and peptides that can be divided into two groups. The first group, the non-hydrophobic fraction, consists of peptides resulting from natural proteolysis of caseins and identified as components PP5, PP8-fast and PP8-slow, highly soluble. The second group of proteins comprises the hydrophobic fractions of glycoproteins whose main component is called PP3⁴⁹. The techno-functional and biological properties of proteose-peptone fraction are thermal stability, emulsifying and foaming properties, inhibition of spontaneous lipolysis in milk and antibacterial, antiviral and anticarcinogenic activities⁵⁰. The good emulsifying capacity of proteose-peptone fraction as functional ingredient in ice-cream production allows the reduction or total elimination of the use of emulsifiers such as monoglycerides and diglycerides⁵¹. It has been reported that the purified PP3 has a higher emulsifying activity than the total unpurified proteose-peptone fraction⁵². Faure et al.⁵³ patented a method for the industrial production of proteose-peptone enriched extract from whey protein concentrate or whey protein isolate as opposed to skimmed milk, a more expensive raw material. They proposed several uses of these extracts, e.g. in food products and supplements, in nutritional, pharmaceutical and/or cosmetic compositions, as emulsifier or as foaming agent, and/or for low fat products.

3.3. Enzyme modification of milk proteins

Enzymatic tailoring of proteins by hydrolysis or cross-linking is attempted in order to improve their functionality and stability.

3.3.1. Cross-linking of milk proteins

Cross-linking of food proteins can be catalyzed by enzymes such as peroxidase, tyrosinase, transglutaminase, and laccase⁵⁴. The susceptibility of a protein to cross-linking depends on its macromolecular structure. Individually, both the caseins and whey proteins are good substrates, but in a mixed solution caseins are more susceptible to cross-linking than native whey proteins⁵⁵. Some structural modifications of whey proteins, such as heat denaturation or chemical modification, make them more suitable to be cross-linked, either with caseins or other whey proteins^{56,57}.

Transglutaminase (TG) is one of the most widely used proteins for cross-linking of milk proteins. In a generic TG reaction, the γ -carboxamide groups of the glutamine residues act as acyl donors, and the ϵ -amino groups of lysine residues act as acceptors. Enzymatic cross-linking of individual milk proteins and other proteins with TG has been shown to increase heat stability and has a considerable effect on their emulsifying properties^{58,59}.

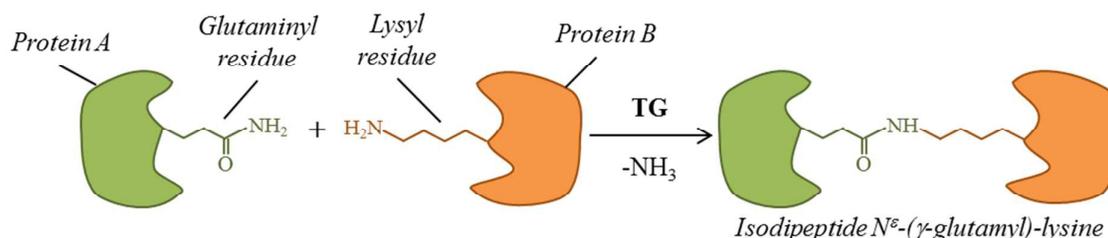


Figure 8 Transglutaminase-catalyzed protein cross-linking

In the study of the effect of the degree of enzymatic cross-linking of milk proteins⁶⁰, higher emulsion stabilities were observed at low degrees of cross-linking compared to native proteins or extensive cross-linking. On the other hand, creaming stability of emulsions was improved even at high degrees of cross-linking compared to native proteins; this was attributed to the increased viscosity of the medium or changes in the adsorbed layer. It has also been reported that properties of emulsions containing cross-linked α -lactalbumin were influenced by the sequence of cross-linking and emulsification⁶¹. While cross-linking before emulsification decreased the stability of the emulsion, due to a lower rate of protein adsorption and restricted ability of protein to unfold at the oil–water interface, this stability was improved when cross-linking was carried out after emulsification. In addition, due to the partial unfolding of globular proteins when adsorbed at the oil–water interface, cross-linking after emulsification is easier and a higher increase in surface viscosity is reached⁶². Conversely, in sodium-caseinate-stabilized emulsions, it was found that cross-linking by transglutaminase before emulsification significantly improved the emulsion stability, while cross-linking after emulsification produced a great loss of long-term stability due to droplet coalescence⁶³.

