

Trends in whey protein fractionation

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Received: 13 February 2011 / Accepted: 8 March 2011 / Published online: 19 March 2011
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Abstract Whey is a by-product of cheese manufacture that is normally treated as a waste. However, it contains a mixture of proteins with important nutritional and biological attributes. To extract these valuable proteins, whey fractionation has been developed using three main techniques; namely chromatographic (e.g., ion-exchange and hydrophobic adsorption), membrane (e.g., traditional pressure-driven and electro-separation)-, or combined methods. Recently, new promising techniques have been introduced such as aqueous two-phase separation (ATPS) and magnetic fishing. This article reviews the use of these techniques together with an evaluation of their performance regarding the yield and purity of two major proteins in whey.

Keywords Whey protein · Fractionation · Review · Chromatography · Membrane separation · New techniques

Introduction

Whey is the principal by-product of cheese and casein manufacture. It is a multi-component protein mixture containing proteins of high nutritional value and versatile functional properties. Whey proteins are therefore widely used in the food and pharmaceutical industries. In general, whey is a dilute solution composed of lactose, a variety of proteins, minerals, vitamins and fat. Whey proteins, constituting about 0.7% (w/v) of the total weight of whey, include beta-lactoglobulin (BLG), alpha-lactalbumin (ALA), bovine serum albumin (BSA) and immunoglobulins (IgG). ALA and BLG alone comprise about 70% (w/w) of the total whey proteins and are responsible for the hydration, gelation, emulsifying and foaming properties of whey. Minor proteins are also present: lactoferrin (LF), lactoperoxidase (LP), enzymes, protein components of the MFGM (milk fat globule membrane), proteose-peptone components and glycomacropeptide (only found in sweet whey) (Gonzalez-Martinez et al. 2002; Strange et al. 1992; Perez et al. 2006). The problem with whey utilization is that a very large volume of whey is produced worldwide each year and it contains only dilute concentrations of these valuable proteins and other biochemicals.

There are three main routes of whey utilization: animal feedstocks, lactose production and the production of whey powders and individual whey proteins (Sienkiewicz 1990). The lack of consistency in the gross composition and functionality of whey

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powders has limited their acceptance by the food processing industry (Morr and Ha 1993). Commercial whey protein concentrates can also develop a stale off-flavour due to the presence of lipid and protein impurities (Morr and Ha 1991). In addition, the unique nutritional, therapeutic, and functional characteristics of the individual whey proteins are largely unrealised in these whey products due to interactions between components and degradation during processing. There has thus been considerable commercial interest in the production of individual (purified) whey proteins with well-characterised functional and biological properties (Zydney 1998). There is also an increasing need to produce individual whey proteins on a large scale without damaging protein structure or their functional properties.

This article reviews the different methods adopted for whey protein fractionation with focus on the two major established techniques namely; chromatography and membrane separation. To the best of the authors' knowledge, whey protein fractionation has not been reviewed elsewhere except by a couple of authors (Zydney 1998; Datta et al. 2008) who focused only on the membrane filtration technique.

Fractionation techniques

Since the 1950s, numerous techniques have been adopted for the purification of whey proteins. Some of the early techniques proved to be impractical and can only be applied for lab-scale purposes. Examples of these are salting out, precipitation which is based on selective solubility in presence of solvents, and acid/heat separation which utilises the differences in thermal stability in acidic conditions. A technical process should be simple, rapid, non-denaturing and must guarantee a high yield and product quality. None of the above cited procedures fulfil these prerequisites for successful scaling-up.

Several processes have been proposed for commercial-scale production of whey protein fractions. These processes fall into two main categories: (1) chromatography using selective adsorption or selective elution and (2) membrane separation. Membrane separation 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. For

dilute protein solutions such as whey, large volumes of liquid must be processed to recover a fixed mass of protein. Chromatographic processes are less volume dependent because adsorbent capacity depends mostly on the mass of protein recovered, not the volume of liquid processed (Doulton et al. 2004), as well as the concentration of the adsorbed species. Other methods were introduced recently including aqueous two-phase separation and magnetic fishing. The feasibility of these methods for scale-up processing is still under investigation. The following review will focus on the two commonly used techniques namely column chromatography and membrane separation, with emphasis on the trends that have been investigated in the past 20 years or so. Additional promising methods will also be presented.

Chromatographic methods

These include: ion exchange chromatography in which polyvalent, charged proteins bind to oppositely charged ion exchangers; affinity chromatography which exploits the specific interactions that can occur between proteins and immobilized ligands; and a more general class normally referred to simply as adsorption chromatography where the mechanism of adsorption is often uncertain. Examples of the latter class include the use of hydroxyapatite where adsorption is due to hydrophilic interactions, and other non-charged adsorbents where hydrophobic interactions are implicated in the adsorption process. This class will be referred to in the text as 'miscellaneous'. In what follows, the three classes of chromatography will be reviewed in detail.

