Accepted Manuscript

A review of magnetic separation of whey proteins and potential application to whey proteins recovery, isolation and utilization.

Paula Nicolás, María Luján Ferreira, Verónica Lassalle

PII:	S0260-8774(18)30456-4
DOI:	10.1016/j.jfoodeng.2018.10.021
Reference:	JFOE 9439
To appear in:	*Journal of Food Engineering*
Received Date:	13 April 2018
Accepted Date:	19 October 2018

Please cite this article as: Paula Nicolás, María Luján Ferreira, Verónica Lassalle, A review of magnetic separation of whey proteins and potential application to whey proteins recovery, isolation and utilization., *Journal of Food Engineering* (2018), doi: 10.1016/j.jfoodeng.2018.10.021

A review of magnetic separation of whey proteins and potential application to whey proteins recovery, isolation and utilization.

Paula Nicolás[a]*, María Luján Ferreira[b] and Verónica Lassalle[a]

[a]Instituto de Química del Sur (INQUISUR), Departamento de Química, Universidad Nacional del Sur, CONICET. Avda. Alem 1253, Bahía Blanca, Argentina.

[b]Planta Piloto de ingeniería Química (PLAPIQUI), Universidad Nacional del Sur, CONICET. Camino La Carrindanga km 7, Bahía Blanca, Argentina.

*Corresponding author: paula.nicolas@uns.edu.ar

Abstract

Cheese whey (CW) is a voluminous effluent generating environmental and economic impact in milk producing countries. Proteins from CW are useful for biotechnological applications. Available procedures to purify CW are complex and expensive. Magnetic nanotechnology emerges as an alternative to attain this goal. Magnetic nanoparticles are easily and economically prepared and can be formulated to selectively bind proteins in whey. Magnetic decantation allows simple and fast protein isolation by means of a magnet. The extra advantage is the possibility to regenerate and reuse the magnetic material in successive cycles. In this contribution, competitiveness of magnetic nanodevices is reviewed as a potential tool for the valorization and remediation of milk industry wastes. A critical analysis of recompiled data is included comparing magnetic nanomaterials with the current technologies intended for CW treatments. The purpose is to determine the most important factors that carry towards an effective recovery of proteins for diverse applications.

Keywords

Cheese Whey, Whey Protein, Magnetic Separation, Magnetic Nanoparticles, Protein Isolation, Protein Purification

Abbreviations' list

BSA……...	bovine serum albumin
CW……	cheese whey
Ig……...	immunoglobulin
LA…….	lactalbumin
LF…….	lactoferrin
LG…….	lactoglobulin
LPO……...	lactoperoxidase
LYS……...	Lysozyme
MAG…….	magnetite
MNPs……	magnetic nanoparticles

1.Introduction

Dairy products industry plays an important role in economic activity in many emerging and developed countries. For instance, in 2016 nearly 10 billion liters of milk were produced in Argentina ("Agroindustria.gob.ar," n.d.). Over 40 percent of the total milk production is destined to cheese elaboration (Paez et al., n.d.). From this process, cheese whey (CW) emerges as a voluminous effluent constituted by proteins, lactose, vitamins and mineral salts. Around 0.9 L of whey are generated per L of milk. A considerable fraction of lactose and milk proteins remain solubilized after precipitation by rennet or acids. CW is a large reserve of dietary proteins that remains outside the channels of human consumption when reutilization or revalorization procedures are not implemented. It is a substance of high nutritional value, but very polluting on contact with natural water and expensive to process. The limited management of CW brings a high indirect environmental problem. As a matter of fact, many researchers point out how polluting raw whey is due to its large biochemical and chemical oxygen demand impact. Efforts are made to clean the whey and enable its safe discarding (Ganju and Gogate, 2017; Remón et al., 2016; Yadav et al., 2015) and to include the proteins in cheese, as one of the viable options to decrease the impact (Masotti et al., 2017).

The current treatments are almost restricted to the use of membrane technologies, i.e.ultrafiltration, nanofiltration, microfiltration and inverse osmosis. From these procedures products as whey powder, demineralized whey powder, permeate powder, food grade lactose and WPC (whey protein concentrate) can be obtained in Argentina. Protein powders are considered to provide high quality protein that may be quickly available to enhance post-exercise recovery (Hogan et al., 2016). These alternatives are feasible for companies that are able to process large volumes of whey and have an important investment capacity. As a result, the valorisation of CW is a limiting issue for small and medium businesses. The conversion of the whey into a valuable and exportable product means a positive economic impact, the preservation of the environment (as it industrializes a residue of cheese processing) and a contribution to regional and national development. In this context, magnetic nanotechnology emerges as a valuable tool for the recovery of protein based on the magnetic separation principle. A special interest is focused on magnetic nanoparticles based on iron oxide magnetite/maghemite with a functionalizing coating able to bind specific molecules (Mehta, 2017).

MNPs have been thoroughly studied for biomolecules separation; however, their specific application to CW proteins isolation and purification has been barely reported. Combination or even substitution of filtration membrane technology by magnetic separation would be not only cost-effective but also would simplify whey process design. In this contribution, isolation of proteins from milk, whey or model systems is reviewed, focusing on magnetic supports, and more specifically in magnetic nanomaterials as materials to assess the recovery. The purpose is to gain insight into the feasibility of whey proteins separation and purification providing value to an extensive residue derived from one of the most important industries in Argentina. Besides the procedures proposed here, involving the magnetic nanomaterials may be useful in any milk-producing country and could be extrapolated to other milk-based food residues.

2. Generalities on cheese whey: Composition and available treatments

The products derived from CW are combination of diverse nutrients. Each bovine protein possesses *separately* interesting functionalities that require a high degree of purification. Lactoferrin, β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoperoxidase and bovine serum albumin (BSA) are all of interest. Lysozyme is also present in cheese whey although in extremely low concentrations (< 1 mg/L) (Vasavada and Cousin, 1993). Practically all publications dealing with the characterization of cheese whey include detailed information about physicochemical properties of each protein (such as molecular structure, weight or isoelectric point). Hence, it will not be repeated here because of the different focus proposed for

this manuscript (Kinsella and Whitehead, 1989; Yadav et al., 2015). A brief description of the most relevant proteins is presented as follows:

- Lactoferrin has shown anti-microbial and anti-viral activity (Embleton et al., 2013; Wakabayashi et al., 2014), anti-cancer properties (Lim et al., 2015; Song et al., 2017), tissue regeneration capacity (Shi et al., 2017) and other important functions (García-Montoya et al., 2012).
- Purified β-lactoglobulin can be used for the detection of cow milk protein intolerance (Aich et al., 2015). A concrete function of this milk protein is not yet found, however it is capable of binding to hydrophobic ligands like cholesterol and vitamin D2 (Hochwallner et al., 2014).
- α-Lactalbumin is appreciated as a source of peptides with numerous bioactivities such as anti-cancer, which may be utilized in the production of functional foods (Kamau et al., 2010). Bovine immunoglobulins improve symptoms, nutritional status, and various biomarkers associated with intestinal pathologies (Petschow et al., 2014).
- Lactoperoxidase is a natural enzyme of the mammals' host defense system carrying antimicrobial and antiviral properties (Madureira et al., 2007).
- BSA has been historically used as a protein concentration standard and *in vitro* biochemical assays.

The most efficient separation and purification techniques available for CW protein extraction are filtration membranes (Ganju and Gogate, 2017; Saxena et al., 2009) and chromatography (in column or membranes) (Chase and El-sayed, 2011). Variable pore sizes are then employed depending on the required selectivity. Membrane processes are volume-dependent separation methods, wherein the equipment capacity and cost of manufacture is proportional to the volume of solution processed and not to the mass of product. Filtration using membranes occurs by allowing molecules of certain size to pass through and excluding others.

Organic polymer membranes are the leading type for industrial applications. Nevertheless, they are prone to chemical or bio-fouling (Mikhaylin and Bazinet, 2016; Steinhauer et al., 2015), low fluxes, low mechanical strength and restricted chemical and thermal stability (Ishak et al., 2017). Ceramic membranes are considered the best candidates to replace polymeric membranes. Due to the high amount of flux, separation efficiency, long lifetime, and a continuous decrease in fabrication costs, research on ceramic membranes is increasing fast (Qiu et al., 2017).

Chromatographic separation of proteins consists in their selective adsorption on a functionalized solid (packed column or a membrane) and subsequent elution with a liquid phase. The interaction between the biomolecule and the stationary phase may present a diverse nature: hydrophobic (Santos et al., 2011), ionic (Li et al., 2017), or other specific affinity like dye binding (Urtasun et al., 2017) or transition-metal binding (Li et al., 2008).