The modification of whey protein using an immobilized form of microbial transglutaminase, besides the well-known benefits of immobilized enzymes such as an easy separation of catalyst and substrate reutilization of the enzyme or no need for a downstream inactivation treatment; allowed to control the extent of cross-linking. Additionally, the combination of recombinant fusion proteins and immobilized enzyme technologies was also studied. In both cases, intrinsic and apparent viscosity increased, gel point temperature decreased, and stronger, more brittle gels were formed^{64,65}.

Regarding applications, cross-linking may be used for stabilizing products such as yogurt, whipping cream, fresh cheese and novel milk products. Yoghurt from lactoperoxidase-, laccase- glucose oxidase- or transglutaminase-treated milk was characterized by a minor acidity and whey drainage as well as by a more soft, homogeneous and creamy consistency showing better sensory characteristics than yoghurt from untreated milk^{66,67}, indicating that cross-linking may be a very useful tool for low-fat fermented products. Improved gel strength and increased viscosity as a result of improved water-holding properties have also been reported⁶⁸.

3.3.2. Enzymatic hydrolysis of milk proteins

Food-grade proteolytic enzymes are obtained from different sources (animal, bacterial, fungal or plant), having different pH and temperature optima and hydrolyzing a variety of peptide bonds. Enzymatic hydrolysis of milk proteins modifies the surface hydrophobicity, the emulsifying and foaming properties, and solubility⁶⁹. It has also influence on the digestibility and allergenicity. The enzymatic hydrolysis of milk proteins enables tailoring their functional properties to meet individual requirements of food formulations. Partial hydrolysis of proteins may improve their functionality, but excessive hydrolysis could negatively affect it. It has been reported that higher hydrolysis degrees produce bitterness⁶⁹ and can result in adverse effects on the emulsifying functionality⁷⁰. Controlled hydrolysis may enhance some functional properties and simultaneously be detrimental to others⁷¹. For all those reasons, the control of the hydrolysis degree is crucial. To achieve that, the selection of the right proteolytic enzyme (such as pepsin, trypsin, neutrase, chymotrypsin, and plasmin), time, environmental conditions and extent of hydrolysis is crucial. Caseins, because of their flexible random structures, are more susceptible to hydrolysis compared to the whey proteins, with compact globular structure⁶⁹.

Milk proteins concentrates have limited use in some applications, especially in beverages, because of their poor solubility, which is an important factor in determining their functional properties. Hydrolysates show improved solubility what broaden its range of applications⁶⁹. In general, the hydrolysates have a higher rate of diffusion to the oil/water interface and cover a larger area of the interface than the native protein^{72, 73}. Furthermore, peptides have a high nutritional value, are easy to digest and absorb, and are less allergenic.

The influence of hydrolysis on several applications has been studied. For example, the neutrase treatment of milk proteins to prepare yoghurt showed an improvement in body, texture and flavor, a faster rate of acid development and a reduction in the production time⁷⁴. Protein hydrolysates, from casein and whey protein, are increasingly finding commercial applications in a number of formulated foods such as; high-energy supplements, geriatrics foods, weight-control foods and hypoallergenic infant formulas. The growing trend towards low-calorie and low-fat diets has led to the use of modified proteins as fat replacers⁷¹. WPI hydrolysates have been used as emulsifiers to produce nanoemulsions for food applications⁷⁵.