Affinity chromatography

There are many examples of selective adsorption and selective elution processes for whey proteins. In case of affinity chromatography, Gurgel et al. (2000) used the immobilized hexapeptide, WHWRKR, for ALA adsorption, while Gambero et al. (1997) separated the same protein from bovine milk whey using chemically modified silica with β -diketoamine groups. For BLG adsorption, Wang et al. (1993) and Noppe et al. (1998) used immobilized retinal on Celite. Vyas et al. (2002) employed trans-retinal immobilized on calcium bio-silicate to separate BLG from sweet whey.

They applied three different modes of operation: packed-bed column, stirred tank and fluidized bed column, and obtained BLG recovery of 80, >95 and >95%, respectively. The third mode was considered the best suited for further scale-up. Puerta et al. (2002) studied the adsorption kinetics of BLG on a polyclonal immunochromatographic support. Konecny et al. (1994) used thiophilic chromatography on a T-gel (thiophilic gel composed of divinylsulfone activated-agarose coupled with 2-mercaptoethanol) to purify IgG from sweet cheese whey and found this method suitable for large-scale whey IgG isolation. Ounis et al. (2008) used heparin affinity chromatography to extract minor proteins from whey protein isolates. The results suggested that this method can be used to concentrate the minor cationic proteins such as LF and other growth factors. Table 1 summarizes the above-mentioned applications.

Miscellaneous adsorption chromatography

Using hydrophobic chromatography, Chaplin (1986) separated bovine whey proteins and caseins on a fast protein liquid chromatography column, phenyl-superose; Hahn et al. (2003) compared different hydrophobic interaction media regarding dynamic binding capacity, recovery and mass transfer properties; and Conrado et al. (2005) described the operating parameters related to the use of Streamline phenyl for the recovery of ALA from cow milk whey in an expanded-bed adsorption mode of operation using a theoretical model. ALA at a purity of 79% was obtained using this method.

By means of hydroxyapatite chromatography, Schlatterer et al. (2004) separated BLG from acid

whey with NaF as a displacer. About 50–55% of the BLG was recovered at a purity of 96%.

Ion-exchange chromatography

More focus is given to this class as it has been extensively employed by numerous workers for whey protein purification purposes. First, work done on pure systems of model whey proteins will be reviewed versus work done on whey protein systems.

A. Systems involving pure whey proteins

Some studies have been performed on the two-component, competitive adsorption of model protein systems involving the two major whey proteins, ALA and BLG. Flashner et al. (1983) studied the high performance anion-exchanger chromatographic separation of four proteins cytochrome c, α_1 -acid glycoprotein, ovalbumin, and β -lactoglobulin on polyethyleneimine (PEI)-silica gel column using a 20 min linear gradient from 0.025 to 0.50 M potassium phosphate at pH 6.8. Wahlgren et al. (1993) investigated the adsorption of a mixture of BLG, lactoferrin and lysozyme on hydrophilic silica surfaces. BLG (negatively-charged) was adsorbed in small amounts while lactoferrin and lysozyme (both positively-charged) were adsorbed in higher quantities. They deduced that adsorption from binary mixtures of oppositely charged proteins was affected by protein–protein interactions. According to them, there was either a cooperative adsorption leading to higher amounts of adsorbed proteins or a competitive adsorption depending on the solution conditions, the protein and the surface. James (1994) investigated both the single and binary adsorption/desorption equilibrium

Table 1 Applications of affinity chromatography techniques for whey protein fractionation

Adsorbent	Protein	Reference
Silica gel modified with beta-diketoamine groups	ALA	Gambero et al. (1997)
Amino resin followed by WHWRKR resin	ALA	Gurgel et al. (2000)
Immobilized retinal	BLG	Wang et al. (1993) Noppe et al. (1998)
Calcium biosilicate	BLG	Vyas et al. (2002)
Polyclonal immunochromatographic support	BLG	Puerta et al. (2002)
T-gel	IgG	Konecny et al. (1994)
Heparin	Minor proteins	Ounis et al. (2008)

and dynamics of lactoferrin and lactoperoxidase, purified from raw whey solutions, using three different sepharose cation-exchangers. Outinen et al. (1996) evaluated the adsorption characteristics of ALA and BLG on eleven strongly-basic anion-exchange adsorbents by performing frontal analysis with sweet whey (pH 6.5). Significant fractionation of the two proteins was obtained only with Diaion HPA, and optimum fractionation conditions (pH 6–7, BLG load of 16–20 mg per cm³ of resin, degree of demineralization 0–50) were determined in batch adsorption experiments in which 75–80% recovery of ALA was obtained. Kim and Kuga (2002) studied the separation of the two variants of BLG (A and B) using a cellulose-based anion-exchanger bearing water-soluble polycations. In all of the above column-chromatography studies, the method of protein separation was based on selective elution following adsorption rather than selective adsorption.

