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Industrial Proteins from Potato Juice. A Review

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ABSTRACT

Industrial proteins constitute proteins of nutritious and functional values obtained from animal and plant sources, and recovered commercially on a large scale. Among the plant crop proteins, potato has up to recently been regarded only as a nutritious supplement to feeds, unsuitable in human foods due to low technical quality. Major potato proteins characterized by molecular size, are the 40-42 kDa patatin group (35-40%) and the protease inhibitor group of 7-21 kDa (25-50%). Although well-balanced in amino acid composition, and possessing valuable protein functionality, food utilization has been limited due to high levels of total glycoalkaloids (TGA) and phenolic compounds. Potato juice (PJ) from starch manufacturing is the major source of protein extraction. The voluminous and dilute protein solution (1-2%) has a complex composition and is high in organic matter; hence, the rigorous demands on the effluent require efficient recovery of protein and peptide fractions. The recovery methods from PJ that are reviewed here include precipitation with various acids, membrane separation and chromatographic adsorption. Precipitation with various acids, often in combination with heat coagulation, gives high recoveries but destroys functionality. Membrane separation (reverse osmosis, ultrafiltration) is better, but ultrafiltration is often difficult to perform and uneconomical due to filter fouling. As a gentle processing alternative, expanded bed adsorption (EBA) has been demonstrated as a scalable chromatographic method with the ability to separate the major protein groups from phenolic compounds and TGA, resulting in highly functional protein preparations. EBA, as well as other improvements in established technologies are now gradually moving potato proteins into a better position as an industrial protein.

Keywords: adsorption, expanded bed chromatography, glycoalkaloid, membrane separation, patatin, phenolic compounds, precipitation, protease inhibitor

CONTENTS

INDUSTRIAL PROTEINS

The global need for protein is increasing with the world's population growth and increasing incomes. In the global food supply, several sources of proteins exist of both animal and vegetable origin. Consumer preferences are not only governed by quality, but also by, for example, price, allergies, if the product contains genetically modified ingredients/feedstock and the environmental impact during production. The increased commercial importance of vegetable proteins as a food ingredient is a result of some of these facts (Smil 2002).

Industrial proteins are defined as "proteins produced and/or processed on a comparatively large (i.e. industrial) scale" (Voragen 2005). They include proteins of animal, plant, and microbial origin. Among the animal sources of proteins, milk whey, beef muscle collagen and egg white dominate. Of the four major global plant crops (corn, rice, wheat, potato), three of them including corn, wheat and potato are also sources of industrial proteins. Vegetable proteins come mainly from seed or storage parts like legume pods, grain, corn and seeds. Legume seed constitute a large part of the world's protein source. Among well known legume seed plants are soybeans, lupin, pea, and fababean.

Today, potato tuber is the world's No. 4 food crop, with production in 2005 of more than 320 million tones (www.fao.org). China has become the world's leading commercial potato producer in terms of volume, and just under one third of all is now harvested in China and India alone. Over the next decade, most of the world's potatoes will likely be grown in Asia, Africa and Latin America.

To be regarded as an attractive food protein, it should possess high nutritive quality or exhibit functional properties, or even better, both. The term protein quality mainly reflects the amino acid composition and digestibility of the protein. High-quality proteins have been defined as those

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that contain all the essential amino acids at levels greater than FAO/WHO/UNU (1985) reference levels, and digestibility is defined as the proportion of food nitrogen that is adsorbed after ingestion and must be comparable to or better than those of egg-white or milk proteins. Because they are more completely digested, proteins of animal origin are generally of higher quality than those from vegetables. Quality is ultimately affected by the protein conformation, which in turn is dependant on the separation method and processing conditions including pH, ionic strength, and heat during the recovery process.

Protein functionality is defined as "those physical and chemical properties which affect the behavior of proteins in food systems during processing, storage, preparation and consumption" (Kinsella 1976). Factors related to functional properties of proteins are hydrophobicity, cross-links (e.g. disulfide bonds), secondary, tertiary and quaternary structure, and molecular flexibility/ rigidity. Moure and co-authors (2006) suggested classifying functional properties according to their mechanism of action on three main groups: hydration properties (e.g. solubility), protein structure and rheology (e.g. gelation), and properties related to protein surface (e.g. emulsifying and foaming activities).

In general, industrial proteins come from biomass sources where they are abundant, often as defined fractions. Whether protein fractions are based on size, solubility or physical processing, high volume availability has made these proteins useful in many applications. The challenge for industrial proteins from plant sources are the varying levels of phytochemicals. These, often toxic compounds, are undesired in products for food and feed applications, and thus must be removed during protein recovery.

About 1-2% of the human population has allergies related to food, with egg, gluten, soy, fish and nuts being among the most common. These are all important food protein sources, and many are being used as emulsifiers, gelling and foaming agents in food systems. Allergy towards potato protein is much less common (Castells *et al.* 1986), and potato protein concentrates can therefore be an interesting replacement for these proteins as food hydrocolloids.