Packed-column chromatography steps are effective and specific in the enrichment of bioactive peptides within downstream processes. On the other hand, they are time-consuming when applied using conventional laboratory methods. These processes are expensive, difficult to apply and, in some cases, may affect the secondary structure of peptides, resulting in the possible alteration or elimination of their bioactive characteristics. In view of this, industrial scale-up is not economically viable for the food industry (Dullius et al., 2018).

In opposition to column-filling materials, MNPs offer the alternative of solid-liquid extraction in batch mode. The adsorbents with a magnetic core do not need to be packed into cartridges, and the centrifugation steps may be substituted by the magnetic decantation mediated by an external magnetic field to achieve solid–liquid separation (X. Ding et al., 2015). Another disadvantage of standard column liquid chromatography procedures is related to the impossibility of to cope with samples containing particulate or suspended material –like raw cheese whey- so they are not suitable for work in early stages of the isolation/purification process. In this case magnetically modified two-phase systems have shown their usefulness (Safarik and Safarikova, 2004).

Table 1 summarizes the current trends in whey protein fractionation and the fundamentals of these techniques.

Table 1. Working principles of whey protein purification techniques (based on Chase and El-sayed, 2011).

Chromatography		
Ion exchange	*Affinity*	*Unspecific adsorption*
Net charge-based interactions -Anion exchange (Santos et al., 2012) -Cation exchange (Doultani et al., 2004) -Mixed ion exchange (Saufi and Fee, 2011)	Selective adsorption/ elution -Specific interactions between proteins and immobilized ligands like metals (Carvalho et al., 2014), dyes (Baieli et al., 2014), bio-affined molecules (Lai et al., 2013) and others.	-Hydrophobic interactions (Conrado et al., 2005), other non-specified

Filtration membranes	
Transversal or tangential flow. Size-based separation. Variable pore size membranes -Ultrafiltration (Chamberland et al., 2017) -Microfiltration (Hernández and Harte, 2009) -Nanofiltration (Cohen et al., 2017) -Reverse osmosis (Yorgun et al., 2008)	-Electroseparation -Electrodialysis: for the elimination of ions. Selective migration of ions through a membrane under an electric field (Mikhaylin et al., 2018) -Electroacidification: bipolar membranes that dissociate water molecules at their interfaces (Bazinet et al., 2004)

In practice, two or more techniques are combined in a deliberate order for whey treatment. Scheme 1 represents examples for obtaining WPI (whey protein isolate) and WPC (whey protein concentrate).

Scheme 1. Examples of WPC and WPI obtaining pathways.

As stated, these technologies demand a strong investment. Therefore, it is necessary to develop alternative separation techniques affordable to local and small-scale producers. MNPs, especially those based in magnetite (Fe_3O_4) have been used for immobilization of molecules including drugs (Hauser et al., 2015), enzymes (Netto et al., 2013), other proteins (Iype et al., 2017), genes (Vosen et al., 2016), antibodies (Gu et al., 2016) and even whole cells (Chen et al., 2016). MNPs have proven to be useful in the purification of proteins from their native medium (Gädke et al., 2017). Actually, MNPs can be considered as a particular type of stationary phase in liquid affinity chromatography. Simple batch systems are perfectly suitable for sample treatment in small or large scale. Applying the appropriate elution conditions, the immobilized species can be recovered in pure form, whereas the MNPs can be reused in several purification cycles (Zhou et al., 2018).

3. Magnetic nanomaterials useful for protein separation

The structure of MNPs, independently of their use, consists generally in an inorganic magnetic core (usually magnetite/maghemite) coated with a shell, of varied nature, i.e small molecules (organic or inorganic) or polymeric moieties, depending on the desired applications. Their preparation may be achieved through several methodologies. The simplest one is co-precipitation of magnetite from Fe^{2+} and Fe^{3+} salts by adding a base like NaOH in presence of a surfactant or functional molecule under nitrogen atmosphere and magnetic stirring (Figure 1). This particular technique makes MNPs a very low-cost material (Azcona et al., 2016; Nicolás et al., 2014, 2013). Commercial magnetite is also available in the market at an accessible price. For example 97 % purity magnetite nanopowder (Aldrich) costs 903 dollars/250 g. Materials like Poly(N-isopropylacrylamide) cost 782 dollars/10 g, and a carboxymethyl-functionalized cation exchanger is 1188 dollars/ 250 mL (Supelco). All this information is valid for Argentina in the Sigma-Aldrich website. Scanning the prices of the traditional beads it is clear that market value of iron oxide nanopowder is sensibly lower.

Figure 1. Reactor disposition for coprecipitation of MNPs.

Due to their nanosize (5-20 nm), MNPs have the particular property of superparamagnetism. This behavior allows to direct the position of the particles with an external magnetic field and additionally, no magnetization remains after removing such field (null remanence). Hydrodynamic diameter (often measured by dynamic light scattering) may be significantly larger than the one observed by electronic microscopy since the particles tend to aggregate. The aggregation of the MNPs in a liquid medium is highly dependent on their surface charge. Zeta potential (ζpot) is a good indicator of the stability of MNPs in suspension. Its sign and magnitude may be adjustable by controlling the pH of the medium if the particles contain protonated/deprotonated groups (Liu et al., 2018; Vega-Chacón et al., 2017).

The magnetic core is chemically modified in order to provide them with a selective affinity towards the target protein. Normally the mechanisms operating to bind to the proteins are the following:
-Mostly electrostatic, dependent on the surface charge of both the particle and the target molecule. These charges are tunable by adjusting the pH of contact medium.
-Mostly hydrophobic, interactions between hydrophobic moieties on the carrier with hydrophobic regions or domains on the protein. Thus, the pendant hydrophobic groups dominate the surface chemistry of the carriers used for hydrophobic adsorption.
-Mostly chemical, dependent on the affinity of functional groups in the coating with the exposed protein amino acid residues. Scheme 2 shows the main types of MNPs useful for protein adsorption.

Scheme 2. Structure of MNPs used for protein immobilization.

An in-depth description of MNPs applied to protein separation are presented in next section as a function of their surface chemistry.

3.1. Iron oxide@metal-metal oxide

Some proteins contain exposed amino acid residues capable of coordinating with transition metals. Histidine (His) acts as an electron donor group and is present in the surface of lactoferrin (Iyer et al., 1994), immunoglobulins (Al-Mashikhi et al., 1988), lactalbumin (Berliner+ and Kaptein, 1980), lactoglobulin (Olsen et al., 2015) or BSA (Alaiz and Gir6n, 1994) in variable amounts. The affinity of His for copper ions has been utilized in column chromatography for whey protein extraction with relative success. For example, lactoferrin and immunoglobulin were separated from cheese whey by Cu-affinity chromatography (Al-Mashikhi et al., 1988). Cu^{+2} ions were immobilized on 1,4-butanediol diglycidyl ether-iminodiacetic acid and Sepharose 6B, packed into a column. Immunoglobulin adsorbed strongly, unlike other proteins, and could be recover with 53 to 77 % purity. Better results were

obtained under the same chromatographic system for B-lactalbumin, which eluted after immunoglobulin. A purity of 90% and recovery of 80% was achieved, but it was necessary to reload the eluted protein on Cu-free matrix to clean it from leached metal (Blomkalns and Gomez, 1997). Cheese whey lactoferrin was directly captured with Cu^{2+} on polyacrylamide cryogel-iminodiacetic acid. This was possible after ultrafiltration of the whey to get rid of the lighter proteins. Lactoperoxidase was a possible contaminant but it passed straight through the Cu^{2+}-cryogel, while lactoferrin was retained and could be eluted with high purity (Carvalho et al., 2014). Artificially tagging proteins with this His or other amino acids is a common technique for adsorption enhancement (Wijekoon et al., 2016).

Regarding MNPs, transition metals have been attached onto diversely functionalized magnetic materials in order to potentiate the protein binding according to the previously mentioned antecedents. Cu^{+2} ions were immobilized through EDTA on MAG and then contacted with a 2.0 $mg.mL^{-1}$ bovine hemoglobin (BHb) solution, a His-rich protein. Adsorption was maximum at pH 8 (1200 mg protein.g of $carrier^{-1}$ in one hour) and no significant effect was observed by variation of ionic strength. Specificity towards BHb was evaluated in a mixture together with lysozyme (Lyz) and BSA, which possess less surface-exposed His residues. 89.8% of BHb was removed from the mixture, whereas only 19 % of BSA and 15,9 % of Lyz attached to the particles (C. Ding et al., 2015). His-tagged Green Fluorescent Protein (GFP) could also be recovered by the same material up to 120 mg.g $carrier^{-1}$ per hour (Fraga García et al., 2015).