In addition to column-chromatography studies, a few relevant, competitive adsorption studies have been performed on ion-exchange membranes. Weinbrenner and Etzel (1994) studied the competitive adsorption of ALA and BSA to a sulphopropyl ion-exchange membrane. Competitive adsorption caused displacement of bound BSA monomer by the more strongly bound BSA dimer, illustrating that even apparently single protein systems may display multicomponent competitive behaviour. In the two-protein experiment, ALA was competitively displaced by the more strongly bound BSA monomer and dimer, indicating that the binding strength was in the order: BSA dimer > BSA monomer > ALA. Goodall et al. (2008) constructed binary solution breakthrough curves to show that ALA and BSA were displaced from the strong and weak anion-exchanger membranes by BLG. One limitation of this method was the low protein binding capacities observed. El-Sayed and Chase (2009a, b) studied the single- and two-component cation-exchange adsorption of ALA and BLG onto SP sepharose FF. They also presented a novel method for pinpointing the isoelectric point of the protein based on Langmuir data (El-Sayed and Chase 2009b). In the two-component system, there was strong evidence for competitive adsorption as ALA displaced and eluted all of the BLG from the column in a pure form. ALA was able to displace BLG in spite of the lower affinity of the former protein to the adsorbent. This

unexpected finding was corroborated using confocal laser scanning microscopy results (El-Sayed and Chase 2009c). In addition, a simple lumped kinetic model was used by the same workers to simulate the relevant breakthrough curves for the adsorption of ALA and BLG (El-Sayed and Chase 2010a).

B. Whey systems

Several attempts have been made to separate and purify the different components of whey by means of either column or membrane ion-exchange techniques. A few adsorption studies involving whey proteins have been performed on ion-exchange membranes. Weinbrenner and Etzel (1994) studied the competitive adsorption of ALA and BSA to a sulphopropyl ion-exchange membrane. Lucas et al. (1998) extracted ALA selectively from acid casein whey protein concentrate using anion-exchange membrane chromatography. The inorganic membrane employed was chemically modified by a polyethylene amine coating that carries positive charges in order to increase the membrane selectivity for ALA. Goodall et al. (2008) constructed rennet whey breakthrough curves to show that ALA and BSA were displaced from the strong and weak anion-exchange membranes by BLG. The limitation of this method was the low protein binding capacities observed.

The majority of study has been devoted to column chromatography. Workers in this field employed either anion-exchangers, cation-exchangers or a combination of both to separate whey proteins.

Anion-exchange Flashner et al. (1983) separated BLG on a polyethylene imine (PEI)-silica gel anion-exchange column using linear gradient elution with potassium phosphate (0.025–0.50 M) at pH 6.8. Colby et al. (1996) studied the effect of compression on the scale-up of a commercial packed-bed ion-exchange process to manufacture a whey growth factor extract using lactoperoxidase and lactoferrin as model substances. Carrere et al. (1996) studied the recovery of alpha-lactalbumin and beta-lactoglobulin from sweet whey protein using a fluidized ion exchange chromatographic process. They lumped both components as one entity in the pore-diffusion adsorption model as well as in a subsequent lumped kinetic elution model. Vogt and Freitag (1997) investigated the suitability of anion-exchange and hydroxyapatite displacement

chromatography for the processing of dairy whey. Lan et al. (2002) employed a liquid–solid circulating fluidized bed ion-exchange extraction system for continuous protein recovery from cheese whey. Pedersen et al. (2003) developed a consistent set of chromatographic data, including isotherms and transport properties, for the adsorption of whey proteins onto several anion-exchangers.

Cation-exchange Less work has been done on the cation-exchange chromatography of whey proteins. Hahn et al. (1998) developed a fractionation scheme for IgG, lactoferrin and lactoperoxidase based on the cation-exchangers S-HyperD F, S Sepharose FF, Fractogel EMD-S 650 (S) and Macro-Prep High S. They investigated the binding capacities for IgG and the different elution behaviours observed when sequential step gradients with NaCl buffers were applied. Since the process used salt for elution rather than changes in pH alone, several resin equilibration steps were needed to wash out the salt from the preceding step before elution of the next protein in the sequence. These extra steps took additional time and required additional buffer, hence reducing the productivity of the process and increasing the cost. Andersson and Mattiasson (2006) employed the simulated moving bed technology (SMB) to chromatographically separate LP and LF from whey protein concentrate using the cation-exchanger streamline-SP. SMB technology proved to be more advantageous when compared to conventional batch chromatography in terms of raised productivity, product concentration, reduced buffer consumption, as well as more efficient use of raw material. Lozano et al. (2008) employed cation-exchange chromatography only as a final purification step after isolating BLG from bovine whey by means of precipitation. Doultani et al. (2004) developed a cation-exchange column chromatography process that used selective elution to fractionate proteins from whey. Three different elution procedures were used as follows: (a) a single elution buffer to obtain whey protein isolate (WPI); (b) two elution buffers to obtain ALA and WPI depleted in ALA; and (c) four elution buffers to obtain ALA, WPI depleted in ALA, lactoperoxidase and lactoferrin. ALA was obtained with a recovery of more than 90%, while almost all of the BLG was recovered but with a low purity as it was mixed with substantial amounts of BSA and IgG.