The impetus for this review on protein recovery is the fact that potato proteins, for long only suitable as animal feed supplements, are making their way into the food supply, with the potential as replacement ingredient to soy, whey, and caseinates in food formulations. The knowledge on potato protein behavior and its impact on recovery have increased considerably over the last decade.

THE MAJOR POTATO PROTEINS

Potato (*Solanum tuberosum* L.) proteins represent an interesting group of vegetable proteins. Soluble potato proteins in potato tubers have been classified into three groups (Pots 1999), namely the patatins, the protease inhibitors and other proteins of higher molecular weights (**Table 1**). The quantity of each group varies in different potato cultivars, but in general around 35-40% is patatin, 25-50% is protease inhibitors and the rest belongs to the high molecular weight group.

Patatin is a family of glycoproteins with molecular masses around 40-42 kDa and pIs from 4.8-5.2 (Racusen *et al.* 1980; Park *et al.* 1983). The primary sequence of patatin (362 amino acids) shows neither extended hydrophilic or hydrophobic amino acid sequences. The positive and negative side chain charges are randomly distributed over the sequence (Pots 1999).

Patatin is considered a storage protein in the potato because of its high accumulation in the tuber (Racusen *et al.* 1980). It is speculated whether the physiological function of patatin in potato is associated with wound or defence response (Dennis *et al.* 1974; Andrews *et al.* 1988), or signal transduction (Senda *et al.* 1996). It has a lipid-acyl hydrolase activity (LAH), and catalyzes the non-specific hydrolysis of phospholipids, glycolipids, mono- and diacyl glycerols, and esters of long chain fatty acids (Galliard 1971; Racusen *et al.* 1980, 1984; Andrews *et al.* 1988). Rydel and co-authors (2003) found that patatin lacks a flexible lid usually present in lipases that shields the active site. Consequently, to provide interaction with lipophilic substrates, patatin has hydrophobic patches available on the surface. The native form is a dimer, whereas it unfolds to monomers in the presences of dissociating media (Racusen *et al.* 1984). Patatin is considered to have a well balanced amino acid composition, high in the essential amino acid lysine (Knorr 1978).

Table 1 Composition of protein in PJ (adapted from Pouvreau and co-authors 2001).

Protein		% of total*
Patatin		35-40
Protease inhibitors:		25-50
	Potato Inhibitor I (PI-I)	4.5
	Potato serine protease inhibitor (PSPI/ PI-2)	22.3
	Potato cysteine protease inhibitor (PCPI)	11.5
	Potato aspartate protease inhibitor (PAPI)	6.1
	Potato Kunitz-type protease inhibitor (PKPI)	3.6
	Potato carboxypeptidase protease inhibitor (PCI)	0.9
	"other serine protease inhibitors" (OSPI)	1.5
Other	e. g. starch synthetase, polyphenol oxidase, potato multicystatin inhibitor	12

* May vary between potato cultivars

The protease inhibitors make up a heterogeneous group of proteins with molecular masses ranging from 7-21 kDa and pIs from 5.0-8.0. Protease inhibitors neutralize proteolytic enzymes from several microorganisms and insects, as a part of the plant defense mechanisms (Jongsma 1995). The protease inhibitor group has gained new focus because of the finding of possible anticarcinogenic and positive dietary effects (Hill *et al.* 1990; Kennedy 1998). The application of the so-called Protease Inhibitor II-proteins (21 kDa) as a therapeutic satiety signal through the hormone cholecystokinin (CCK) was described in a patent by Ausich and coworkers (2003). Plant protease inhibitors are generally classified based on the active site of the protease they inhibit, and the protease inhibitors have been divided into seven families on the basis of their specificity (Pouvreau *et al.* 2001) (**Table 1**). The most abundant of these groups in PJ are PSPI and PCPI, representing 22 and 12% of the total protein in PJ in cv. 'Elkana' (Pouvreau *et al.* 2001). PSPI is a Kunitz-type inhibitor showing activity against serine proteases like trypsin and chymotrypsin.

FUNCTIONAL PROPERTIES

A well recovered potato protein has satisfactory solubility, significant foaming and emulsifying properties. This is the general conclusion from several studies based on carefully prepared proteins (Knorr *et al.* 1977; Holm *et al.* 1980; Knorr 1980). Holm and Eriksen (1980) studied the emulsifying capacity of potato protein concentrates and found that undenatured potato protein was superior to a commercial soy protein product. Defined fractions of potato proteins give highly stable emulsions compared to the stability of emulsions made with the original, raw composition of the potato protein (Ralet *et al.* 2000). Stable emulsions were prepared both at pH 6 and 4 from protein obtained by adsorption chromatography (Løkra *et al.* 2008).

POTATO JUICE

The production of potato starch leads to large amounts of proteinaceous waste water, PJ. The amount of PJ obtained per ton tuber depends on the processing technology, but can be in the range 0.7-7 m^3/ton (Natu *et al.* 1991). About 11% of the global potato crop is utilized for starch manufacture, mainly within the EU.