Some studies have been carried out to elucidate the functional and biological properties of hydrolysates obtained from hydrolysis of milk proteins, including interactions between native proteins and their hydrolysates⁷⁶. The stabilizing properties of polypeptides from α_{s1} -casein can in principle outperform the properties of the protein. However, the hydrolysis of the protein also produces undesired polypeptides that interfere with the optimal function of the desired ones. The purification of the desired fragments enhances their emulsion stabilizing properties⁷⁷. It is clear that a deeper understanding of the structure-function relationship of hydrolysates behavior both in model and real food systems is essential to increase the use of enzymes in enhancing the functionality of food proteins⁷¹.

4. Value-added products from milk proteins

4.1. Milk proteins as a source of bioactive peptides

The concept of bioactive peptides derived from food proteins was developed in the 1980s, since then, a worldwide interest for bioactive peptide is growing in the scientific community. This suggests the potential use of biopeptides as nutraceuticals and ingredients of functional foods to promote health and reduce the risk of diseases. Bioactive peptides, defined as “specific protein fragments that have a positive effect on body functions or conditions and might ultimately influence health”, can be released from their parent protein by enzymatic hydrolysis using food-grade enzymes, during microbial fermentation by proteolytic cultures or during gastrointestinal digestion⁷⁸. Moreover, recombinant DNA technologies are being investigated for obtaining large quantities of highly purified peptide fractions⁷⁹.

The most common way to produce bioactive peptides is through enzymatic hydrolysis of proteins; this process is analogous to that stated above for the enzymatic modification of proteins. For that reason, the bioactive properties of the peptides are influenced by the same factors (enzymes used, hydrolysis time, degree of hydrolysis, pretreatment of the protein,...). In order to obtain high-yield peptides with high bioactivity further processing, such as fractionation and concentration of the hydrolyzed protein, is needed^{76,80}.

Major milk proteins (caseins, α -lactalbumin, β -lactoglobulin) constitute an important source for bioactive peptides with a wide range of physiological properties. Milk-derived bioactive peptides were shown to have antihypertensive, antithrombotic, antimicrobial, antioxidant, immunomodulatory, antipyretic, opioid, mineral-binding properties and anticarcinogenic activities. Numerous fragments of caseins and whey proteins have been identified as bioactive peptides and categorized according to their particular health beneficial potentials^{79, 81-83}. Phelan et al. published a comprehensive review regarding biological effects of casein-derived bioactive peptides, their application in industry and safety aspects and regulations⁷⁸.

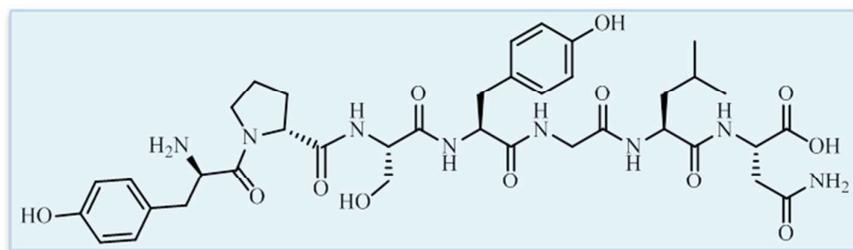


Figure 9 Chemical structure of a bioactive peptide; casoxin A derived from κ -casein, fragment (35-41), with opioid antagonist activity. Amino acid sequence: Tyr-Pro-Ser-Tyr-Gly-Leu-Asn⁸⁴

Food products containing bioactive peptides are commercially available in Japan, Europe, and the United States or under commercial development in these countries⁸⁰. Calpis[®] (Japan) and Evolus[®] (Finland) are used by their antihypertensive properties. Other commercially available bioactive peptides are whey protein hydrolysate BioZate (USA), containing fragments derived from β -lactoglobulin, and C12 peptide[®] (Holland), used as a food additive and enhanced antihypertensive peptide obtained by hydrolysis of casein. Other casein derived peptides (phosphopeptides, β -casomorphins) have already found interesting applications as dietary supplements and pharmaceutical preparations^{78,81}.