Very little work has been done on cation-exchange adsorption of the two major whey proteins, BLG and ALA. In addition, all of the column-chromatography whey purification studies reported in the literature were based on selective elution rather than selective adsorption. Recent work has investigated the purification of ALA and BLG from whey concentrate mixtures using cation-exchange chromatography (El-Sayed and Chase 2010b). A novel separation process based on selective adsorption was developed that produced high yields and purities of both proteins. Almost all of the BLG in the feed was recovered, with 78% being recovered at 95% purity and a further 20% at 86% purity. In addition, 67% of ALA was recovered, 48% at 54% purity and 19% at 60% purity. In addition, El-Sayed and Chase (2010a) employed a simple lumped kinetic model to simulate the breakthrough curves for the cation-exchange adsorption of ALA and BLG in whey concentrate mixtures.

Anion-cation-exchange A number of workers performed a combination of cation- and anion-exchange steps to purify these proteins. Gerberding and Byers (1998) described a preparative ion-exchange chromatographic process for the separation and recovery of the four major proteins ALA, BLG, BSA and IgG as well as lactose from sweet dairy whey. In that study, it was found that an anion-exchange step was most effective in separating BLG from the whey feed mixture of pH 5.8 while a cation-exchange step was used to further recover the IgG. The obtained yields for ALA, BLG, IgG and BSA were 36, 94, 6 and 21%, respectively. The low recovery and incomplete separation observed made the process impractical for commercial application. Ye et al. (2000) used cation-exchange chromatography to recover lactoperoxidase and lactoferrin by applying salt-gradient elution using 0–0.55 M NaCl in 50 mM Tris/HCl, pH 6.5. The effluent was adjusted to pH 8.5 and passed through an anion-exchange column to recover ALA and BLG. ALA was eluted with 0–0.15 M NaCl in 50 mM Tris/HCl, pH 8.5. Next the buffer irrigating the column was adjusted to pH 6.8, and BLG was eluted with 0–0.2 M NaCl in 50 mM Tris/HCl, pH 6.8.

A few studies have been made on the modelling of whey protein adsorption. Carrere et al. (1996) studied the recovery of ALA and BLG from sweet whey protein by a fluidized ion exchange chromatographic

process. They lumped both components as one entity in the pore-diffusion adsorption model as well as in a subsequent lumped kinetic elution model.

Table 2 shows the most recent work that has been performed on whey protein fractionation via the foregoing ion-exchange chromatography techniques.

Membrane separation methods

The membrane techniques used for the separation of whey proteins fall into the following three main categories:

Conventional filtration

Included here are the ultrafiltration and diafiltration modes. Cheang and Zydney (2004) examined the use of a two-stage tangential flow filtration system for the purification of ALA and BLG from whey protein isolate. Separation was achieved through 100 and 30 kDa membranes in series. ALA was obtained at 90% yield but the recovery of BLG was more challenging. Almecija et al. (2007) investigated the potential of membrane ultrafiltration for the fractionation of clarified whey. A 300 kDa tubular ceramic membrane was used in a continuous diafiltration

mode, and the effect of pH was evaluated. The highest permeate yields for ALA and BLG were obtained at pH 9 and were 56 and 33%, respectively, while BSA, IgG and LF were mostly retained at the working pH values. Muller et al. (1999) investigated the separation of ALA using different ultrafiltration modes of operation. They developed model equations for the continuous, discontinuous concentration or diafiltration modes (single or combined). They showed that continuous concentration up to a high volume reduction ratio (11 ± 15) or combined continuous concentration- diafiltration helped to obtain a fraction with both an enhanced purity and a satisfactory yield of ALA of, up to 90%, in the permeate.

Ion-exchange membrane chromatography

This technique has been reviewed in the previous section on chromatography. However, it is included in Table 3 for comparison purposes.

Electro-separation

This group of processes include electro-dialysis and electro-acidification. The latter is a recent technology that provides the coupled effect of the dimineralization and acidification by using the properties of

Table 2 Applications of ion-exchange chromatography techniques for whey protein fractionation

Adsorbent	Protein	Reference
Anion-exchange		
Spherosil QMA	ALA, BLG	Carrere et al. (1996)
Polystyrene-divinylbenzene copolymer, basic silica and polystyrene resin	ALA, BLG	Outinen et al. (1996)
Q-sepharose FF	ALA, BLG (A, B), BSA	Pederson et al. (2003)
Source 30Q		
Ceramic Q-hyper DF		
Merck fractogel EMDTMAE 650 (s)		
Cation-exchange		
SP sepharose big beads	WPI, ALA, LP, LF	Doultani et al. (2004)
SP sepharose FF	ALA, BLG	El-Sayed and Chase (2010a)
Anion/cation-exchange		
Pharmacia biotech model XK 50/30	BLG, IgG	Gerberding and Byers (1998)
Diethylaminoethyl-Toyopearl	BLG	Ye et al. (2000)
Quaternary aminoethyl-Toyopearl	ALA, BLG	
Sulphopropyl-Toyopearl	LP, LF	