The principal steps in starch production and its by-products are presented in **Fig. 1**. First, the potatoes are washed

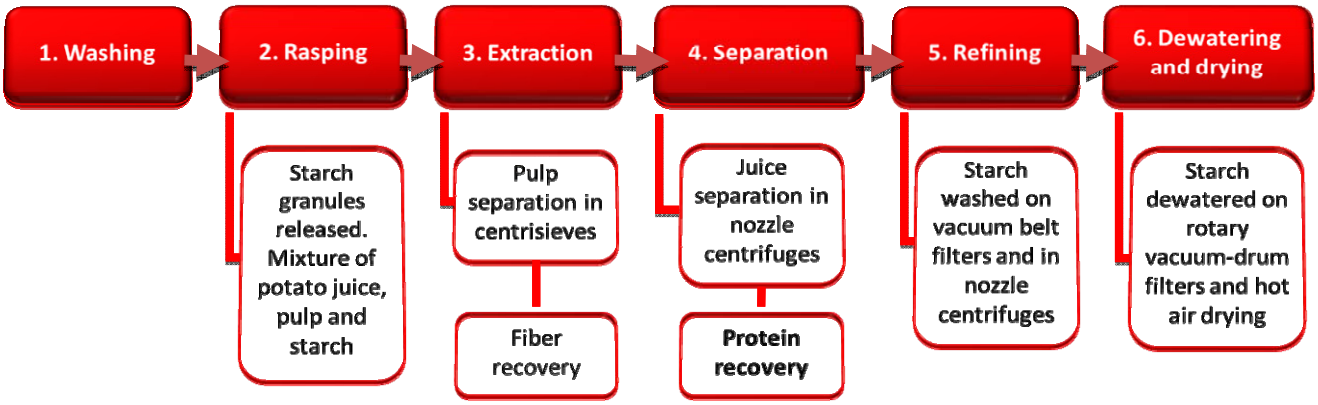

Fig. 1 The principal steps in the manufacturing process from whole potatoes to dry starch product. The schematic process line is shown with pulp extraction before juice separation (the *Gösta Larson*-method), but steps 3 and 4 may be interchanged in other layouts. Typical reclamation and reuse of process water is not shown in this diagram.

Table 2 Average composition of potato juice (adapted from van Koningsveld 2001).

Component	% of dry matter
Protein (N x 6.25)	26.8
Peptides (N x 6.25)	4.4
Amino acids and amides (N x 5.13)	9.6
Other N- containing compounds	1.8
Sugars	15.8
Lipids	2.2
Organic acids	13.2
Chlorogenic acid	0.4
Caffeic acids	0.1
Potassium	11.2
Phosphorus	1.0
Other components	10.1

to remove soil, dirt and other impurities by fluming through a water channel. Next, the potatoes are grinded (rasping) to open the tuber cells and release the starch granules. The result is a mixture of pulp, PJ and starch. Sodium bisulphite is added to this mixture to prevent discoloration by oxidation of phenolic compounds present in potatoes. In subsequent steps, the proteins and fibers can be recovered from side streams of the process. The sequence of unit operations and the technology used may vary between processing plants, but all are focused on minimizing the environmental burden of the waste.

PJ is a complex mixture of several components, making it a challenging feedstock for protein isolation. **Table 2** provides an overview of the major components of PJ. Undiluted PJ contains up to 5% dry matter, and around 20-25% of this dry matter is protein (Knorr *et al.* 1977). The rest consists of free amino acids, glycoalkaloids, sugars, lipids, organic acids, phenolic compounds, minerals and other components (van Koningsveld 2001). The pH of the juice is in the range 5.6-5.9.

The glycoalkaloids and the phenolic compounds are of special interest, because they can cause problems for the subsequent use of potato proteins for human consumption. The most abundant glycoalkaloids present in PJ are α-solanine and α-chaconine (Lisinska *et al.* 1989). Glycoalkaloids have a bitter taste and are toxic to humans and animals at certain levels. Therefore, the protein concentrates have to comply with upper limits for the content of glycoalkaloids to be acceptable for either food or feed use. The other compounds of specific interest are the phenolic compounds (Friedman 1997). Plant phenolic compounds interact with proteins and can alter their surface charge and solubility properties (Rawel *et al.* 2002). Chlorogenic acid (5-*O*-caffeoylquinic acid, CQA), an ester of caffeic acid and quinic acid, is the major phenolic compound in PJ along with caffeic acid. CQA can be oxidised to quinones enzymatically by polyphenol oxidase or non-enzymatically at alkaline pH. The resulting quinones are highly reactive and can e.g. interact with lysine residues on the protein (Pierpoint 1969). The often rapid and significant darkening of the juice which goes from beige, over orange to brown and black, is caused by the polymerization of the dihydroxy phenols in their quinonic state to form water-insoluble melanine type polymers.

