

High-Value proteins from crude whey In a Rhobust® manner

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The competitive benefits that dairy manufacturers are gaining from employing Expanded Bed Adsorption (EBA) chromatography combined with membrane filtration for the purification of high value food protein ingredients from whey is discussed herein.

Trendy whey proteins

One of the main by-products of cheese production is proteins which come in the liquefied state, also known as whey. Whey proteins are in high demand among food and beverage producers after the scientists have discovered their outstanding functionalities i.e. emulsification, foaming, gelation, water binding and unique health benefits. It was established that whey proteins contain all essential amino acids required by the human body and deliver a full spectrum of health benefits such as immune modulation, intestinal health, antibacterial properties against pathogenic bacteria, appetite suppression/weight loss, apoptosis of some types of cancer cells, release of bioactive peptides, and antihypertensive peptides (*Global Industry Analysts, Inc. (March 2008) Protein Ingredients – A Global Strategic Business Report*).

Due to their ability to get metabolized directly into muscle tissue and prevent muscle breakdown, whey proteins have found their first applications in the sports nutrition market where they are used as ingredients for energy bars, isotonic and sports drinks. Further on, high nutritional value and neutral taste of whey proteins resulted in their increased popularity in other markets as well, for example among producers of functional beverages and children beverages.

Not surprisingly, dairy producers are always looking for better, more efficient ways to isolate all these valuable proteins in order to keep up with the market demands for value-added food ingredients which are free from contaminating sugars, fat, cholesterol and off-flavours. The conventional process employed for whey protein recovery today is membrane filtration, which results in the production of whey protein concentrate (WPC) with protein levels ranging from 35% to 85%, or whey protein isolate (WPI) which contains greater than 90% proteins.

Upfront's scientists have developed a new process that combines membrane filtration with its proprietary Rhobust® EBA chromatography and have introduced it to the dairy market. This combination of technologies enables the range of benefits from both technologies to be fully exploited, which includes new high quality products with >95% purity proteins, novel selective processes for specific market needs, plant and process flexibility and low water, energy and chemical consumption. The Rhobust® Whey Refinery is a short cut to higher yields and competitive cost profiles which will allow the companies to stay competitive in the by-product area.

EBA chromatography for higher purity and significant cost savings

Chromatography was traditionally perceived as a complex and expensive technology which was limited to the small scale production of biopharmaceuticals. However, Upfront overcame the initial problems associated with scaling up protein adsorption chromatography and developed a new design of EBA to create the Rhobust® Whey Refinery platform which is available for the commercial production of high purity proteins from industrial side streams, including whey proteins.

The Rhobust® platform was first commissioned and applied at an industrial plant in 2002 by Dairy Farmers, one of the largest Australian cooperatives. Chromatographic columns with a 1.5 m diameter were installed to capture whey bioactive proteins i.e. lactoferrin, lactoperoxidase and immunoglobulin G (IgGs). The plant had the capacity to process several hundred thousand litre of cheese whey per day at a linear flow rate of up to 2000 cm/hr.

The application of Rhobust® in the dairy industry was successfully followed up with a new, major food ingredient industry player, the Dutch company AVEBE/Solanic in 2007, when a new factory was constructed for isolation of proteins from potato starch side streams based on Upfront's novel technology.

Both cases indicate that the Rhobust® Whey Refinery is a very promising tool for the industrial production of value-added by products in the food ingredient and dairy industry.

Rhobust® Whey Refinery process

Upfront's Whey Refinery process combines the Rhobust® EBA chromatography platform with conventional membrane filtration, evaporation and drying units already installed in the dairy facilities. This setup provides a flexible approach to

wey refinery operations allowing the individual wey components to be isolated, concentrated and dried into high value ingredients with reduction of time and process steps.

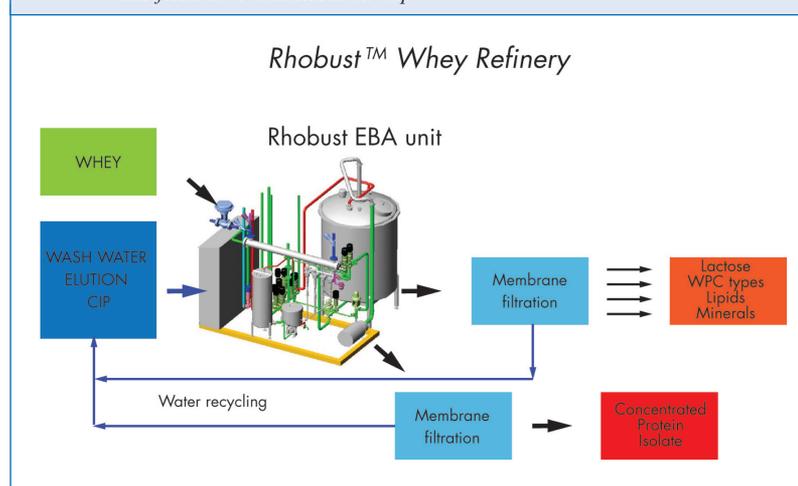
The Rhobust® Wey Refinery platform has been standardized into a unit system that make it easy to install and operate in any dairy facility. The key feature of the unit is that it is automatically operated and contains all the equipment, instrumentation and automation necessary to perform the various chromatography steps.