Table 3 Applications of membrane techniques for whey protein fractionation

Method	Membrane type/MWCO (kDa)	Protein	Reference
Conventional filtration			
UF, DF	Zirconium oxide with alpha-alumina support 300	ALA	Muller et al. (1999)
Two-stage UF	Regenerated cellulose membrane 30, 100	ALA, BLG	Cheang and Zydney (2004)
UF	Ceramic membrane 300	ALA, BLG, BSA, IgG, LF	Almecija et al. (2007)
Ion-exchange chromatography			
Anion-exchange	Zirconium oxide inorganic membrane 10, 15, 50, 150	ALA	Lucas et al. (1998)
Anion-exchange	Quaternary ammonium diethyl amine	ALA, BLG, BSA	Goodall et al. (2008)
Electro-separation			
Electro-acidification	Bipolar membrane CMX-SB cationic membrane	BLG	Bazinet et al. (2004)
Electro-dialysis	CMX-SB cationic membrane, a neosepta AMX-SB anionic membrane, a polyethersulfone UF membrane 500	LF	Ndiaye et al. (2010)

bipolar membranes to dissociate water molecules at their interfaces followed by cation-exchange membranes to demineralise by migration of low molecular weight ionic species. Bazinet et al. (2004) demonstrated the feasibility of bipolar membrane electro-acidification for whey protein separation. This technology allowed the separation of 98% pure BLG with a 44% recovery yield. Ndiaye et al. (2010) evaluated the feasibility of separating LF from whey using electrodialysis with an ultrafiltration membrane system of 500 kDa. The highest migration rate for LF was obtained at pH 3 with a migration yield of 15%.

Table 3 presents the main applications of membrane techniques in the purification of individual whey proteins.

Combined methods

These are based on coupling two commonly used techniques; such as chromatography/membrane separation, chemical treatment/membrane separation, or magnetic fishing/ion-exchange or affinity adsorption.

Xu et al. (2000) separated bovine IgG and GMP from dairy whey. GMP was selectively adsorbed on a polystyrene anion-exchanger IRA93 at pH 4.7. Afterwards, IgG was concentrated in whey by selectively removing ALA, BLG and BSA using the same anion-

exchanger at pH 7.0 followed by a 100 kDa Amicon YM membrane. Konrad et al. (2000) isolated BLG from whey through a peptic treatment of whey and membrane UF. BLG was produced with a recovery of 67% and a purity of 94%. Bhattacharjee et al. (2006) purified BLG from whey protein concentrate via two-stage UF with flat disk membranes in a stirred rotating disk module, followed by an anion-exchange membrane chromatography; BLG at a purity of 87.6% was obtained in the permeate. Konrad and Keinschmidt (2008) isolated ALA from sweet whey by means of UF followed by a tryptic treatment of the permeate, and then a final UF/DF step of the hydrolysate. Up to 15% overall recovery of ALA was achieved with a purity of 90–95%.

One of the relatively recent techniques for whey protein purification is magnetic fishing. It involves the use of magnetic particles bearing: either an immobilized affinity ligand, a hydrophobic ligand, or ion-exchange groups, as well as magnetic biopolymer particles that have affinity to the desired component. The target protein binds to these particles forming a complex which is easily and rapidly removed from the sample with an appropriate magnetic separator. After washing out the contaminants, the isolated target compound(s) can be eluted. Heeboll-Nielsen et al. (2004) used supermagnetic ion-exchangers to fractionate whey proteins. Crude bovine whey was

allowed to contact with a supermagnetic cation-exchanger to adsorb LF and LP and then the supernatant was contacted with a supermagnetic anion-exchanger to adsorb BLG. The adsorbed proteins were then salt eluted. LF was also recovered from crude whey by adsorption of the protein to magnetic micro cation-exchanger particles followed by elution involving high-gradient magnetic separation (Meyer et al. 2007). The same protein was isolated from acid whey by magnetic affinity separation, employing supermagnetic polyglycidyl methacrylate particles derivatized with heparin. This technology proved to be fast, and has a potential for scaling-up (Chen et al. 2007). Table 4 summarizes the various combined techniques reported up to date.

Additional methods for the separation of individual whey proteins have been introduced recently.

Aqueous two-phase separation (ATPS) is a technique that is widely used for the separation of biological material, because of its high water content which provides mild extraction conditions. It is based on the incompatibility of two different hydrophilic polymers in the common solvent water or aqueous solutions of polymers and salts above certain critical concentrations. The technique was first used for whey protein purification by Chen (1992) and Coimbra et al. (1994). A polymeric-saline two-phase system composed of poly (ethylene glycol) (PEG) and potassium phosphate was used for the isolation and separation of BLG and ALA from whey. The technique was further

developed by Jara and Pilosof (2010) who employed a two-phase system composed of whey protein concentrate and polysaccharide hydroxypropyl methylcellulose at pH 6.5 to study the relative partitioning of ALA and BLG.

Size exclusion chromatography (SEC) is usually used as a final purification step following ion-exchange and affinity methods. El-Sayed and Chase (2009) suggested using SEC as a polishing step to increase the purity of ALA obtained from a two-stage ion-exchange chromatography process. Preliminary studies showed that the purity could be increased from 60% to over 90%. Rojas et al. (2004) used SEC to purify cheese whey ALA and BLG produced, respectively in the polymeric and saline phases of ATP systems. They obtained a high degree of protein purification of 99.7% for the saline phase and 99.6% for the polymeric phase.