Although a lot of information exists on the production, processing, potential health benefits and mechanisms of action of milk protein-derived peptides^{80, 83}, aspects such as health-

promoting effects, allergy, intolerance, molecular mechanisms of action and bioavailability in humans should be more deeply evaluated in the near future. Those aspects strongly depend on the concentration in bioactive peptides; very low concentration might not be sufficient to provoke the desired benefit, while too high concentration might cause negative effects or be unsafe. Additionally, most of the studies to identify milk bioactive peptides as well as to study their bioavailability and molecular mechanisms of action have been carried out *in vitro*^{80, 81, 85}. The main problem is that *in vivo* pharmacological effects of peptides would hardly be equivalent to their *in vitro* bioactivity because other variables come into play, such as absorption, bioavailability, and degradation of peptides by physiological enzymes⁸⁶. Microencapsulation and nanoemulsion have been explored for the administration of bioactive peptides, to enhance and control their stability and absorption^{75, 87}.

Moreover, setting specific legal regulations is also an aspect to be developed. The current regulatory framework is still deficient. Japan was the first country to adopt regulations for allowing claims on functional foods in 1991 (FOSHU: Foods for Specific Health Use). In United States, the Food and Drug Administration (FDA) is charged with the responsibility for the regulation of dietary supplements produced before 1994, whereas the safety of those marketed after 1994 is the responsibility of the manufacturer. In Canada, bioactive peptides could be sold as natural health products or as an ingredient of functional food. These products fall under the Natural Health Products Regulations of the Food and Drugs Act which came into effect in 2004. In the European Union, there is no appropriate legislation for functional foods or nutraceuticals as a distinct category of foods. The general food law regulations are applicable to all foods. Only regulations concerning the use of casein and caseinates in the manufacture of cheeses have been established by the European Commission 760/2008 of July 31, 2008⁸⁸.

Much of the work to date has been focused on milk, cheese and other dairy products as sources of bioactive proteins and peptides, still, interest in other sources of food-derived bioactive peptides is growing. Some of these sources are fish species such as sardines, tuna, bonito and salmon, as well as other animal products such as blood, eggs and gelatin. Plants are also potential sources, for instance: wheat, rice, soya, pumpkin and mushrooms⁸². A Quantitative structure–activity relationship (QSAR)-based *in-silico* method was proposed for the prediction of food protein sources that can yield bioactive peptides⁸⁰. To achieve that goal, details of the structure-function properties of active sequences should be known. The biological effects attributed to the consumption of bioactive peptides and their possible sources are numerous and continuously increasing.

4.2. Use of whey proteins to obtain bioemulsifiers

Bioemulsifiers are surface-active agents synthesized by microorganisms: bacteria, fungi and yeast. These substances are characterized by their environmentally friendly nature, since they are easily biodegradable and have low toxicity. Moreover, they have good surface activity, emulsifying ability and antimicrobial properties. For all those reasons, the interest in these bioemulsifiers has been increasing as an alternative to chemical surfactants⁸⁹. The pathogenic nature of many microorganisms to produce bioemulsifiers restricts their range of uses, especially in the food industry. Byproducts from microorganisms with the GRAS status can be applied in the food and pharmaceutical industries. Therefore, bioemulsifiers obtained from GRAS microorganisms would not represent a risk, hence being of great interest in food and medicine applications.⁹⁰

One of the most interesting aspects of bioemulsifiers for food industries is the possibility of using their by-products or residues as substrates. The main alternative sources for bioemulsifier production comprise oily residues, milk and distillery wastes, and carbohydrate-rich residues⁸⁹. Whey milk and cheese whey have been reported as potential substrates in the production of bioemulsifiers by diverse surfactant-producing microorganisms⁹¹⁻⁹³. Cheese whey as a waste product from cheese production is known for being a pollution problem in the dairy industry, so its valorization through the production of bioemulsifiers represents both a solution for the environmental problem and an economic incentive.