RECOVERY TECHNOLOGIES FOR INDUSTRIAL PROTEINS

Largely, the processing strategy determines whether a protein can be utilized for feed or for food. A gentle isolation method can preserve functional properties crucial for food use whereas protein intended for feed often can be isolated by harsher and less expensive methods. The price of the protein on the marketplace is strongly dependant of the purity of the protein and the product titer in the starting material (Brunt 1988). Typically, market prices for feed protein is in the range 0.5-1.0 US dollars per kg, whereas estimates for food protein is in the range 8-10 US dollars per kg for whey protein and potato protein (Olander 2008). This is 4-7 orders of magnitude less than the cost of bioactive proteins for the biopharmaceutical market which require a purity of 99.9%, and thus considerably more extensive means of purification.

Generally, the separation processes available for industrial proteins (10-100 g l^{-1}) fall into three categories: *Precipitation* by adjusting physical properties of the solution, *membrane separation* and *chromatographic separation*. These processes are used both separately and in combination. Typically, precipitation is preferred for proteins for feed/ food use, whereas chromatographic methods, often high resolution, are used for refining proteins further for pharmaceutical use. Foam fractionation is a method for recovery of protein using gas bubble flotation that does not fall into the three categories stated above. It is not widely used commercially, and will not be discussed further.

The following gives an overview of some of the most common recovery methods for potato proteins (**Table 3**). A comprehensive review on the composition, recovery and functional properties was also given by Ralet and Gueguen (Ralet *et al.* 1999). The potato starch industry has evolved various innovative methods to recover protein from their by-product streams. These processes are to some extent proprietary and insights are limited, but the technologies rely on the general principles discussed here. However, since 1997 a number of patents have been issued on the improved recovery and separation of potato proteins. They will be referred to where appropriate. A good transparent example of a novel industrial process is offered through the NewPotatoPro process implemented by the Karup Potato Starch factory, Denmark (newpotatopro.dk).

Table 3 Examples of methods for total protein isolation from potato juice.

Method	Conditions	References
Heat coagulation and acid precipitation	Steam injection at 104-121°C, alternatively heat exchanger at 80-95°C. No pH adjustment (5.6-5.9). Centrifugation or decanting. Adjustment to pH 3-4 with mineral acids (HCl, H_2SO_4, H_3PO_4) or citric acid, followed by heating to 80-90°C. Centrifugation or decanting.	Strolle *et al.* 1973; Meister *et al.* 1976; Knorr *et al.* 1977; Rosenau *et al.* 1978; Knorr 1980; Knorr 1982; Kemmekroonsberg *et al.* 1997; van Koningsveld *et al.* 2001; Ausich *et al.* 2003; Bárta *et al.* 2008
Metal salt precipitation	Addition of $FeCl_3 \cdot 6H_20$, $ZnCl_2$, $Al_2(SO_4)_3$ to reach acidic pH (3-5). Precipitation at room temperature or cold. Washing of pellet.	van Koningsveld *et al.* 2001; Bárta *et al.* 2008
Organic solvent precipitation	20% final concentration of absolute ethanol, methanol, acetone or isopropanol. Incubation ~1 hr, room temperature or cold (0 or 4°C), pH 5.0. Washing of pellet.	Ryan *et al.* 1999; van Koningsveld *et al.* 2002a; van Koningsveld *et al.* 2002b; van Koningsveld *et al.* 2006; Bárta *et al.* 2008
CMC complexation	Carboxymethyl cellulose; 0.1-0.15 g/g protein, pH 2.5 or 4.5.	Wojnowska *et al.* 1981; Gonzalez *et al.* 1991; Vikelouda *et al.* 2004
Membrane separation	Concentration 5-10X by UF or RO; protein retention using molecular weight cut-offs in the range 3-150 kD; membranes of cellulose acetate, polyethersulphone, polyacrylnitrill, various holders.	Eriksson *et al.* 1976; Oosten 1976; Wojnowska *et al.* 1981; Boruch *et al.* 1989; Edens *et al.* 1997; Rüffer *et al.* 1997; Zwijnenberg *et al.* 2002; Giuseppin *et al.* 2008a
Expanded bed adsorption (EBA) chromatography	Expanded bed chromatography; protein adsorption from crude juice at pH 7.5 (patatin) or 4.5-4.8 (total protein), elution at pH 10-12 or pH 6.0. Flow rates 150-600 cm/h.	Strætkvern *et al.* 1999; Strætkvern *et al.* 2002; Strætkvern *et al.* 2005; Claussen *et al.* 2007; Giuseppin *et al.* 2008a

Precipitation

Precipitation with salts, organic solvents or by changing pH is widely used to recover and fractionate industrial proteins. High concentrations of salt (often ammonium sulphate for proteins) lead to the process of salting out. Previously, it was assumed that the salt competed with protein for the water molecules. Now, it is more widely accepted that the precipitation of protein in high salt concentrations is due to the removal of water molecules from the hydrophobic surface of the protein (Scopes 1987). The proteins can then interact hydrophobically, and precipitate out of solution.