Nickel is another His-binding metal. NiO-decorated MNPs were successfully applied to His-tagged proteins with MAG-SiO_2 or MAG-Al_2O_3 as starting materials (Li et al., 2015; Mirahmadi-Zare et al., 2016). Ni^{2+} cation was immobilized on maghemite-glicidilpropyl-trimethylsilane-N,N.carboxymethyl-lysine. Upon repeated contact with a cell lysate from which to extract a His-tagged protein, the loss of particles was very high after the fourth cycle, leading to a low adsorption efficiency in the fifth cycle (Gädke et al., 2017).

A comparison between the lysozyme binding ability on carboxymethyl-chitosan-coated MAG modified with Fe^{+3}, Zn^{+2} or Cu^{+2}, respectively, was reported. The higher efficiency was found employing Fe at pH 6 (232.56 mg/g vs 200 for Zn and 185 for Cu). Fe (III) ions have a strong affinity for oxygen containing functional groups such as carboxylic and phenolic oxygen, so the aspartic acid, glutamic acid and tyrosine residues which on the surface of the lysozyme molecules provides affinity-binding sites for Fe (III) ions through carboxylate and phenolic functional groups.

The elution strategy was, in principle, the increase of ionic strength using an aqueous solution of NaCl 0.2M. As it was not enough to achieve elution, imidazole was utilized as well It was found that using 0.2 M imidazole containing 0.2 M NaCl solution could recover more than 95% of the bound proteins (Sun et al., 2011a).

Unpublished own data confirms the results achieved by other authors regarding the synergic effect of Cu on MNPs structure. In our case, citric-acid-coated MNPs (CA-MAG) remove about 60 % of total whey proteins. Retreating the CA-MAG-processed whey with Cu^{2+}-CA-MAG this percentage raised to 80 %. SDS-PAGE analysis showed that CA-MAG adsorption system exhibited a larger affinity for positively charged lactoferrin and lactoperoxidase. The reason for choosing Cu over Fe is merely cost-effective, since copper sulphate is about 30 % cheaper than iron(II) sulphate (prices in Argentina).

The articles dealing with transition-metal modified MNPs show the modifier as a suitable one to assess an efficient whey protein extraction. It is worth mention that few studies have been found in open literature evaluating diverse metals on different MNPs. From the analysis of those, hardly any conclusions can be drawn on this topic. It is necessary to plan more systematic research devoted to evaluate the adsorption capacity and selectivity of transition metals on the same magnetic matrix. Selection of the organic phase as coating of MNPs should

ponder a series of conditions such as high chemical and mechanical resistance in whey environment, stable metal uptake and low cost.

3.2.Iron oxide@polymers

The coating of MNPs with polymeric moieties is one of the most widespread strategy to increase the versatility of the magnetic systems providing specific properties to attain the desired applications. In the field of protein separation/immobilization and/or isolation a wide gamma of polymers, natural and synthetic, have been employed to magnetic phase functionalization. In general terms, aminated and/or carboxylated polymers are the preferred because of their affinity with protein moieties.

One of the most employed polymers is chitosan, a versatile biopolymer extensively used in the biomedical and the industrial fields for its biocompatibility and non-cytotoxicity (Choi et al., 2016). Magnetic chitosan composites have been widely studied for several applications, including proteins capture. In previous own works we have reported the efficient adsorption of BSA onto Fe_3O_4-chitosan particles (Nicolás et al., 2013). Similar findings were earlier achieved by employing magnetite chitosan and magnetite cellulose composites to assess the immobilization of BSA and insulin by simple adsorption mechanism. Proteins and chitosan (CS) particles presented opposite surface charge at the selected pH (5.7, distilled water). Electrostatic interactions are then expected to govern the BSA and insulin (negatively charged) adsorption on CS based supports (positive). On the other hand, cellulose particles are also negatively charged, hence not electrostatic but mainly physical and hydrophobic interactions (plus other Van der Waals forces such as H-bonding) are expected to maintain the proteins linked to those supports (Lassalle et al., 2011)

Other possibilities include the use of a crosslinking molecule to form covalent bonds, such as carbodiimide (Sadeghi et al., 2016). In this case, most of the offered BSA was incorporated but no elution tests were performed. This is a complex situation since the creation of covalent bonds between the protein and the carrier would require a harsh chemical treatment to reverse the reaction and recover the target biomolecule, impairing its integrity.

Carboxymethyl chitosan provided an appropriate compatibility with lysozyme. The biopolymer, anchored on MAG-poly-ethylene glycol particles, reached a 256.4 mg/g loading capacity. Due to the small diameter, the adsorption equilibrium was reached within barely 20 min and fitted well with the Langmuir model (Sun et al., 2011b).

MAG coated with carboxymethyl cellulose was compared to chitosan-MAG in a peroxidase purification process. Both supports have a strong positive charge under the applied experimental conditions (pH=5), being even higher for chitosan-MAG. Nevertheless, carboxymethyl cellulose composite demonstrated to be the most efficient in terms of purification yield. The authors assigned this result to the larger surface area than the underivatized chitosan (Zengin Kurt et al., 2017).

Carboxyl -modified magnetic particles were prepared by polymerization of acrylamide with N, N´-methylenebisacrylamide in presence of magnetite particles under sonication. The resulting polymer exposed $–COO^-$ groups. Combinations of magnetic fishing and aqueous two-phase extraction (ATPE) were tested. The liquid extraction medium contained water, PEG4000 and ammonium sulphate forming two phases after centrifugation. A mixture of BSA, lysozyme, cytochrome C and myoglobin (Mb) were treated in two different ways, exposing the proteins to magnetic fishing followed by extraction and vice versa (Gai et al., 2011). When magnetic particles were added to the proteins mixture, Cyt C and Lyz were adsorbed completely and BSA and Mb remained in solution. Then, adding the mixture of PEG and sulphate into the remaining solution, the top and bottom phase were formed. BSA and Mb were fully allocated in the bottom phase, and their concentrations were significantly higher than the original solution. It indicated that Cyt C and Lyz could be separated by magnetic particles adsorption directly, and the remained BSA and Mb were enriched by ATPE.

Superparamagnetic microspheres (3.2 μm) were obtained by coating magnetite with poly(glycidyl methacrylate) and further functionalized with ethylenediamine, exposing $–NH_3^+$ groups. Upon contact with a pure BSA solution at pH 7-7.5 over 150 mg of protein adsorbed per gram of carrier. More than 90 % of the attached BSA could be recovered by elution with 0.6 M NaCl (Liu et al., 2016).

In another extensive research, 12 different magnetic ion-exchangers were created by linking polyethyleneimine (PEI), trimethylamine or diethylamine ethyl chloride on MAG particles. The PEI support presented high loading capacity towards BSA (337 mg/g) in a model system, so it was selected for crude bovine whey treatment. The process consisted of using first a magnetic cation-exchanger (MAG-poly-glutaraldehyde-epichlorohydrin-sulphite) to adsorb basic protein, and the supernatant was then contacted with the PEI anion-exchanger. Desorption selectivity was subsequently studied by sequentially increasing the concentration of NaCl in the elution buffer. In the initial cation-exchange step quantitative removal of lactoferrin and lactoperoxidase was achieved with some simultaneous binding of immunoglobulins. The immunoglobulins were separated from the other two proteins by desorbing them with a low concentration of NaCl (≤0.4 M), whereas lactoferrin and lactoperoxidase were co-eluted in significantly purer form, e.g. lactoperoxidase was purified 28-fold over the starting material, when the NaCl concentration was increased to 0.4–1M. The anion-exchanger adsorbed β-lactoglobulin selectively allowing separation from the remaining protein (Heebøll-Nielsen et al., 2004).

Numerous studies were carried out in the application of MNPs coated polymers to assess protein purification and immobilization. However, the specific separation and isolation of cheese whey proteins is more limited. Although cationic polymers seem to be more suitable, certainly any clear trend may be visualized because of the limited information in available literature.

3.3.Iron oxide@bioaffinity ligand

Diverse biomolecular moieties are currently employed as extraction sorbents. Such biomolecules may be linked to the magnetic surface by either covalent coupling and/or non-covalent interactions. These bioaffinity sorbents can be prepared using, for example, antibodies or aptamers, and may operate by a retention mechanism during the extraction based on molecular recognition. Other types of biological molecules present high affinity for a determined part of a molecule. For instance, Concanavalin A, a kind of protein with specificity to sugars, was immobilized on MNPs through carbodiimide. Since lactoferrin is a glycoprotein (contains oligosaccharide chains covalently attached to the polypeptide), the particles bonded selectively to it in presence of BSA: 58.12 mg lactoferrin/g vs. 0.8 of albumin. Elution was performed with a Tris buffer containing D-methylglucoside and NaCl (Lai et al., 2013).