Regardless of their interesting properties, the use of bioemulsifiers has still not reach a large scale since many regulations concerning the approval of new food ingredients are required by governmental agencies, currently there are not specific legal regulations for this substances. Their eventual toxicological aspects should be clearly identified before declaring them safe for food utilization⁸⁹. Nevertheless, an increasing number of patents claiming their use as additives for food, cosmetics and pharmaceutical products demonstrates the global interest in their exploitation⁹⁴.

5. Alternative non-food applications of milk proteins and dairy by-products

Although this review has been focused on the uses of milk components in the food industry, it is worth taking into consideration other milk non-food applications. Commercial non-food applications of casein proteins include textile fibers, adhesives, packaging films, biomaterials, plastics and additives in paints, concrete, cement, cosmetics and rubber. Compared to caseins, whey proteins are not so exploited in non-food industries, surfactants in cosmetology and pharmacology, and manufacture of protective films or coatings are some examples⁹⁵. Other minor milk proteins, such as lactoferrin and lactoperoxidase, have been studied for their potential application in pharmaceuticals and personal health care products⁹⁶.

In the current desired green framework, the most important aspect of non-food applications is the valorization of dairy co-products, hence reducing waste streams to compliance with safety and environmental regulations. Cheese whey, by-product of cheese production, is rich in whey proteins and lactose and hence, as have been stated above, have a high BOD which makes it one of the most polluting food byproduct streams²³. Considerable efforts have been made in order to solve this environmental problem, besides its applications in the food area, cheese whey also finds some value-added non-food applications⁹⁷. Those are mainly in the manufacture of fermented products reducing considerably the BOD and COD of whey⁹⁸. Those products are: biogas, hydrogen, biofuels (ethanol or butanol), polyhydroxyalkanoates, organic acids (lactic, acetic, propionic, and citric), exopolysaccharides, amino acids (glutamic, lysine, and threonine), vitamins (B12 and B2), biopolymers (xanthan gum), antibiotics, enzymes and single cell proteins^{22, 95, 99-102}. Most of the organic acids are used as substrates to obtain different products. For example, acetic acid is used to produce calcium magnesium acetate and polyhydroxyalkanoates. The production of lactic acid, in particular, is an important application that has been studied extensively²⁴. Lactic acid demand has been increased for last decade especially because of the growing development of the polylactic acid market, the biodegradable alternative to synthetic polymers derived from petroleum resources. Lactic acid is also used to make propylene oxide, propylene glycol and acrylic fibers⁹⁵. One of the most recent applications is the production of hydrogen by anaerobic fermentation from

cheese whey (reviewed by Prazeres et. al⁹⁷), producing also CH₄. COD reductions around 80 - 90% and sugar consumption between 86 and 97% can be reached.

A clinical application of cheese whey has also been proposed using it as a source of growth factors and antimicrobial agents¹⁰³. Those substances have been proved to be effective in preventing tissue damage and stimulating wound repair. The direct production of electricity through microbial fuel cells is another option for cheese whey valorization, yet it still presents several drawbacks for industrial application⁹⁷. Blends composed of whey proteins and gelatin (a byproduct of the leather industry) have been reported to work as fill jug agents in the leather industry. That represents a cheaper source of protein (\$1.05/lb) than current sources such as sodium caseinate (\$5.8/lb) and gelatin (\$2.6/lb)¹⁰⁴.