Precipitation with water-miscible organic solvents such as ethanol is common. The solvent associates much stronger with water than do the proteins, thus removing the water molecules from the protein surface (Englard *et al.* 1990). The proteins then interact by attractive electrostatic and dipole forces. Organic solvents also decrease the dielectric constant of water, which in effect makes the interactions between charged groups on the protein surface stronger. Older literature classified potato proteins according to their solubility in solvents (water, dilute salt, aqueous alcohol and dilute alkali), giving rise to fractions of albumins, globulins, prolamins and glutelins (Knorr 1978). This classification, however, often gave ambiguous results, and is now abandoned in favor of more defined characterization based on analytical methods.

Potato proteins were successfully isolated by ethanol in lab-scale preparations by van Koningsveld and co-authors in several publications (van Koningsveld *et al.* 2002a, 2002b, 2006). Recently, Bárta and co-workers (2008) also isolated potato proteins from PJ by ethanol, and found higher yield when precipitated at ice cold conditions. In a patent, Ryan *et al*. (1999), describes the extraction in aqueous ethanol of the protease inhibitors. The mixture was first heated either to 50 or 70°C, and then cooled to initiate precipitation. The inhibitor proteins were precipitated from the soluble phase by dialysis against dilute formic acid.

Precipitation by adjusting the pH is commonly used for isolating proteins. The majority of proteins has an isoelectric point at acidic pH, and may be precipitated by an adjustment of pH, often in combination with heat treatment. At the isoelectric point, there is little electrostatic repulsion between molecules (Scopes 1987).

The commercial method for isolating protein from industrial PJ is heat coagulation and acid precipitation (Strolle *et al.* 1973; Meister *et al.* 1976; Knorr *et al.* 1977; Rosenau *et al.* 1978; Knorr 1980, 1982; Ausich *et al.* 2003). Although heat coagulation combined with acid precipitation result in high yields, the protein is of poor quality with low solubility, having discoloration and off-flavor produced by the harsh treatment, mainly caused by associated phenolic compounds and by the oxidation of lipoproteins. The dried protein product is therefore used as feed ingredient.

A comparison of citric acid vs. HCl for precipitation of potato proteins form PJ revealed that citric acid at pH 4 gave higher yield than HCl at pH 3 (Knorr 1982). van Koningsveld and co-authors (2001) also investigated several acids at pH 2.5-5.5, and observed that the yield decreased when increasing the pH from 3 to 5. Because the isoelectric points for most potato proteins are between 4.5 and 6.5, it was not surprising that protein precipitated in this region. They found it, however, surprising that the maximum precipitation occurred at pH values below 4. They concluded that the solubility behavior is different in purified forms of patatin compared to that in PJ, and that precipitation of potato proteins does not seem to be determined only by their isoelectric point. The same authors also compared the yield and solubility of potato proteins precipitated with various metal salts, and found that addition of $FeCl_3$ gave the best resolubility compared with $FeCl_2$ and $ZnCl_2$. Addition of metal salts gave lower yield than precipitation with acid, but resulted in higher proportion of resolubility.

Complexation of potato proteins with charged polymers like carboxymethylcellulose (CMC) has also been investigated (Vikelouda *et al.* 2004). Proteins were complexed with CMC at pH 2.5 from PJ and they found that low concentration of CMC present in the protein precipitate lead to increased solubility and improved emulsion stability.

Membrane separations

Membrane cross flow filtration is now widely employed in the food processing and dairy industries, for clarification, concentration, demineralization, and for wastewater treatment (for reviews, see Daufin *et al*. 2001 and Rausch 2002). Ultrafiltration (UF) is the typical method used for obtaining functional preparations of soy, wheat gluten and whey proteins. UF membranes offer a wide range of molecular cut-off sizes (5-300 kDa), and can be used both to concentrate the protein and to remove minerals and small-size molecules from the product by diafiltration.

Early reports on UF to treat potato starch effluents for protein recovery were published by Oosten (1976) and by Eriksson and Sivik (1976). Since then, UF under various conditions has been reported in only a few publications (Wojnowska *et al.* 1981; Boruch *et al.* 1989; Zwijnenberg *et al.* 2002). The problem for large scale applications has been the extensive membrane fouling reducing permeate flux, and cleaning problems mainly caused by the fibers in the PJ (Eriksson *et al.* 1976). However, this difficulty has apparently been minimized by first inducing flocculation of the pectin fiber at room temperature with addition of $CaHPO_4$. In a patented method, the Ca-flocculated pectin and other insoluble particles were removed before the soluble phase was concentrated by UF and the concentrate then