For non-antibody proteins, often no natural partner is known with suitable properties for use as an affinity reagent (Béhar et al., 2016). The structure-based design of chemicals and peptidic mimetics is then an alternative to obtain artificial ligands for biological targets. Specifically, proteins artificially tailored for this purpose are called *affitins*. Few manuscripts have been published on this topic since it's a relatively new concept. Applications include purification of antibodies and non-immunoglobulin proteins (Béhar et al., 2016) and purification and detection of conformational changes of other proteins (Krehenbrink et al., 2008). Efforts are being oriented to the design of stable and functional affitins (Béhar et al., 2014; Miranda et al., 2011).

Anti-lysozyme and anti-IgG affitins were immobilized onto magnetic particles to assess their potential for protein purification by magnetic fishing. The optimal lysozyme and human IgG binding conditions yielded 58 mg lysozyme/g support and 165 mg IgG/g support, respectively. The recovery of proteins was possible in high yield (≥95%) and with high purity, namely ≥95% and 81%, when recovering lysozyme from *Escherichia coli* supernatant and IgG from human plasma, respectively (Fernandes et al., 2016).

The described entities may preferentially bind to protein A/G, lectins, enzymes, as well as molecular receptors sensitive to molecular interactions with certain groups of molecules. Hence, they can also be used as selective tools in extraction procedures (Pichon et al., 2012).

3.4. Miscellaneous magnetic nanomaterials

The classification regarding the nature of the magnetic phase modifier correlates to the compounds most extensively used in open literature for these purposes. However other modifiers/functionalizers less commonly reported are summarized in this section.

Table 2 lists different magnetic nanomaterials with uncommon or unspecific modifications that have been tested for protein recovery.

Table 2. Uncommon magnetic nanomaterials applied to protein extraction

Material	Target proteins	Medium	Contact conditions	Results	Ref.
MAG-SiO_2-$HS_2C_3H_6$-Si-$(EtO)_3$	BSA Lysozyme	Artificial solutions	10 mg particles + 1 mL protein (5µM) in buffer (100 mM, pH 4.65, 7.5, and 11)	Both proteins adsorb. Amount of each pH-dependent	(Lee et al., 2012)
MAG-Au-organic acid	Lysozyme	Egg white	2 mg of Fe_3O_4/Au-acid NPs + 1 mL of diluted egg white, pH=10. 10 min at room T with shaking	346 µg protein /mg NPs. Total elution and reusability	(Zhu et al., 2016)
MAG-SiO_2-3Cl-triazine	BSA	Artificial solution	4 mg NPs +1 mL BSA 0.5 mg/mL room T 1-8 h	130 mg BSA/g NPs when 1 mg BSA is offered to 4 mg NPs	(Bordbar et al., 2014)

According to the contribution of Lee et al. (2012), low-molecular-weight proteins (i.e., LYZ) are earlier retained at the surface and are later displaced by relatively high-molecular-weight ones. Besides this partial selectivity, in these particular systems, proteins were not completely separated from particles even using 3 M NaCl. Treatment with 0.1 M glycine solution (pH 2.3) was necessary to partially desorb them. Thus, -SH modified materials should be ruled out as efficient whey proteins adsorbents.

MAG-gold NPs demonstrated suitable adsorption and desorption capacity for lysozyme (Zhu et al., 2016). However Lyz concentration in whey is markedly lower than in egg white (< 1mg/L vs. 2.2 to 4.5 mg/mL (Vidal et al., 2005)), hence this behavior may not be straightforwardly extrapolated to the CW residue. MAG-Au-organic acids particles are another alternative deserving interest and not yet explored in real whey samples, for which its selectivity towards other proteins is unknown. Nevertheless, the high fabrication costs associated to gold nanomaterials is an important limitation when intending their application in large-scale whey processing.

In the case of MAG-triazine particles (Bordbar et al., 2014), immobilization occurs through a strong covalent bond. Recovery of pure protein would then imply a harsh chemical treatment not suitable concerning the retention of whey proteins biological functions.

4. Elution methodologies and recovery results

In general terms, the elution of proteins from MNPs or other adsorbent material consists of displacing the biomolecule by other chemical species with a stronger affinity for the solid support. The requirements of this process are devoted to detach the protein, as well as to maintain its integrity (structure, biological function) and purity for further eventual applications.

Different strategies are implemented to assess this goal. The most widely employed are ionic strength increase, pH modification and ligand exchange with amino acids fractions, among others less common. Sometimes, combinations of these methods are needed for a proper and efficient elution. It is worth mention that the adopted strategy would completely depend on the mechanism of adsorbent-protein retention.

Table 3 compares the efficiency of different elution methods for a particular protein from several magnetic and not magnetic materials.

Table 3. Elution methods of whey proteins from solid supports

Protein	Elution method	Efficiency of elution % *	Adsorbent Support(s)	Reference
Lactoferrin	1 M NaCl in 20 mM sodium phosphate, pH 6	61,9	Both Lewatit MP500 anionic resin and Sepharose cationic resin into ethylene vinyl alcohol base polymer	(Saufi and Fee, 2011)
β-Lactoglobulin		76.5		
A-Lactoalbumin		14.9		
BSA		0		
Immunoglobulin G		72,6		
Lactoferrin	0.5 M NaCl in Phosphate buffer pH 6	90	Superparamagnetic polyglycidyl methacrylate particles coupled with heparin	(Chen et al., 2007)
	20mM acetic buffer, pH 4.0	65		
Lactoperoxidase	2 M NaCl in 20 mM acetate buffer pH 5.0	92	Sepharose 6B-reactive red 4 dye	(Urtasun et al., 2017)
Lactoperoxidase	Stepwise elution: 0.075 M NaCl, 0.15 M and 1 M in 10 mM phosphate buffer pH 5.8	92	Cation exchange composite cryogel embedded with cellulose beads	(Pan et al., 2015)
β-Lactoglobulin	NaF 0.6 M in 10 mM phosphate buffer pH 6.25	100	Ceramic hydroxyapatite	(Schlatterer et al., 2004)
Lactoferrin	2 M NaCl in 25% ethylene glycol pH 7	99	Composite polymer membrane with Red HE-3B dye	(Wolman et al., 2007)
		80	Agarose beads- Red HE-3B dye	

Lactoferrin	Temperature drop from 40 to 4 °C	76	Resin based on *N*-iso-propylacrylamide	(Maharjan et al., 2016)
BSA	50 mMTris/HCl, 1M NaCl, pH 8.0 50 mM sodium citrate, pH 3.5 (2 cycles each)	88 54	MAG-polyethyleneimine anion exchanger	(Heebøll-Nielsen et al., 2004)
Lactoferrin and Immunoglobulin G	0.05 M Tris-acetic acid containing 0.5 M NaCI, pH gradient 8.0 to 2.8	Not reported	Sepharose 6B-organic ligands-Cu^{2+}	(Al-Mashikhi et al., 1988)

*Percentage of eluted protein considering only the adsorbed amount, not the offered initial one.

Proteins are complex amphoteric molecules containing both negative and positive charges, their net charge may be controlled by conveniently adjusting the pH of surrounding media. This means that proteins can be separated on both anion and cation exchangers by selecting the suitable pH of the used buffer. To achieve good separation the buffer pH should be at least one pH unit above or below the isoelectric point of the protein (Abd El-Salam and El-Shibiny, 2017). This procedure results efficient since the protein is linked to the adsorbent by electrostatic interactions involving protonated/deprotonated groups. From Table 3 it is evident that this is the main type of protein-MNP interaction.

6. Comparison between different proteins adsorbents employed in different media

In this section, an integral comparison involving from retention to elution procedure is presented considering available procedures for whey proteins isolation.

Table 4. Practical parameters of interest of different whey protein recovery methods

Separation method	Final product	Elution method	Recovery results	Ref.
UF membrane (polyethersulfone MW cut-off 10 kDa) + 4 cycles DF	Whey protein concentrate (mixture of all proteins)	Not applicable	72% protein product. No loss in permeate	(Baldasso et al., 2011)
Sepharose-iminodiacetate-Cu^{+2}	Mostly A-LA some B-LG	Acetate buffer* in steps: pH 5.5, 5.0, 4.5, 3.8	80 % yield, 90% purity	(Blomkalns and Gomez, 1997)
Phenyl- modified hydrophobic resin	Mainly α-LA, some B-LG and casein	50 mM Tris 1.5 mM $CaCl_2$ pH 7.5	79 % purity	(Conrado et al., 2005)
$(NH_4)_2SO_4$ precip. + Anion exchanger (Diethylamino-ethyl cellulose)	α-LA, BSA and B-Ig	NaCl 0.1-0.4 M in 0.05 M Tris	Comparable to commercial standards (qualitatively	(Neyestani et al., 2003)

+		pH 6.5 (removes Ig)	estimated by PAGE)	
Gel filtration (Sephadex G-50)		20 mM PO_4^{-3} buffer, pH 8.6 (separates BSA from LA)		
MAG-GLUT-epichlorohydrin-SO_3^-	Mostly LPO and some LF	1 M NaCl in 10 mM PO_4^{-3} buffer pH 7	>90% LPO capture, purified 36-fold and concentrated 4.7-fold.	(Heebøll-Nielsen et al., 2004)
Sepharose 6B-Red 4 dye	LPO, minimum LF	2 M NaCl in 20 mM acetate buffer pH 5.0	86.5 % yield, >80 % purity	(Urtasun et al., 2017)
Cation exchange composite cryogel embedded with cellulose beads	LPO	Stepwise: NaCl 0.075 M, 0.15 M and 1 M in 10 mM phosphate buffer pH 5.8	>98% purity	(Pan et al., 2015)
MAG-carbodiimide-Concanavalin A	LF	0.02M Tris buffer with 0.2 M α-D-methylglucoside and 0.5 M NaCl pH 7.4	Highly selective in presence of BSA	(Lai et al., 2013)
MAG-Anti-lysozyme affitin	Lysozyme (from cell lysate)	100 mM glycine-HCl buffer, 0.15 M NaCl, pH 2.5	≥95% yield ≥95% purity	(Fernandes et al., 2016)

412 UF=ultrafiltration DF=diafiltration

413 *not clear if buffer contains NaCl

Surprisingly, no information about reuse of the adsorbents is reported in the cited publications, although all of them have that potential.