Finally, cheese whey can be used as a source of lactose which can be purified by crystallization¹⁰⁵. Lactose is a raw material for diverse lactose derivatives in pharmaceutical and cosmetic formulations (drug carriers, coating agents, lactulose, lactitol, lactobionic acid, adhesives, foams, etc)^{95, 99}. Alternative applications are based on the direct fermentation of lactose or the fermentation of the glucose and galactose obtained from the hydrolysis of lactose, though their principal applications are in the food industry, in infant formulas or as a sweetener^{95, 106}.

From an economic perspective, an analysis of the added value of whey valorization on the overall valorization of raw milk has been carried out. Results from the integral dairy valorization model showed that the valorization of byproducts increases the profit by 24.3%, while an additional profit can be achieved when two valorization processes are integrated. With an increasing in the demand of whey-based products of the 25%, significant benefits can be produced¹⁰⁷.

6. Conclusions

Growing concerns for health and environment characterize today's society, which increases the acceptability of food ingredients manufactured from milk, recognized by the consumer as "natural". This can be one of the reasons why dairy ingredients are becoming more and more important in food product innovation and production. Moreover, the continuous technological and research advances in the milk industry make it possible to capture and tailor the inherent biological and nutritional properties of milk ingredients and transfer them to a range of food and non-food applications. Fractionation techniques allow isolating milk ingredients with diverse ranges of purity and there is no doubt that they are critical in the development of new products and ingredients that drive the demand for dairy products. A wide variety of dairy ingredients is already commercially available and used not only in dairy foods but in many other products such as confectionery, beverages, cereals, sauces, dressings and sport complements.

Milk proteins are the most maximized components, through the obtaining of numerous formulations of MPCs, WPCs and caseins, with different compositions and applications in a variety of food and pharmaceutical industries. Additionally, the properties of these products are enhanced by their enzymatic modification, hydrolysis and cross-linking increasing their functionality and possible applications. In addition, their range of applications is boosted by the production of bioactive peptides, a field with unlimited research potential. Concerning WPCs, which can be produced from skim milk, are mainly obtained as a by-product in the

cheese industry, so their utilization as a valuable product has relevance to environmental concerns relating to whey disposal and hence to the cost of the product. In addition, whey protein from the cheese industry is valorized in the production of bioemulsifiers.

Alternative applications of milk proteins in the non-food area are also essential from the green perspective. The possibility of full valorization of those is first and foremost the main focus of interest. Additionally the green factor is part of the most innovative processes, for example transferring all this knowledge and dairy technology to vegetable counterparts.

Nevertheless, some final considerations regarding the use of concentrated or modified milk ingredients in the food industry should be mentioned. Even though milk is a safe product, processing or concentration of its components to obtain the desired beneficial properties, might eventually cause some adverse health effects. It is clear that a deeper understanding of the functional and nutritional properties of these ingredients will be essential to meet consumer expectations and avoid subsequent problems. As specific regulations for these new ingredients are insufficient, the development of standard and regulated in vitro and in vivo procedures to assess their functional and nutritional claims, purity, applications, intolerance, and allergenicity, would be essential for the establishment of those regulations.

Knowing those challenges, as mentioned before the full valorization of proteins is associated with the necessity to achieve cross-industry innovation. This may take a disruptive path given the paradigm or orthodoxy that derives from the presumed lack of relationship, in practical terms, between food and vegetable proteins platforms. We have shown in the introduction that this is not the case, indeed the two platforms are connected from a bigger data standpoint as shown on illustration 1. Now what are the tools at disposal to breakdown this types of orthodoxy.

A good basis to build the most appropriate toolbox is probably to start from some principles and illustration from other domains of significant industrial science and technology impact, e.g.:

“Innovation, a subject of massive interest” almost the next BUZZ call, said Rebouillat⁶, and to underline that “Innovation...

- is stronger-than-logic,
- is in essence not predictable,
- requires to realize that all the best people do not work for the same company,
- cannot be “cloned” since innovation cultures are hardly transferable,
- is moving towards fundamental co-creation with intense purposeful networking,
- is happening collaterally with the advent of Big and Bigger Data where word are now worth a million images,
- has to preferably happen with a business model even if innovation generally starts with a niche market ...”