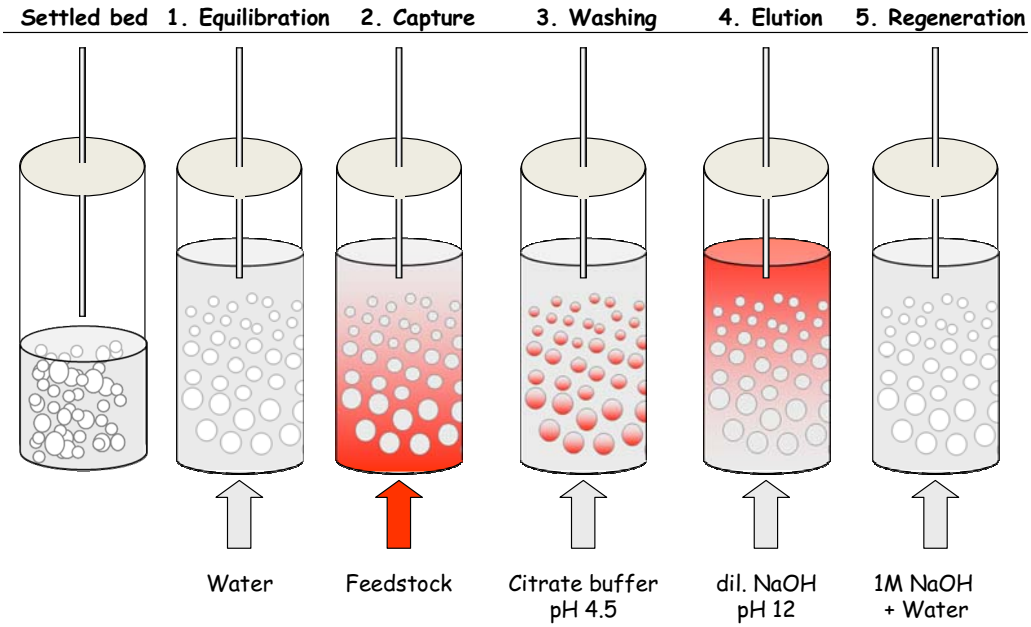

Fig. 2 The sequence of steps in EBA chromatography of potato proteins from PJ, from a settled bed to the elution of proteins and regeneration. The spheres illustrate the disperse resin eventually forming a stable bed of classified particles. Under the column is indicated the choice of buffer for that step. All steps are carried out in the expanded mode.

diafiltrated to remove salts from the native protein product (Edens *et al.* 1997).

In order to conserve protein functionality, membrane processing is a far better option than precipitation although the proteins are susceptible to denaturation by shear. In a lab scale study, ultrafiltration was compared to polyelectrolyte coagulation and cryoconcentration, and the ultrafiltration concentrate gave the highest yield and best functional properties (Wojnowska *et al.* 1981). Zwijnenberg and co-workers (2002) reported on the functional properties of potato protein concentrates, and compared different pore sizes and membrane configurations, both in lab and pilot scale trials. Although little experimental details are described in their report, they concluded that the protein had very good emulsifying and foaming properties. In addition to membrane fouling, efficient separation and reduction of undesirables like glycoalkaloids and phenolic compounds is difficult, partly due to low solubility and adsorption to the protein fraction (Wojnowska *et al.* 1981). Diafiltration is therefore required to improve the product quality (Edens et al. 1997; Zwijnenberg *et al.* 2002).

Reverse osmosis (RO) on the other hand, in which the membranes also retain minerals and small molecules have been employed successfully for several years to pre-concentrate PJ before heat coagulation (Rüffer *et al.* 1997; Rausch 2002). The volume reduction obtained through multistage RO is energy efficient, increases protein yields and improves water re-usage within the whole starch production, but the process is not intended for functional protein recovery.

Adsorption chromatography

Adsorption to stationary supports or resins is well described in chromatography. Typical general purpose adsorbents for macromolecules are ion exchangers and hydrophobic interactions, or more recently combinations of these chemistries (mixed modal). Simulated Moving Bed technology applied to the adsorption chromatography of proteins from PJ was recently reported (Andersson *et al.* 2008). The lab-scale SMB configuration captured approximately 80% of the feed protein using an anion exchanger resin, with a minimum use of extra water.

Expanded bed adsorption (EBA) is a comparatively new chromatographic technology for recovering protein from crude feedstock, and most of the research on EBA has been conducted on capture of biopharmaceutical protein products from fermentation broths (reviewed by Anspach *et al*. 1999). EBA is also possible for other applications, and Barnfield and co-workers (1997) demonstrated that EBA chromatography is a scalable technology. The technology has been demonstrated on a commercial scale for the industrial processing of immunoglobulins from whey (Noel *et al.* 2007), processing about 200,000 L of whey per day resulting in 100 kg immunoglobulins. EBA was first demonstrated by Strætkvern and co-workers (1999) as a promising method for the capture of potato proteins from industrial PJ. An industrial process for obtaining native potato protein fractions by EBA is described in a patent by Giuseppin and co-workers (2008a). Patatin and protease inhibitors can be isolated in separated fractions by manipulating the pH-conditions on the column. The eluted fractions are concentrated further by ultrafiltration in a process which also facilitates removal of glycoalkaloids.