7. Conclusions and prospects

Extracting whey proteins by means of magnetic fishing represents an attractive alternative among the traditional ones because of several advantages such as:

-Simplification of reactor design
-Facile and time-saving magnetic decantation (indistinctly of the volume of whey) vs. long loading times related to large volume of whey passing through narrow columns or membranes.
-Low-cost starting materials and simple preparation methods of MNPs
-Chemical and mechanic resistance enabling potential long-term reusability

A great volume and more exhaustive, experimental and theoretical, studies are currently required in order to assess the real potential of this novel technology to provide added value to a large residue such as the cheese whey. The purpose of this review is to raise awareness on the topic establishing the starting point for the exploration of favorable experimental conditions and improvements related to use and optimization of performance in protein separation. The real interest is in revalorizing the isolated proteins from wastes by low cost, simple and green-made nanotechnological materials.

The take-home message of this review is that, currently, the most effective techniques for whey protein recovery include the combination of molecular-size-based membrane filtration and affinity chromatography. Membranes involve thorough maintenance to avoid fouling. Specific columns are not suitable for industrial-scale volumes of whey, not to mention their elevated price.

MNPs arise as a cheap, versatile and reusable material for the rational design of whey protein adsorption systems. Besides, their production and final disposition are environmentally friendly.

There are still vacancy areas: there is no single procedure able to treat whey and isolate proteins. The main necessity is to develop ***low-cost*** magnetic adsorption systems for whey proteins recovery optimizing elution conditions to maximize selectivity and yield.

11. Acknowledgements

The authors wish to thank ANPCyT (National Agency for Scientific and Technological Promotion) and CONICET (Nacional Council of Scientific and Technic Investigations) for their financial support.

12. References

Abd El-Salam, M.H., El-Shibiny, S., 2017. Separation of Bioactive Whey Proteins and Peptides, in: Alexandru Mihai Grumezescu, Alina Maria Holban (Eds.), Ingredients Extraction by Physicochemical Methods in Food. Elsevier, pp. 463–494.

Agroindustria.gob.ar [WWW Document], n.d. URL http://www.agroindustria.gob.ar//sitio/areas/ss_lecheria/estadisticas/_01_primaria/_archivos/PPV018.php

Aich, R., Batabyal, S., Joardar, S.N., 2015. Isolation and purification of beta-lactoglobulin from cow milk. Vet. world 8, 621–4.

Al-Mashikhi, S.A., Li-Chan, E., Nakai, S., 1988. Separation of Immunoglobulins and Lactoferrin from Cheese Whey by Chelating Chromatography. J. Dairy Sci. 71, 1747–1755.

Alaiz, M., Gir6n, J., 1994. Modification of Histidine Residues in Bovine Serum Albumin by Reaction with (E)-2=0ctenal. J. Agric. Food Chem 42, 2094–2098.

Azcona, P., Zysler, R., Lassalle, V., 2016. Simple and novel strategies to achieve shape and size control of magnetite nanoparticles intended for biomedical applications. Colloids Surfaces A Physicochem. Eng. Asp. 504, 320–330.

463 Baieli, M.F., Urtasun, N., Miranda, M.V., Cascone, O., Wolman, F.J., 2014. Isolation of
464 lactoferrin from whey by dye-affinity chromatography with Yellow HE-4R attached to
465 chitosan mini-spheres. Int. Dairy J. 39, 53–59.

466 Baldasso, C., Barros, T.C., Tessaro, I.C., 2011. Concentration and purification of whey proteins
467 by ultrafiltration. Desalination 278, 381–386.

468 Bazinet, L., Ippersiel, D., Mahdavi, B., 2004. Fractionation of whey proteins by bipolar
469 membrane electroacidification. Innov. Food Sci. Emerg. Technol. 5, 17–25.

470 Béhar, G., Pacheco, S., Maillasson, M., Mouratou, B., Pecorari, F., 2014. Switching an anti-IgG
471 binding site between archaeal extremophilic proteins results in Affitins with enhanced pH
472 stability. J. Biotechnol. 192, 123–129.

473 Béhar, G., Renodon-Cornière, A., Mouratou, B., Pecorari, F., 2016. Affitins as robust tailored
474 reagents for affinity chromatography purification of antibodies and non-immunoglobulin
475 proteins. J. Chromatogr. A 1441, 44–51.

476 Berliner+, L.J., Kaptein, R., 1980. Laser Photo-CIDNP Detection of Surface Aromatic Residues
477 in Dissociating Bovine a-Lactalbumin at Submillimolar Concentrations* 255, 3261–9262.

478 Blomkalns, A.L., Gomez, M.R., 1997. Purification of Bovine α-Lactalbumin by Immobilized
479 Metal Ion Affinity Chromatography. Prep. Biochem. Biotechnol. 27, 219–226.

480 Bordbar, A.K., Rastegari, A.A., Amiri, R., Ranjbakhsh, E., Abbasi, M., Khosropour, A.R.,
481 2014. Characterization of modified magnetite nanoparticles for albumin immobilization.
482 Biotechnol. Res. Int. 2014, 1–6.

483 Carvalho, B.M.A., Carvalho, L.M., Silva, W.F., Minim, L.A., Soares, A.M., Carvalho, G.G.P.,
484 Da Silva, S.L., 2014. Direct capture of lactoferrin from cheese whey on supermacroporous
485 column of polyacrylamide cryogel with copper ions. Food Chem. 154, 308–314.

486 Chamberland, J., Beaulieu-Carbonneau, G., Lessard, M.-H., Labrie, S., Bazinet, L., Doyen, A.,
487 Pouliot, Y., 2017. Effect of membrane material chemistry and properties on biofouling
488 susceptibility during milk and cheese whey ultrafiltration. J. Memb. Sci. 542, 208–216.

489 Chase, H.A., El-sayed, M.M.H., 2011. Trends in whey protein fractionation. Biotechnol. Lett.
490 33, 1501–1511.

491 Chen, G., Liu, J., Qi, Y., Yao, J., Yan, B., 2016. Biodiesel production using magnetic whole-cell
492 biocatalysts by immobilization of Pseudomonas mendocina on Fe3O4-chitosan
493 microspheres. Biochem. Eng. J. 113, 86–92.

494 Chen, L., Guo, C., Guan, Y., Liu, H., 2007. Isolation of lactoferrin from acid whey by magnetic
495 affinity separation. Sep. Purif. Technol. 56, 168–174.

496 Choi, C., Nam, J.-P., Nah, J.-W., 2016. Application of chitosan and chitosan derivatives as
497 biomaterials. J. Ind. Eng. Chem. 33, 1–10.

498 Cohen, J.L., Barile, D., Liu, Y., de Moura Bell, J.M.L.N., 2017. Role of pH in the recovery of
499 bovine milk oligosaccharides from colostrum whey permeate by nanofiltration. Int. Dairy
500 J. 66, 68–75.

501 Conrado, L.S., Veredas, V., Nóbrega, E.S., Santana, C.C., 2005. Concentration of α-
502 Lactalbumin from cow milk whey through expanded bed adsorption using a hydrophobic
503 resin. Brazilian J. Chem. Eng. 22, 501–509.

504 Ding, C., Ma, X., Yao, X., Jia, L., 2015. Facile synthesis of copper(II)-decorated magnetic
505 particles for selective removal of hemoglobin from blood samples. J. Chromatogr. A 1424,
506 18–26.

507 Ding, X., Wang, Y., Wang, Y., Pan, Q., Chen, J., Huang, Y., & Xu, K., 2015. Preparation of
508 magnetic chitosan and graphene oxide-functional guanidinium ionic liquid composite for
509 the solid-phase extraction of protein. Anal. Chim. Acta 861, 36–46.