Combined ideas drive innovation timelessly using ideas from the last century discoveries and inventions; often pooled and combined with ideas generated by the most advanced invention algorithms. On that matter, the Illustration 2 below does not require more explanations.

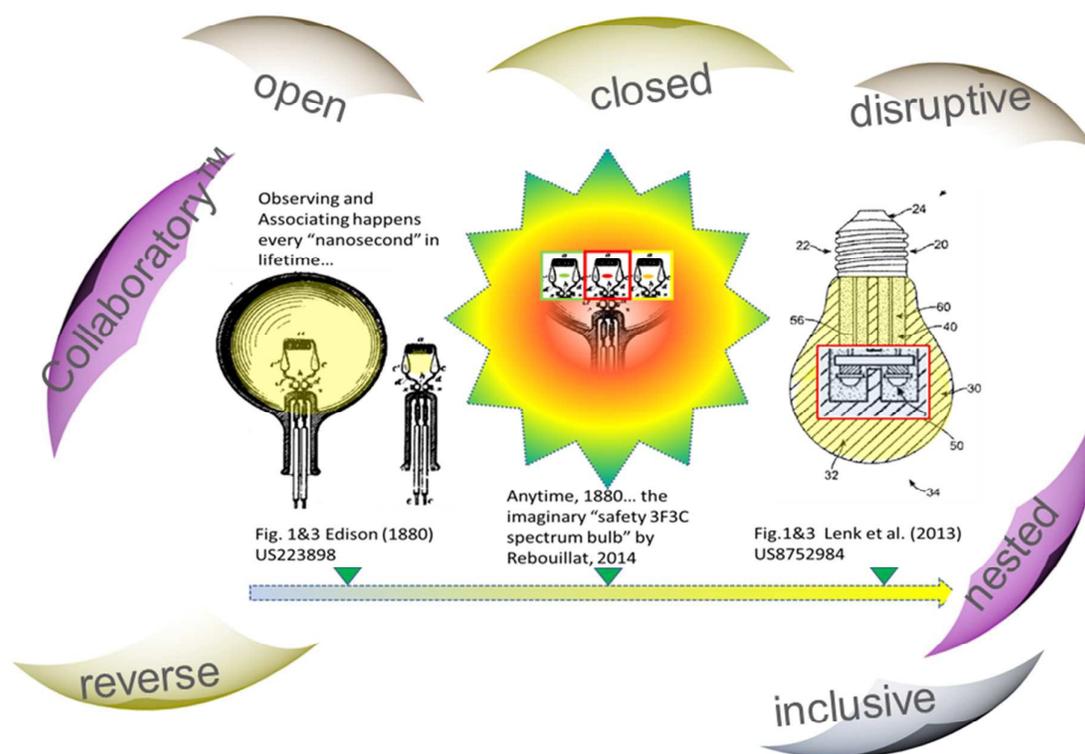


Illustration 2⁶: Combinatorial, associative or intersecting approaches, as illustrated above are part of the Disruptive Innovation Path. For example between the Edison's monoflament bulb (left) and the Lenk's multiLED light emitting systems (right), there are multiple possible concepts (Rebouillat's 3F3C spectrum bulb - middle) simply deriving from observation and visualization; i.e. from spontaneous 'visual analytics'.

Reviews are "immensely" inspiring and provocative in terms of innovation, although they tend to miss the adjacent technology analysis (ATA) that disruptive innovation mostly derives from. One objective of the current review was to promote innovation from a public literature analysis and some specific science and technology interpretations; this for educational illustrative purpose. Bigger Data analysis, ATA, and innovation principles have been largely covered by Rebouillat (in a series of 5 reviews such as Rebouillat⁶ and preceding or subsequent chapters) on innovation and neighboring aspects.

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