# **Filtration & Separation**



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How to make whey into high value functional ingredients? Natalya Clark of Upfront Chromatography explains

Ver the past few decades whey has shifted from being an unwanted waste stream generated during cheese production to the starting material for producing high value functional food ingredients. The exceptional functional and nutritional properties of whey proteins has led to food formulators crowning it the "king protein." With a protein content of approximately five to seven g/l whey has an abundance of bioactive ingredients, including  $\alpha$ -lactalbumin, immunoglobulins, lactoferrin and lactoperoxidase, which are renowned for their high nutritional properties and ability to enhance the human immune defence system.

Unsurprisingly, dairy producers are always looking for better, more efficient ways to isolate all these valuable proteins so that they can make their ideal food ingredients free from contaminating sugars, fat, cholesterol and off-flavours. Membrane filtration is the most common processing technology used for whey protein recovery and produces proteins either in the form of whey protein concentrate (WPC) with protein levels ranging from 35 per cent to 85 per cent, or whey protein isolate (WPI) which contains greater than 90 per cent proteins.

"We have challenged the conventional membrane filtration methods by bringing chromatography to dairy manufacturers. Our Rhobust Whey Refinery chromatography platform allows these manufacturers to process all proteins from whey to quality WPI products with superior standards," notes Allan Lihme, CTO of Upfront Chromatography.

## Higher purity

Chromatography was traditionally seen as a complex and expensive technology, which was limited to the small scale production of biopharmaceuticals. Upfront overcame the initial problems associated with scaling up expanded Rhobust Whey Refinery platform, which is available for the commercial production of high purity proteins from industrial side streams including high value added food ingredients from whey. The low manufacturing costs that arise from using it has enabled higher profit margins. The Rhobust platform was first commissioned and

bed adsorption (EBA) chromatography to produce the

The Rhobust platform was first commissioned and applied at a large-scale plant in 2002 by Dairy Farmers, one of the largest Australian cooperatives. Chromatographic columns with a 1.5m diameter were installed to capture whey bio-active proteins ie lactoferrin, lactoperoxidase and immunoglobulin G (IgGs). The plant had the capacity to process several hundred thousand litres of cheese whey per day at a linear flow rate of up to 2,000 cm/hr.

"Our success with Dairy Farmers and other companies has led us to believe that the Rhobust Whey Refinery will continue to be widely used as an industrial tool for largescale dairy production," Lihme says.

#### The process

The refinery process combines the EBA chromatography platform with conventional membrane filtration,

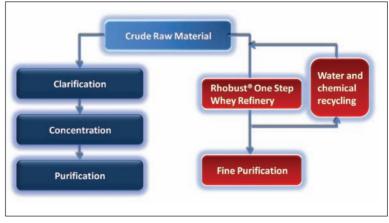


Figure 1. The Rhobust Whey Refinery eliminates traditional pre-treatment steps

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evaporation and drying units that are already used in dairy facilities. This provides a flexible approach to whey refinery operations allowing the individual whey components to be isolated, concentrated and dried into high value products.

The platform has been standardised into a unit system that makes it easy to install and operate. A key feature of the unit is that it is built on a frame that contains all the equipment, instrumentation and automation necessary to perform the numerous and various chromatography steps.

Rhobust EBA technology ensures, as the name implies, a robust one-step process that eliminates traditional centrifuge and protective filtration steps. Instead, crude whey is treated directly in the chromatographic column and various particulates of cheese whey such as fat micelles and small cheese particles can pass freely together with lactose, minerals, fat globules and various low molecular weight substances. The resin adsorbs the proteins, which are later eluted with buffers at different pH values.

The EBA process design eliminates the pre-treatment of whey required in conventional membrane processing, which still produces a fat-containing by-product with limited applications.

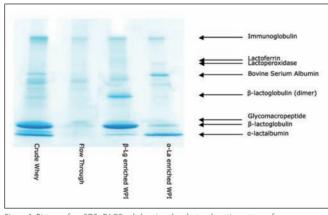


Figure 2. Picture of an SDS\_PAGE gel showing the electrophoretic pattern of  $\alpha$ -lactoglobulin and  $\beta$ -lactalbumin enriched WPI

### Separating $\alpha$ -lactalbumin and $\beta$ -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  $\alpha$ -lactalbumin, a major human breast milk protein, rich in essential amino acids required for a baby's growth and development, from unwanted  $\beta$ -lactoglobulin, which is allergenic.

The Rhobust Whey Refinery provides the perfect solution for producing higher purity, allergen-free, infant formula enriched with higher concentrations of desirable  $\alpha$ -*lactalbumin* – mimicking the natural human milk product. At the same time the technology allows isolation of highly concentrated  $\beta$ -*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 whey refinery based on Rhobust EBA ensures extremely advanced levels of selectivity allowing isolation of individual whey proteins in much higher concentrations than any other existing method," Lihme states. "It is an excellent tool for the dairy industry to manufacture multiple value-added protein products."

Results on the use of Rhobust whey refinery have been compiled in a white paper by Werner Kofod Nielsens and with research from Niels-Kristian Østergaard at Upfront (available on request). They show that the ratio of  $\alpha$ -lactalbumin to  $\beta$ -lactoglobulin in the end product obtained with EBA equals 4:1 to 6:1 versus 1:1 to 2:1 for other methods. Figure 2 shows a picture of a SDSMPAGE gel that has been stained to detect proteins: the electrophoretic pattern of crude whey (lane 1), flow through (lane 2) and  $\beta$ -lactoglobulin enriched WPI (lane 3) and  $\alpha$ -lactalbumin (lane 4) enriched WPI are shown. (Each protein in the mixture is separated according to its electrophoretic mobility and the intensity of staining signifies the product concentration.) With relevance to infant formulas, lane four shows that the product contains large quantities of the highly desirable  $\alpha$ -lactalbumin and very little of the  $\beta$ -lactoglobulin.

Overall, the flexibility afforded by the Rhobust whey refinery design allows high purification levels to be attained for a number of products, including but not limited to: +95 per cent WPI, high purity  $\alpha$ -lactalbumin and  $\alpha$ -lactalbumin enriched WPI;  $\beta$ -lactoglobulin depleted WPI; +95 per cent  $\beta$ -lactoglobulin, immunoglobulin enriched WPI; glycomacropeptide and lactoperoxidase.

#### Saving costs

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The recycling of water and buffer chemicals is an excellent way to save costs and protect the environment. A major economical and environmental concern when purifying whey protein fractions is that water consumption can increase by up to 70 per cent of the total volume of processed raw material. An additional advantage of Rhobust EBA Whey Refinery is that it reduces water consumption.

EBA chromatography also requires low concentrations of buffers, which minimise the use of chemicals; these can also be partially recovered by means of conventional membrane filtration. Thus, 80 per cent of buffer chemicals including NaCl are re-used in the process of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin production equating to a saving of 67 per cent in chemical costs.

"We are happy to bring our low cost route to production of highly purified differentiated value added products with multiple functionalities," concludes Lihme. DDD

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