Evaluation of established techniques with respect to ALA and BLG

Table 5 compiles the reported values of the yield and purity of the two major whey proteins obtained via chromatography and membrane techniques. As can be seen, ion-exchange chromatography methods produce higher yields but lower purity as compared to other chromatography methods like affinity, hydrophobic or hydroxyapatite. As for membrane

Table 4 Applications of combined methods for whey protein fractionation

Methods	Specifications	Protein	Reference
Peptic treatment/UF, DF	Alumina-based ceramic membrane (15 kDa)	ALA	Konrad et al. (2000)
Anion-exchange chromatography/UF	Polystyrenic exchanger (IRA93)/100 (kDa)	IgG	Xu et al. (2000)
ATPS/SEC		ALA, BLG	Jara and Pilosof (2010)
Supermagnetic anion/cation-exchanger		LP, LF, IgG, BLG	Heeboll-Nielsen et al. (2004)
Magnetic affinity	Supermagnetic polyglycidyl methacrylate particles coupled with heparin	LF	Chen et al. (2007)
Magnetic micro ion-exchange		LF	Meyer et al. (2007)
UF/tryptic hydrolysis	100, 150 (kDa)	ALA	Konrad and Kleinschmidt (2008)
Two-stage UF/anion-exchange chromatography	10, 30 (kDa)	BLG	Ndiaye et al. (2010)

Table 5 Yields and purity for ALA and BLG obtained using column chromatography and membrane separation techniques

Method	% Yield	% Purity	Protein	Reference
Column chromatography				
Ion-exchange	36		ALA	Gerberding and Byers (1998)
	94		BLG	
Ion-exchange	>92	Low	ALA	Doulton et al. (2004)
	100		BLG	
Affinity		80–95	BLG	Vyas et al. (2002)
Affinity	47.9	90.6	ALA	Gurgel et al. (2000)
	35.2	100		
Hydrophobic		79	ALA	Conrado et al. (2005)
Hydroxyapatite	50–55	96	BLG	Schlatterer et al. (2004)
Membrane				
UF	>90		ALA	Cheang and Zydney (2004)
UF	56	Low	ALA	Almecija (2007)
	33		BLG	
UF/peptic treatment	15	90–95	ALA	Konrad et al. (2000)
UF/tryptic treatment	67.3	94.1	BLG	Konrad and Kleinschmidt (2008)
UF/ion-exchange		87.6	BLG	Bhattacharjee et al. (2006)
Membrane electro-acidification	44	98	BLG	Bazinet et al. (2004)

techniques, pressure-driven separation methods such as UF and DF produce reasonable yields, but low % purities. However, higher purity levels are achieved when these techniques are coupled with other techniques such as ion-exchange chromatography and chemical treatment. Alternatively, the purity increases when an additional driving force is added such as the potential difference/bipolarity effect in electro-acidification.

Conclusion

This review has covered the different techniques that have been used for the purification of the individual whey proteins. These include conventional chromatography and membrane techniques, in addition to other techniques that have been recently introduced for whey protein purification such as electro-separation, magnetic fishing and ATPS. It was found that column chromatography and membrane separation remain the most commonly used techniques for whey protein fractionation. However, when using a single method per se, there is always a trade off between yield and purity. Therefore, there is now a growing interest in coupling these two techniques, or coupling

one of them with a third technique such as chemical treatment and magnetic fishing. Better results particularly in terms of purity levels, were obtained with the coupling approach involving a combination of more than one technique. Magnetic fishing, electro-separation and ATPS proved to be the more promising techniques for whey protein fractionation particularly when coupled with other traditional techniques as affinity and ion-exchange adsorption. However, their suitability for scale-up is yet to be investigated. The technical and economical feasibility of these coupled systems will probably be one of the major future challenges facing the whey protein fractionation industry.

References

- Almecija MC, Ibanez R, Guadix A, Guadix EM (2007) Effect of pH on the fractionation of whey proteins with a ceramic ultrafiltration membrane. *J Membr Sci* 288:28–35
- Andersson J, Mattiasson B (2006) Simulated moving bed technology with a simplified approach for protein purification separation of lactoperoxidase and lactoferrin from whey protein concentrate. *J Chromatogr A* 1107:88–95
- Bazinet L, Ippersiel D, Mahdavi B (2004) Fractionation of whey proteins by bipolar membrane electro-acidification. *Innovat Food Sci Emerg Technol* 5:17–25