The following products are currently purified by the Rhobust® Wey Refinery:

- WPI (Wey Protein Isolates) >95 % purity;
- high purity α -lactalbumin and α -lactalbumin enriched WPI;
- β -lactoglobulin depleted WPI;
- β -lactoglobulin >95 % purity;
- Immunoglobulin enriched WPI;
- Glycomacropeptide (GMP)
- Lactoferrin

All protein products have improved functionality for food ingredient usage, whilst the gentle EBA purification process conserves the biological activity of protein intended for nutraceutical applications. They are largely free of lipids, carbohydrates and minerals and have a neutral taste profile.

Figure 1 The combination of Upfront's Rhobust® unit with advanced membrane techniques enables industrial utilization of adsorption chromatography and the EBA format enables direct adsorption from crude feed stocks without prior clarification or concentration steps



Greater value – separating α -lactalbumin and β -lactoglobulin

EBA chromatography helps with solving product purity challenges. This is particularly important for infant formula producers who struggle to find ways to obtain a higher quality product. One of the challenges they face is connected with improving the separation of α -lactalbumin, a major human breast milk protein, rich in essential amino acids required for a baby's growth and development, from unwanted β -lactoglobulin which is allergenic.

The Rhobust® Wey Refinery provides the perfect solution for producing higher purity, allergen-free, infant formula enriched with higher concentrations of desirable α -lactalbumin i.e. mimics the natural human milk product. At the same time the technology allows isolation of highly concentrated β -lactoglobulin, which shows excellent foam formation, water-binding and gelation properties and is ideal as an egg white replacer, fat reducer and a source for proteins in clear flavoured drinks.

The Wey Refinery based on Rhobust® EBA ensures extremely advanced levels of selectivity allowing isolation of individual wey proteins in much higher concentrations than any other existing method. It is an excellent tool for the dairy industry to manufacture multiple value-added protein products.

It removes the need for harsh heat treatment or clarification steps, which means that the native structure of the protein is preserved while costs are significantly reduced.

Results show that the ratio of α -lactalbumin to β -lactoglobulin in the end product obtained with EBA is 5:1 to 10:1. *Figure 2* is the image of an SDS PAGE gel that has been stained to detect proteins and represents the electrophoretic pattern of purification of α -lactalbumin and β -lactoglobulin from crude sweet wey. Each protein in the mixture is separated according to its electrophoretic mobility and the intensity of staining signifies the product concentration.

First lane: composition of the crude sweet wey.

Second lane: composition of the flow through without any proteins except GMP.

Third lane: protein pattern for β -lactoglobulin with a purity of > 90 %.

Fourth lane: pattern for the α -lactalbumin fraction with > 50 % α -lactalbumin and < 5 – 10 % β -lactoglobulin. With relevance to infant formulas, lane four shows that the product contains large quantities of the highly desirable α -lactalbumin and very little of the β -lactoglobulin.

Economic with environment-friendly technology

Recycling of water and buffer chemicals is an attractive benefit of the production process because it helps to save costs and protect the environment. EBA chromatography requires low concentrations of buffers which minimise the use of chemicals; these can also be partially recovered by means of conventional membrane filtration. Thus, the majority of buffer chemicals including NaCl are re-used in the process of α -lactalbumin and β -lactoglobulin production equating to a significant saving in chemical costs.

The Rhobust® Whey Refinery is a low cost route to production of highly purified and differentiated value added products with multiple functionalities. It is now ready to face the market expectations for the increased product quality standards.

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Upfront Chromatography in a nutshell

Upfront Chromatography A/S is a Denmark-based biotech company with significant expertise and regulatory experience in the development of customised industrial processes. Upfront develops and manufactures innovative products and technologies for extraction and recovery of functional biomolecules, macromolecular complexes, and even living cells, directly from bioreactors and industrial side-streams. For customised separation services, Upfront offers access to Rhobust®, a universal process platform based on proprietary 2nd Generation EBA chromatography combined with extensive technical and regulatory support. From a feasibility study to commissioning of the final installation, Upfront works with its customers to develop adsorbents, ligand chemistry, columns and other hardware to optimise process performance. Upfront is certified with the requirements of DS/EN ISO 9001:2000.

Figure 2 SDS-PAGE (non-reduced) gel showing the electrophoretic pattern of β -lactoglobulin and α -lactalbumin enriched WPI