510 Doultani, S., Turhan, K.N., Etzel, M.R., 2004. Fractionation of proteins from whey using cation
511 exchange chromatography. Process Biochem. 39, 1737–1743.

512 Dullius, A., Goettert, M.I., de Souza, C.F.V., 2018. Whey protein hydrolysates as a source of
513 bioactive peptides for functional foods – Biotechnological facilitation of industrial scale-
514 up. J. Funct. Foods 42, 58–74.

515 Embleton, N.D., Berrington, J.E., McGuire, W., Stewart, C.J., Cummings, S.P., 2013.
516 Lactoferrin: Antimicrobial activity and therapeutic potential. Semin. Fetal Neonatal Med.
517 18, 143–149.

518 Fernandes, C.S.M., dos Santos, R., Ottengy, S., Viecinski, A.C., Béhar, G., Mouratou, B.,
519 Pecorari, F., Roque, A.C.A., 2016. Affitins for protein purification by affinity magnetic
520 fishing. J. Chromatogr. A 1457, 50–58.

521 Fraga García, P., Brammen, M., Wolf, M., Reinlein, S., Freiherr von Roman, M., Berensmeier,
522 S., 2015. High-gradient magnetic separation for technical scale protein recovery using low
523 cost magnetic nanoparticles. Sep. Purif. Technol. 150, 29–36.

524 Gädke, J., Kleinfeldt, L., Schubert, C., Rohde, M., Biedendieck, R., Garnweitner, G., Krull, R.,
525 2017. In situ affinity purification of his-tagged protein A from Bacillus megaterium
526 cultivation using recyclable superparamagnetic iron oxide nanoparticles. J. Biotechnol.
527 242, 55–63.

528 Gai, Q., Qu, F., Zhang, T., Zhang, Y., 2011. Integration of carboxyl modified magnetic particles
529 and aqueous two-phase extraction for selective separation of proteins. Talanta 85, 304–
530 309.

531 Ganju, S., Gogate, P.R., 2017. A review on approaches for efficient recovery of whey proteins
532 from dairy industry effluents. J. Food Eng. 215, 84–96.

533 García-Montoya, I.A., Cendón, T.S., Arévalo-Gallegos, S., Rascón-Cruz, Q., 2012. Lactoferrin
534 a multiple bioactive protein: An overview. Biochim. Biophys. Acta - Gen. Subj. 1820,
535 226–236.

536 Gu, J.-L., Tong, H.-F., Lin, D.-Q., 2016. Evaluation of magnetic particles modified with a
537 hydrophobic charge-induction ligand for antibody capture. J. Chromatogr. A 1460, 61–67.

538 Hauser, A.K., Wydra, R.J., Stocke, N.A., Anderson, K.W., Hilt, J.Z., 2015. Magnetic
539 nanoparticles and nanocomposites for remote controlled therapies. J. Control. Release 219,
540 76–94.

541 Heebøll-Nielsen, A., Justesen, S.F.L., Thomas, O.R.T., 2004. Fractionation of whey proteins
542 with high-capacity superparamagnetic ion-exchangers. J. Biotechnol. 113, 247–262.

543 Heebøll-Nielsen, A., Justesen, S.F.L., Hobley, T.J., Thomas, O.R.T., 2004. Superparamagnetic
544 Cation–Exchange Adsorbents for Bioproduct Recovery from Crude Process Liquors by
545 High-Gradient Magnetic Fishing. Sep. Sci. Technol. 39, 2891–2914.

546 Hernández, A., Harte, F.M., 2009. Isolation of caseins from whey proteins by microfiltration
547 modifying the mineral balance in skim milk. J. Dairy Sci. 92, 5357–5362.

548 Hochwallner, H., Schulmeister, U., Swoboda, I., Spitzauer, S., Valenta, R., 2014. Cow's milk
549 allergy: from allergens to new forms of diagnosis, therapy and prevention. Methods 66,
550 22–33.

551 Hogan, S.A., O'Loughlin, I.B., Kelly, P.M., 2016. Soft matter characterisation of whey protein

552 powder systems. Int. Dairy J. 52, 1–9.

553 Ishak, N.F., Hashim, N.A., Othman, M.H.D., Monash, P., Zuki, F.M., 2017. Recent progress in
554 the hydrophilic modification of alumina membranes for protein separation and
555 purification. Ceram. Int. 43, 915–925.

556 Iyer, S., Yip, T.T., Hutchens, T.W., Lonnerdal, B., 1994. Lactoferrin-receptor interaction. Effect
557 of surface exposed histidine residues. Adv. Exp. Med. Biol. 357, 245–52.

558 Iype, T., Thomas, J., Mohan, S., Johnson, K.K., George, L.E., Ambattu, L.A., Bhati, A.,
559 Ailsworth, K., Menon, B., Rayabandla, S.M., Jesudasan, R.A., Santhosh, S., Ramchand,
560 C.N., 2017. A novel method for immobilization of proteins via entrapment of magnetic
561 nanoparticles through epoxy cross-linking. Anal. Biochem. 519, 42–50.

562 Kamau, S.M., Cheison, S.C., Chen, W., Liu, X.-M., Lu, R.-R., 2010. Alpha-Lactalbumin: Its
563 Production Technologies and Bioactive Peptides. Compr. Rev. Food Sci. Food Saf. 9,
564 197–212.

565 Kinsella, J.E., Whitehead, D.M., 1989. Proteins in Whey: Chemical, Physical, and Functional
566 Properties. Adv. Food Nutr. Res. 33, 343–438.

567 Krehenbrink, M., Chami, M., Guilvout, I., Alzari, P.M., Pécorari, F., Pugsley, A.P., 2008.
568 Artificial Binding Proteins (Affitins) as Probes for Conformational Changes in Secretin
569 PulD. J. Mol. Biol. 383, 1058–1068.

570 Lai, B.H., Chang, C.H., Yeh, C.C., Chen, D.H., 2013. Direct binding of Concanvalin A onto
571 iron oxide nanoparticles for fast magnetic selective separation of lactoferrin. Sep. Purif.
572 Technol. 108, 83–88.

573 Lassalle, V.L., Zysler, R.D., Ferreira, M.L., 2011. Novel and facile synthesis of magnetic
574 composites by a modified co-precipitation method. Mater. Chem. Phys. 130, 624–634.

575 Lee, S.Y., Ahn, C.Y., Lee, J., Lee, J.H., Chang, J.H., 2012. Rapid and selective separation for
576 mixed proteins with thiol functionalized magnetic nanoparticles. Nanoscale Res. Lett. 7,
577 279.

578 Li, M., Li, Y., Yu, L., Sun, Y., 2017. Characterization of poly(allylamine) as a polymeric ligand
579 for ion-exchange protein chromatography. J. Chromatogr. A 1486, 103–109.

580 Li, X., Zhao, W., Gu, J., Li, Y., Li, L., Niu, D., Shi, J., 2015. Facile synthesis of magnetic core-
581 mesoporous shell structured sub-microspheres decorated with NiO nanoparticles for
582 magnetic recyclable separation of proteins. Microporous Mesoporous Mater. 207, 142–
583 148.

584 Li, Y., Chung, T.-S., Chan, S.Y., 2008. High-affinity sulfonated materials with transition metal
585 counterions for enhanced protein separation in dual-layer hollow fiber membrane
586 chromatography. J. Chromatogr. A 1187, 285–288.

587 Lim, L.Y., Koh, P.Y., Somani, S., Al Robaian, M., Karim, R., Yean, Y.L., Mitchell, J., Tate,
588 R.J., Edrada-Ebel, R., Blatchford, D.R., Mullin, M., Dufès, C., 2015. Tumor regression
589 following intravenous administration of lactoferrin- and lactoferricin-bearing dendriplexes.
590 Nanomedicine Nanotechnology, Biol. Med. 11, 1445–1454.

591 Liu, J., Dai, C., Hu, Y., 2018. Aqueous aggregation behavior of citric acid coated magnetite
592 nanoparticles: Effects of pH, cations, anions, and humic acid. Environ. Res. 161, 49–60.

593 Liu, Z., Yang, L., Dong, T., Li, W., Sun, X., Zhu, M., Duan, Z., Liu, Q., Liu, H., 2016. Gas-
594 assisted magnetic separation for the purification of proteins in batch systems. Particuology
595 24, 170–176.

596 Madureira, A.R., Pereira, C.I., Gomes, A.M.P., Pintado, M.E., Xavier Malcata, F., 2007. Bovine

whey proteins – Overview on their main biological properties. Food Res. Int. 40, 1197–1211.