- Bhattacharjee S, Bhattacharjee C, Datta S (2006) Studies on the fractionation of beta-lactoglobulin from casein whey using ultrafiltration and ion exchange membrane chromatography. *J Membr Sci* 275:141–150
- Carrere H, Bascoul A, Floquet P, Wilhelm AM, Delmas H (1996) Whey proteins extraction by fluidized ion exchange chromatography: simplified modelling and economical optimization. *Chem Eng J Biochem Eng J* 64(3):307–317
- Chaplin LC (1986) Hydrophobic interaction fast protein liquid chromatography of milk proteins. *J Chromatogr A* 363(2):329–335
- Cheang B, Zydney A (2004) A two stage ultrafiltration process for fractionation of whey protein isolate. *J Membr Sci* 231:156–167
- Chen JP (1992) Partitioning and separation of alpha-lactalbumin and beta-lactoglobulin peg/potassium phosphate two phase systems. *J Bioimaging* 73:360–365
- Chen L, Guo C, Guan Y, Liu H (2007) Isolation of lactoferrin from acid whey by magnetic affinity separation. *Sep Purif Technol* 56:168–174
- Coimbra JR, Thömmes J, Kula MR (1994) Continuous separation of whey proteins with aqueous two-phase system in a Graesser contactor. *J Chromatogr A* 668:85–94
- Colby CB, O'Neill BK, Vaughan F, Middelberg AP (1996) *J Biotechnol Prog* 12:662
- Conrado LS, Veredas V, Nobrega ES, Santana CC (2005) Concentration of α -lactalbumin from cow milk whey through expanded bed adsorption using a hydrophobic resin. *Braz J Chem Eng* 22(4):501–509
- Datta D, Bhattacharjee C, Datta S (2008) Whey protein fractionation using membrane filtration: A review. *J Inst Eng Chem Eng* 89:45–50
- Doultani S, Turhan KN, Etzel MR (2004) Fractionation of proteins from whey using cation exchange chromatography. *Process Biochem* 39:1737–1743
- El-Sayed MH, Chase HA (2009a) Toward separating alpha-lactalbumin and beta-lactoglobulin proteins from whey through cation-exchange adsorption. *IAENG transactions on engineering technologies volume II. American Institute of Physics, New York*
- El-Sayed MH, Chase HA (2009b) Single and two-component cation-exchange adsorption of the two pure major whey proteins. *J Chromatogr A* 1216:8705–8711
- El-Sayed MH, Chase HA (2009c) Confocal microscopy study of uptake kinetics of alpha-lactalbumin and beta-lactoglobulin onto the cation-exchanger SP sepharose FF. *J Sep Sci* 32(18):3246–3256
- El-Sayed MH, Chase HA (2010a) Simulation of the breakthrough curves for the adsorption of alpha-lactalbumin and beta-lactoglobulin to SP sepharose FF cation-exchanger. *J Biochem Eng* 49:221–228
- El-Sayed MH, Chase HA (2010b) Purification of the two major proteins from whey concentrate using a cation-exchange selective adsorption process. *J Biotechnol Prog* 26(1): 192–199
- Flashner M, Ramsden H, Crane LJ (1983) Separation of proteins by high-performance anion-exchange chromatography. *J Anal Biochem* 135(2):340–344
- Gamero A, Kubota LT, Gushikem Y, Airoidi C, Granjeiro JM, Taga EM, Alcantara EFC (1997) Use of chemically modified silica with β -diketoamine groups for separation of α -lactalbumin from bovine milk whey by affinity chromatography. *J Colloid Interface Sci* 185(2):313–316
- Gerberding SJ, Byers CH (1998) Preparative ion-exchange chromatography of proteins from dairy whey. *J Chromatogr A* 808:141–151
- González-Martínez C, Becerra M, Cáfer A, Albors A, Carot J, Chiralt A (2002) Influence of substituting milk powder for whey powder on yogurt quality. *Trends Food Sci Technol* 13:334–340
- Goodall S, Grandison AS, Jauregi PJ, Price J (2008) Selective separation of the major whey proteins using ion exchange membranes. *J Dairy Sci* 91:1–10
- Gurgel PV, Carbonell RG, Swaisgood HE (2000) Fractionation of whey proteins with a hexapeptide ligand affinity resin. *Bioseparation* 9:385–392
- Hahn RP, Schulz M, Schaupp C, Jungbauer A (1998) Bovine whey fractionation based on cation-exchange chromatography. *J Chromatogr A* 795:277–287
- Hahn R, Deinhofer K, Machold C, Jungbauer A (2003) Hydrophobic interaction chromatography of proteins: II. Binding capacity, recovery and mass transfer properties. *J Chromatogr B* 790(1–2):99–114
- Heeboll-Nielsen A, Justesen SFL, Thomas ORT (2004) *J Biotechnol* 113:247–262
- James ER (1994) The application of multicomponent adsorption theory to an ion exchange chromatography system for recovery of whey proteins, Thesis. University of Queensland, Australia
- Jara F, Pilosof AMR (2011) Partitioning of alpha-lactalbumin and beta-lactoglobulin in whey protein concentrate/hydroxypropyl methyl cellulose aqueous two-phase systems. *Food Hydrocoll* 25(3):374–380
- Kim Y, Kuga S (2002) *J Chromatogr A* 955:191–196
- Konecny P, Brown RJ, Scouten WH (1994) Chromatographic purification of immunoglobulin G from bovine milk whey. *J Chromatogr A* 673(1):45–53
- Konrad G, Kleinschmidt T (2008) A new method for isolation of native alpha-lactalbumin from sweet whey. *Int Dairy J* 18:47–54
- Konrad G, Lieske B, Faber W (2000) A large-scale isolation of native beta-lactoglobulin: characterization of physicochemical properties and comparison with other methods. *Int Dairy J* 10:713–721
- Lan Q, Bassi A, Zhu JX, Margaritis A (2002) *J Biotech and Bioeng* 78:157
- Lozano JM, Giraldo GI, Romero CM (2008) An improved method for isolation of β -lactoglobulin. *Int Dairy J* 18:55–63
- Lucas D, Rabiller-Baudry M, Millesime L, Chaufer B, Daufin G (1998) Extraction of alpha-lactalbumin from whey protein concentrate with modified inorganic membranes. *J Membr Sci* 148:1–12
- Meyer A, Berensmeier S, Franzreb M (2007) Direct capture of lactoferrin from whey using magnetic micro-ion exchangers in combination with high-gradient magnetic separation. *React Funct Polym* 67:1577–1588
- Morr CV, Ha EYW (1991) Off-flavours of whey protein concentrates: A literature review. *Int Dairy J* 1:1
- Morr CV, Ha EYW (1993) Whey protein concentrates and isolates: processing and functional properties. *Crit Rev Food Sci Nutr* 33:431–476