The advantage with EBA is the ability to apply crude feedstock without any preliminary clarification or concentration steps. As for conventional liquid chromatography, the EBA apparatus consists of a column filled with a chromatographic resin but without nets. In traditional column chromatography, the resin is packed with a minimum of voids between top and bottom nets. With crude feeds these nets often lead to clogging of the inlet and therefore demands preliminary steps to clarify the feed prior to chromatography. By contrast, the resin in EBA chromatography is fluidized inside the column, providing a more flexible character. **Fig. 2** shows the typical steps of an EBA run of industrial PJ.

Potato proteins isolated with EBA methodology

Of 5% solids in PJ, about 25% is protein. Several studies have presented methods for isolation of potato proteins from PJ (**Table 3**). The problems with these methods are either severe loss of functionality, high cost/ low efficiency not applicable for large scale production, or high levels of toxic compounds such as glycoalkaloids present in the refined protein product. In order to increase the quality of the final product and achieve a native and palatable protein for human consumption, other methods than heat coagulation and acid precipitation have to be explored.

Chromatographic methods are, as we know them from biotechnological applications, cost intensive both on invest-

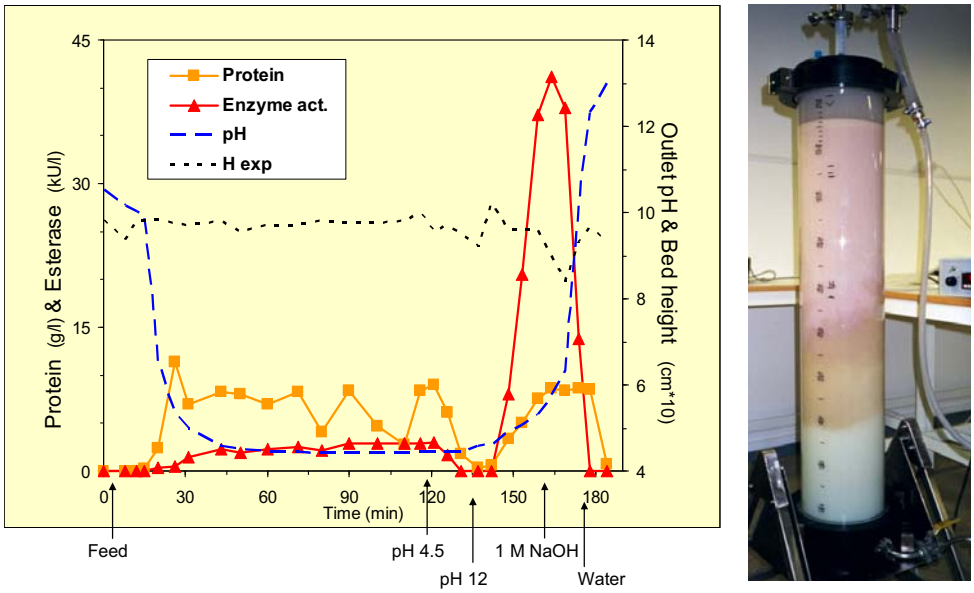

Fig. 3 (**A**) Chromatographic profile of potato protein recovery on pilot scale EBA column (20 cm × 1 m, CV = 17 L mixed mode resin). The feed load was 85 liter PJ (5X CV) at pH 4.5, and the mean flow rate 161 cm h^{-1} in upward direction. The column was washed with 20 mM citric acid pH 4.5, eluted in 10 mM sodium hydroxide (pH 12), and regenerated with a pulse of 1 M sodium hydroxide. The column sequence was run as outlined in **Fig. 2**. The profile includes assays of protein (Biuret) and esterase (patatin), and measurement of pH and expanded bed height (exp). (**B**) Photo of the pilot scale EBA column during desorption of the potato protein: Upper column half shows the protein zone approaching the top outlet; below in the column mid section are colored pigments, and at the bottom, the resin bed is regenerated. (Adapted from Strætkvern *et al*. 2002, ©2008 General Electric Company, Reproduced by permission of the owner).

ment and in operation. However, adsorption chromatography is a gentle isolation technique able to conserve the nativity of proteins. The problem with using conventional chromatography for the recovery of proteins from PJ is due to the complexity and the particulates of the PJ. Mandatory treatment of this effluent prior to feeding on packed bed columns require clarification and dilution before ion-exchange, or adding salts before hydrophobic interaction chromatography. From the use of EBA for potato protein isolation it became clear that this was a feasible and simple method, which needed further exploration (Strætkvern *et al*. 1999). The only pre-conditioning of the crude PJ before adsorption is a pH adjustment. The intention of using EBA as a mean of isolating potato proteins from PJ was to produce a refined protein concentrate for human consumption. It has been argued that the EBA method would be too expensive for industrial applications (van Koningsveld 2001). No studies though have reported on the process economy of EBA on PJ in the published literature.