Maharjan, P., Campi, E.M., De Silva, K., Woonton, B.W., Jackson, W.R., Hearn, M.T.W., 2016. Studies on the application of temperature-responsive ion exchange polymers with whey proteins. J. Chromatogr. A 1438, 113–122.

Masotti, F., Cattaneo, S., Stuknytė, M., De Noni, I., 2017. Technological tools to include whey proteins in cheese: Current status and perspectives. Trends Food Sci. Technol. 64, 102–114.

Mehta, R.V., 2017. Synthesis of magnetic nanoparticles and their dispersions with special reference to applications in biomedicine and biotechnology. Mater. Sci. Eng. C 79, 901–916.

Mikhaylin, S., Bazinet, L., 2016. Fouling on ion-exchange membranes: Classification, characterization and strategies of prevention and control. Adv. Colloid Interface Sci. 229, 34–56.

Mikhaylin, S., Patouillard, L., Margni, M., Bazinet, L., 2018. Milk protein production by a more environmentally sustainable process: bipolar membrane electrodialysis coupled with ultrafiltration. Green Chem. 20, 449–456.

Mirahmadi-Zare, S.Z., Allafchian, A., Aboutalebi, F., Shojaei, P., Khazaie, Y., Dormiani, K., Lachinani, L., Nasr-Esfahan, M.H., 2016. Super magnetic nanoparticles NiFe2O4, coated with aluminum-nickel oxide sol-gel lattices to safe, sensitive and selective purification of his-tagged proteins. Protein Expr. Purif. 121, 52–60.

Miranda, F.F., Brient-Litzler, E., Zidane, N., Pecorari, F., Bedouelle, H., 2011. Reagentless fluorescent biosensors from artificial families of antigen binding proteins. Biosens. Bioelectron. 26, 4184–4190.

Netto, C.G.C.M., Toma, H.E., Andrade, L.H., 2013. Superparamagnetic nanoparticles as versatile carriers and supporting materials for enzymes. J. Mol. Catal. B Enzym. 85–86, 71–92.

Neyestani, T.R., Djalali, M., Pezeshki, M., 2003. Isolation of α-lactalbumin, β-lactoglobulin, and bovine serum albumin from cow's milk using gel filtration and anion-exchange chromatography including evaluation of their antigenicity. Protein Expr. Purif. 29, 202–208.

Nicolás, P., Lassalle, V., Ferreira, M.L., 2014. Development of a magnetic biocatalyst useful for the synthesis of ethyloleate. Bioprocess Biosyst. Eng. 37, 585–591.

Nicolás, P., Saleta, M., Troiani, H., Zysler, R., Lassalle, V., Ferreira, M.L., 2013. Preparation of iron oxide nanoparticles stabilized with biomolecules: Experimental and mechanistic issues. Acta Biomater. 9, 4754–4762.

Olsen, K., Jespersen, B.B., Orlien, V., 2015. Changes of pH in β-Lactoglobulin and β-Casein Solutions during High Pressure Treatment. J. Spectrosc. 2015, 1–6.

Paez, J.C., Roxana, T., Schmidt, M.B., Pirola, E., n.d. Características generales sobre el uso del suero de queso en la Provincia de Santa Fe.

Pan, M., Shen, S., Chen, L., Dai, B., Xu, L., Yun, J., Yao, K., Lin, D.-Q., Yao, S.-J., 2015. Separation of lactoperoxidase from bovine whey milk by cation exchange composite cryogel embedded macroporous cellulose beads. Sep. Purif. Technol. 147, 132–138.

Petschow, B.W., Blikslager, A.T., Weaver, E.M., Campbell, J.M., Polo, J., Shaw, A.L., Burnett, B.P., Klein, G.L., Rhoads, J.M., 2014. Bovine immunoglobulin protein isolates for the

642 nutritional management of enteropathy. World J. Gastroenterol. 20, 11713–26.

643 Pichon, V., Chapuis-Hugon, F., Hennion, M.C., 2012. Bioaffinity sorbents, Comprehensive
644 Sampling and Sample Preparation. Elsevier.

645 Qiu, M., Chen, X., Fan, Y., Xing, W., 2017. Ceramic Membranes, in: Reference Module in
646 Chemistry, Molecular Sciences and Chemical Engineering. Elsevier.

647 Remón, J., García, L., Arauzo, J., 2016. Cheese whey management by catalytic steam reforming
648 and aqueous phase reforming. Fuel Process. Technol. 154, 66–81.

649 Sadeghi, M., Hanifpour, F., Taheri, R., Javadian, H., Ghasemi, M., 2016. Comparison of using
650 formaldehyde and carboxy methyl chitosan in preparation of Fe3O4 superparamagnetic
651 nanoparticles-chitosan hydrogel network: Sorption behavior toward bovine serum
652 albumin. Process Saf. Environ. Prot. 102, 119–128.

653 Safarik, I., Safarikova, M., 2004. Magnetic techniques for the isolation and purification of
654 proteins and peptides. Biomagn. Res. Technol. 2, 7.

655 Santos, M.J., Teixeira, J.A., Rodrigues, L.R., 2012. Fractionation of the major whey proteins
656 and isolation of β-Lactoglobulin variants by anion exchange chromatography. Sep. Purif.
657 Technol. 90, 133–139.

658 Santos, M.J., Teixeira, J.A., Rodrigues, L.R., 2011. Fractionation and recovery of whey proteins
659 by hydrophobic interaction chromatography. J. Chromatogr. B 879, 475–479.

660 Saufi, S.M., Fee, C.J., 2011. Simultaneous anion and cation exchange chromatography of whey
661 proteins using a customizable mixed matrix membrane. J. Chromatogr. A 1218, 9003–
662 9009.

663 Saxena, A., Tripathi, B.P., Kumar, M., Shahi, V.K., 2009. Membrane-based techniques for the
664 separation and purification of proteins: An overview. Adv. Colloid Interface Sci. 145, 1–
665 22.

666 Schlatterer, B., Baeker, R., Schlatterer, K., 2004. Improved purification of β-lactoglobulin from
667 acid whey by means of ceramic hydroxyapatite chromatography with sodium fluoride as a
668 displacer. J. Chromatogr. B 807, 223–228.

669 Shi, P., Wang, Q., Yu, C., Fan, F., Liu, M., Tu, M., Lu, W., Du, M., 2017. Hydroxyapatite
670 nanorod and microsphere functionalized with bioactive lactoferrin as a new biomaterial for
671 enhancement bone regeneration. Colloids Surfaces B Biointerfaces 155, 477–486.

672 Song, M.-M., Xu, H.-L., Liang, J.-X., Xiang, H.-H., Liu, R., Shen, Y.-X., 2017. Lactoferrin
673 modified graphene oxide iron oxide nanocomposite for glioma-targeted drug delivery.
674 Mater. Sci. Eng. C 77, 904–911.

675 Steinhauer, T., Marx, M., Bogend?rfer, K., Kulozik, U., 2015. Membrane fouling during ultra-
676 and microfiltration of whey and whey proteins at different environmental conditions: The
677 role of aggregated whey proteins as fouling initiators. J. Memb. Sci. 489, 20–27.

678 Sun, J., Rao, S., Su, Y., Xu, R., Yang, Y., 2011a. Magnetic carboxymethyl chitosan
679 nanoparticles with immobilized metal ions for lysozyme adsorption. Colloids Surfaces A
680 Physicochem. Eng. Asp. 389, 97–103.

681 Sun, J., Su, Y., Rao, S., Yang, Y., 2011b. Separation of lysozyme using superparamagnetic
682 carboxymethyl chitosan nanoparticles. J. Chromatogr. B 879, 2194–2200.

683 Urtasun, N., Baieli, M.F., Hirsch, D.B., Martínez-Ceron, M.C., Cascone, O., Wolman, F.J.,
684 2017. Lactoperoxidase purification from whey by using dye affinity chromatography.
685 Food Bioprod. Process. 103, 58–65.

686 Vasavada, P.C., Cousin, M.A., 1993. Dairy Microbiology and Safety, in: Hui, Y.H. (Ed.), Dairy
687 Science and Technology Handbook. Vol. 2. VCH, Cambridge.

688 Vega-Chacón, J., Arbeláez, M.I.A., Jorge, J.H., Marques, R.F.C., Jafelicci, M., 2017. pH-
689 responsive poly(aspartic acid) hydrogel-coated magnetite nanoparticles for biomedical
690 applications. Mater. Sci. Eng. C 77, 366–373.

691 Vidal, M.-L., Gautron, J., Nys, Y., 2005. Development of an ELISA for Quantifying Lysozyme
692 in Hen Egg White. J. Agric. Food Chem. 53, 2379–2385.

693 Vosen, S., Rieck, S., Heidsieck, A., Mykhaylyk, O., Zimmermann, K., Plank, C., Gleich, B.,
694 Pfeifer, A., Fleischmann, B.K., Wenzel, D., 2016. Improvement of vascular function by
695 magnetic nanoparticle-assisted circumferential gene transfer into the native endothelium. J.
696 Control. Release 241, 164–173.