- Muller A, Daufin G, Chaufer B (1999) Ultrafiltration modes of operation for the separation of alpha-lactalbumin from acid casein whey. *J Membr Sci* 153:9–21
- Ndiaye N, Pouliot Y, Saucier L, Beaulieu L, Bazinet L (2010) Electro separation of bovine lactoferrin from model and whey solutions. *Sep Purif Technol* 74:93–99
- Noppe W, Haezebrouck P, Hanssens I, De Cuyper M (1998) A simplified purification procedure of alpha-lactalbumin from milk using Ca_2 dependent adsorption in hydrophobic expanded bed chromatography. *Bioseparation* 8:153–158
- Ounis WB, Gauthier SF, Turgeon SL, Roufik S, Pouliot Y (2008) Separation of minor protein components from whey protein isolates by heparin affinity chromatography. *Int Dairy J* 18:1043–1050
- Outinen M, Tossavainen O, Syvaioja EL (1996) Chromatographic fractionation of alpha lactalbumin and beta lactoglobulin with polystyrenic strongly basic anion exchange resins. *Lebensmittel-Wissenschaft Technol* 29(4):340–343
- Pedersen L, Mollerup J, Hansen E, Jungbauer A (2003) Whey Proteins as a model for chromatographic separation of proteins. *J Chromatogr B* 790:161–173
- Pérez OE, Wargon V, Pilosof AMR (2006) Gelation and structural characteristics of incompatible whey proteins/hydroxypropyl methylcellulose mixtures. *Food Hydrocoll* 207:966–974
- Puerta A, Jaulmes A, De Frutos M, Diez-Masa JC, Vidal-Madjar C (2002) Adsorption kinetics of beta-lactoglobulin on a polyclonal immunochromatographic support. *J Chromatogr A* 953(1–2):17–30
- Rojas EG, Coimbra JS, Minim LA, Zuniga AD, Saraiva SH, Minim VP (2004) *Proc Biochem* 39:1751–1759
- Schlatterer B, Baeker R, Schlatterer K (2004) Improved purification of beta-lactoglobulin from acid whey by means of ceramic hydroxyapatite chromatography with sodium fluoride as a displacer. *J Chromatogr B* 807:223–228
- Strange ED, Malin EL, Van Hekken DL, Basch J (1992) Chromatographic and electrophoretic methods used for analysis of milk proteins. *J Chromatogr* 624(1–2):81–102
- Vogt S, Freitag R (1997) *J Chromatogr A* 760:125
- Vyas HK, Izco JM, Jimenez-Flores R (2002) Scale-up of native beta-lactoglobulin affinity separation process. *J Dairy Sci* 85(7):1639–1645
- Wahlgren M, Arnebrant T, Paulson M (1993) *J Colloid Interface Sci* 158:46–53
- Wang Q, Swaisgood HE (1993) Characteristics of beta-lactoglobulin binding to the all-trans-retinal moiety covalently immobilized on Celite™. *J Dairy Sci* 76:1895–1901
- Weinbrenner WF, Etzel MR (1994) *J Chromatogr A* 662: 414–419
- Xu Y, Sleigh R, Hourigan J, Johnson R (2000) Separation of bovine immunoglobulin G and glycomacropeptide from dairy whey. *Proc Biochem* 36(5):393–399
- Ye X, Yoshida S, Ng TB (2000) Isolation of lactoperoxidase, lactoferrin, α -lactalbumin, β -lactoglobulin B and β -lactoglobulin A from bovine rennet whey using ion exchange chromatography. *Int J Biochem Cell Biol* 32(11–12): 1143–1150
- Zydney AL (1998) Protein separations using membrane filtration: new opportunities for whey fractionation. *Int Dairy J* 8:243–250