Fig. 3A shows the chromatographic profile obtained for protein capture on a pilot scale EBA column using PJ at pH 4.5 (Strætkvern *et al.* 2002). In addition to the protein tracing, also the esterase activity of the patatin was analyzed. The chromatographic profile shows that patatin make up a significant part of the breakthrough material, but still the majority of esterase activity is captured on the column at pH 4.5. Protease inhibitors are captured as well. The adsorbed proteins are then eluted at alkaline pH, and the peak coincides with the steep rise in outlet pH. Not evident from the chromatogram during the elution step, but illustrated in **Fig. 3B,** is the separation of proteins from the dark colored pigments (discussed below).

Results from pilot-scale EBA experiments using PJ from a commercial starch plant (Strætkvern *et al.* 2005) showed a recovery of 85.8% patatin esterase activity and capture efficiency of 53.3%. With smaller and denser resin particles, the authors believed the efficiency could be increased. An obvious drawback with EBA is the large consumption of liquid in buffer and washing steps. The process does not solve the need for effluent treatment, but the possibility of obtaining highly refined protein fractions gives an increased value to the byproduct. Reuse of the process water should be carefully considered. The higher the capture efficiency, the more effluent can be treated in one column cycle. This has been successfully demonstrated on commercial scale for whey protein (Noel *et al.* 2007).

Removal of undesirables

Besides obtaining a native protein, chemical characteristics of a protein concentrate ultimately affect functional properties and applications. For feed and food, there are maximum levels for the content of certain compounds. In addition, certain compounds can be safe to consume, but produce off-flavors or discoloration not accepted by the consumer. One of the anticipated benefits of using EBA for recovery of food grade proteins was to exclude phenolic compounds and glycoalkaloids or reduce them to an acceptable level.

α-solanine and α-chaconine constitute the total glycoalkaloids (TGA) in potato, which in mature tubers ranges from 3 to 10 mg/100 g (Lisinska *et al.* 1989). A recommended upper food safety limit of TGA in fresh potato is 20 mg/100 g (200 ppm) (Friedman *et al.* 1997). A ruling from the EU-commission on TGA in potato protein for food purposes set a limit at 150 ppm (Byrne 2002). Apart from the toxicity, 140-170 ppm has been observed as a taste threshold for the glycoalkaloids (Giuseppin *et al.* 2008a).

A level of 48 ppm was obtained in EBA preparations which was a 5-fold reduction compared to an acid and heat-coagulated precipitated commercial preparation (Løkra *et al.* 2008). Only a few publications report on TGA values in potato protein preparations. Wojnowska and co-authors (1981) reported on the glycoalkaloid content of potato proteins isolated by ultrafiltration and cryoconcentration, and found content in the range 360-370 mg/100 g dw. An industry-implemented and patented process of washing heat-coagulated protein with mineral acids reduces the TGA content to < 100 ppm, making the product more acceptable as feed ingredient (Kemmenkroonsberg *et al.* 1997). In a salmon feeding experiment, the commercial product performed satisfactory compared with control feeds (Refstie *et al.* 2003). Active carbon, either by batch adsorption or by passage in a column, is described as a means to reduce TGA below 15 ppm in EBA protein isolates (Giuseppin *et al.* 2008b).

Color pigments present in PJ are also desirable to ex-

clude from the protein concentrate. Not only because of the presumable interaction with protein but also because a white product is more attractive. Colored compounds bind to the EBA column material; Strætkvern and co-authors (1999) described a black-tinting of the adsorbent resin during the loading step of the EBA experiment. In addition, black color is visible in the later stages in elution after the protein fraction from EBA runs with the resin due to differences in retention time (**Fig. 3B**). Because the elution mode is isocratic (alkaline pH), separation of protein and pigments is augmented by the gel sieving effect of the resin. Thus, an increased length of the expanded bed improves resolution.

CONCLUSIONS

In summary, the recovery methods for industrial proteins are numerous, and the choice of method highly affects the later applications of the protein. The most commonly used commercially method is precipitation resulting in high yields, which is also used for potato protein recovery. In order to improve solubility and functional properties of the protein, other methods including membrane separation and chromatographic methods can be employed. One of these methods is EBA chromatography, which has shown promising ability to separate native proteins from crude liquids.

Based on the quality of proteins, EBA can be added to the repertoire of isolation methods for industrial protein. The technology provides preparations higher in protein than many other methods, and provides value-added fractions of improved quality and functionalities (color, phenolics, solubility). Supporting this conclusion was the opening of the potato purification plant at Solanic (a subsidiary of AVEBE; solanic.eu) in 2007 in The Netherlands. The company employs the EBA technology commercially for the recovery of potato proteins, and claims to be able to produce high-value, functional proteins from the sidestream of potato starch production (Olander 2008).

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