697 Wakabayashi, H., Oda, H., Yamauchi, K., Abe, F., 2014. Lactoferrin for prevention of common
698 viral infections. J. Infect. Chemother. 20, 666–671.

699 Wijekoon, C.J.K., Ukuwela, A.A., Wedd, A.G., Xiao, Z., 2016. Evaluation of employing poly-
700 lysine tags versus poly-histidine tags for purification and characterization of recombinant
701 copper-binding proteins. J. Inorg. Biochem. 162, 286–294.

702 Wolman, F.J., Maglio, D.G., Grasselli, M., Cascone, O., 2007. One-step lactoferrin purification
703 from bovine whey and colostrum by affinity membrane chromatography. J. Memb. Sci.
704 288, 132–138.

705 Yadav, J.S.S., Yan, S., Pilli, S., Kumar, L., Tyagi, R.D., Surampalli, R.Y., 2015. Cheese whey:
706 A potential resource to transform into bioprotein, functional/nutritional proteins and
707 bioactive peptides. Biotechnol. Adv. 33, 756–774.

708 Yorgun, M.S., Balcioglu, I.A., Saygin, O., 2008. Performance comparison of ultrafiltration,
709 nanofiltration and reverse osmosis on whey treatment. Desalination 229, 204–216.

710 Zengin Kurt, B., Uckaya, F., Durmus, Z., 2017. Chitosan and carboxymethyl cellulose based
711 magnetic nanocomposites for application of peroxidase purification. Int. J. Biol.
712 Macromol. 96, 149–160.

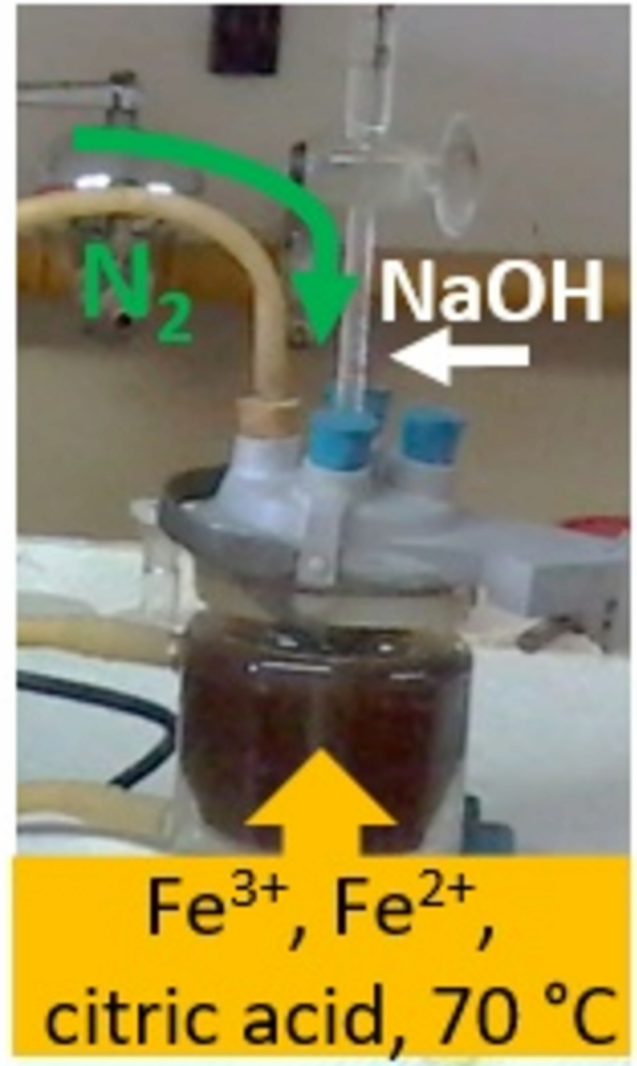
713 Zhou, Y., Yan, D., Yuan, S., Chen, Y., Fletcher, E.E., Shi, H., Han, B., 2018. Selective binding,
714 magnetic separation and purification of histidine-tagged protein using biopolymer
715 magnetic core-shell nanoparticles. Protein Expr. Purif. 144, 5–11.

716 Zhu, X., Zhang, L., Fu, A., Yuan, H., 2016. Efficient purification of lysozyme from egg white
717 by 2-mercapto-5-benzimidazolesulfonic acid modified Fe3O4/Au nanoparticles. Mater.
718 Sci. Eng. C 59, 213–217.

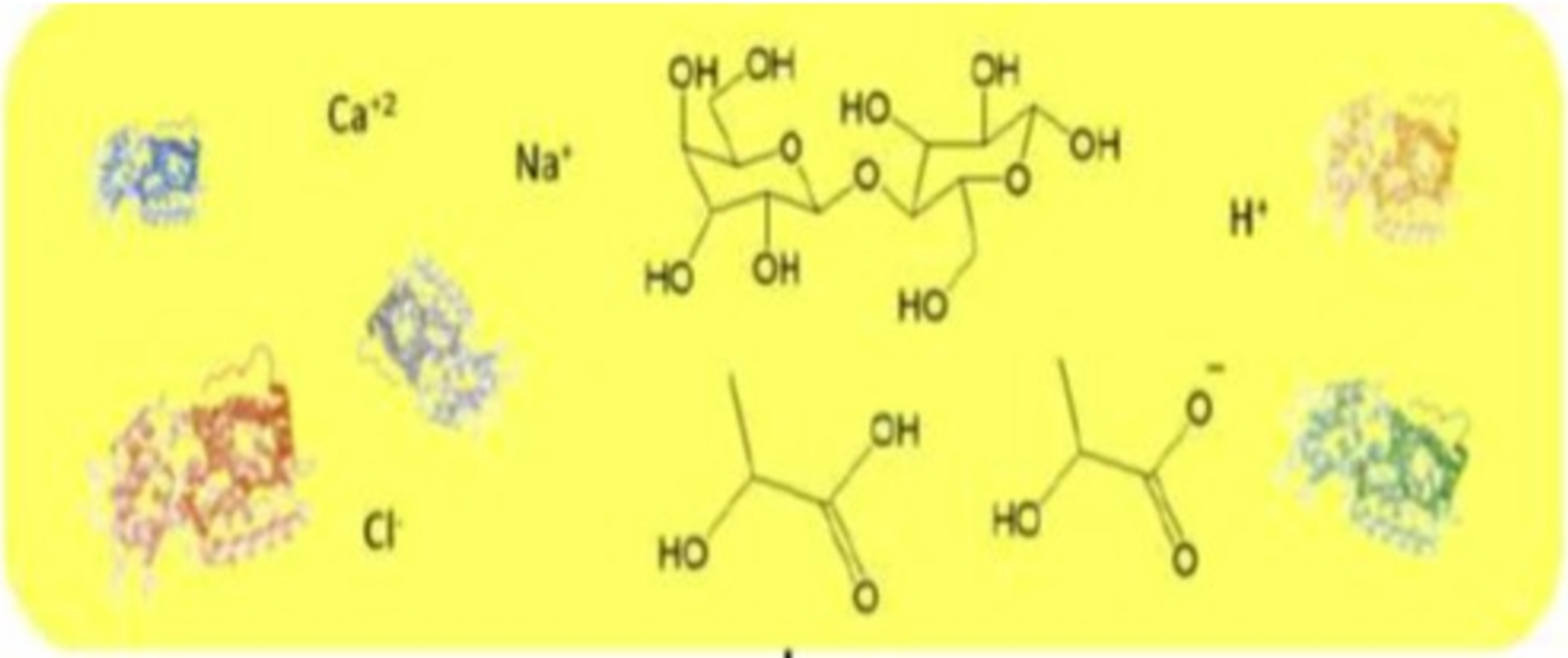
719

Highlights

- Cheese whey proteins isolation is a complex and expensive process
- No single procedure is capable of complete whey treatment
- Magnetic nanoparticles are cost-effective and reusable adsorption systems
- Magnetic decantation enables facile protein separation from whey
- Magnetic adsorption systems are a feasible alternative to filtration or chromatography


N_2
NaOH
Fe^{3+}, Fe^{2+},
citric acid, 70 °C


ELECTROSTATIC
COVALENT
HYDROPHOBIC
NH_3^+
$^-O\text{-}C{=}O$
$H\text{-}C{=}O$
$N{=}C{=}O$
Fe_3O_4



ULTRAFILTRATION

Permeate

Retentate

Diafiltration

Permeate

Retentate

Concentration and spray drying

WPC

MICROFILTRATION

Lipids

Permeate

DIAFILTRATION

WPI

ION EXCHANGE

Desorption

Ultrafiltration

Concentration and spray-drying