Fractionation of milk proteins

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No group of proteins has been as extensively investigated or as well characterized as bovine milk proteins. This can be attributed not only to the importance of milk as a food and as a raw material for a vast processing industry, but also to its convenience as an abundant source of relatively pure protein of varied structure and properties. In spite of the vast range of information available, however, new dairy protein products are a rarity, and the existing range of such products has seen little change in the last 15 years. This is not because of a scarcity of good product ideas, but because of technological and commercial constraints associated with their exploitation. The single most important factor to be considered in any process for separation of milk proteins is scale of operation. Invariably, the requirement is for bulk production and the technical challenges presented by a process involving tonnages of product or hundreds of thousands of litres of process stream are quite different from those familiar to the laboratory scientist. Many of the constraints and requirements are dictated by economic considerations given that, in most cases, the product value is closer to that of commodity protein than of a typical pharmaceutical product. Major factors, therefore, are such things as plant throughput, cost of downstream product recovery and capital cost of separation equipment. Adding to these constraints is the fact that protein damage, during separation, may be no more acceptable than in a conventional biochemical process. Taking these factors into consideration, it is not surprising that relatively few protein fractionation processes have been successfully introduced into the dairy industry. By far the most important are those based on selective precipitation, exploiting the unique properties of the most abundant class of milk proteins, the caseins, which are readily prepared from milk by acid or rennet coagulation. Casein is a major commodity product with world output at approx. 250 000 tonnes and its production represents the only true protein–protein separation process in large-scale commercial use in the dairy industry. Other separation technologies in dairy protein manufacture, such as membrane filtration and adsorption chromatography are largely employed for removal of non-protein components, principally lactose and minerals, from protein. Nonetheless, exciting possibilities for innovative protein products, based on these technologies, do exist. This paper therefore deals with new developments, currently under investigation or just recently introduced into commercial operation.

The main milk protein classes, casein and whey protein, consist of several distinct components with potential in a wide variety of food and pharmaceutical outlets (Table 1). Most attention has been directed to whey proteins since, as globular proteins with unrelated primary structures, they present a wide diversity of functional and biological properties. Of particular interest in this respect are the antimicrobial whey proteins, immunoglobulins, lactoperoxidase (LP) and lactoferrin (LF) which account, respectively, for 15%, 1.5% and 0.5% of total whey proteins. Prospects for the use of these proteins in neonate feed and in health-care products, as protection against pathogenic organisms, have been reviewed [1, 2] and are the subject of several patents and commercial proposals [3–8].

Ion-exchange chromatography is the most favoured method for isolation of whey antimicrobial proteins, being facilitated by the relatively high pl values of these proteins. Several laboratory separations have been described [9–12]. Similarly, interest in the industrial extraction of whey antimicrobial proteins has focused mainly on ion-exchange technology. This technology is not new to the dairy industry, having been originally introduced for demineralization of whey. In more recent times, considerable progress has been made in the development of exchangers for protein adsorption, mainly for the extraction of total whey protein in denatured form, but with the additional objective of achieving protein–protein separations on a large scale. Development of new exchangers for industrial use is necessitated by the unsuitability of most existing laboratory products which fail to meet certain criteria that are vital for commercial viability. The most important of these criteria are listed in Table 2. Of major significance is operating capacity, i.e. the amount of protein adsorbed per unit weight (or volume) of exchanger during actual process operation. This is directly related to the density and accessibility of functional groups and to the kinetics of adsorption. A thorough investigation of this subject has been reported [13, 14]. Decrease in the ratio of exchanger to protein improves capacity, but leads to a reduction in the efficiency of protein extraction. The only meaningful capacity figures, therefore, are those achieved at ratios which allow good protein recovery (at least 70–80%) and where the time for adsorption is acceptable. These and other properties listed in Table 2 determine several factors which are vital to economic viability, such as plant throughput, plant size, concentration of protein in effluent stream and quantity of chemicals used for desorption or pH adjustment. Avoidance of protein denaturation is a further major requirement of industrial exchangers. The impact of denaturation on biological activity of protein is obvious, but it can be equally important for functional properties, i.e. protein characteristics such as gel formation, foam and emulsion stabilization, solubility and water binding capacity, all of which are vital for use of proteins as food ingredients [15].

In the past 10 years, a small number of exchangers have been developed specifically for industrial-scale protein extraction (Table 3). Of these, only three are known to be in or near commercial use, namely Spherosil, Indion and carri-genan/alginate. Spherosil ion-exchangers, developed by Rhone-Poulenc, are made from porous silica beads coated with a polymer containing the desired functional group or used directly in the ungrafted form exploiting the weakly acidic properties of SIOH groups [16–18]. The defined size range of Spherosil beads and their rigidity facilitate their use in a fixed-bed column process. The main use described for Spherosil exchangers is isolation of total whey protein, and with appropriate choice of functional group, direct application to either acid (pH 4.6) or sweet (pH 6.6) whey is
possible. For sweet whey, a two-column process is used which allows the separate isolation of a cationic protein fraction rich in immunoglobulins. Some denaturation of protein appears to occur, at least under laboratory conditions and, as a fuller evaluation of this and its effect on functional or biological properties is required.

Indion S, a high-capacity cation exchanger, is used for whey protein extraction by the Bioisolates process [13]. Two plants have been established, capable of producing several hundred tonnes of product annually, of which one is still in operation [20]. This uses two large exchanger units, operating in tandem, to process 27,500 l/h of whey, and is capable of an output of over 2 tonnes/day of whey protein. The Bioisolates process is a stirred-tank reactor process with a 2 h cycle; adsorption is at pH 3.0 and desorption at pH 9.0, yielding a 2% protein eluant. In laboratory trials, a protein recovery from acid whey of 75% was established [14]. In the same study, a comparison was made between column and batch (i.e. stirred tank) processes using Indion S. The main advantages of the column process were higher operating capacity, smaller reactor size and higher protein concentration in the eluant. These were offset by some disadvantages mainly associated with the slower kinetics of adsorption of a column process. For total whey protein isolation, a batch process was preferred because of its simplicity and shorter cycle time. However, a column process might be preferable for protein-protein separation, but no investigation of this has been reported.

Several ion-exchange processes specifically for large-scale antimicrobial protein separation have been developed. Spring & Peyrouset [23] described a column process applicable to sweet whey, using pulverized silica of particle size 5–500 μm. Selective adsorption of cationic proteins at pH 8.2 followed by step-wise desorption yielded two products, the first an LF concentrate and the second an immunoglobulin/LP mixture. The reported yields of LF suggest poor recovery using this process. LF of good purity is extractable from skimmed milk or whey using the weakly acidic ion exchangers CM-Toyopearl 650M, CM-Sepharose FF and Sepabeads FP-CM13 [24]. Yields are moderate and the exchangers used, which are essentially of the laboratory type, are unlikely to be suitable for industrial scale operation. An immunoaffinity process for LF isolation from milk has also been described [25], but, similarly, its cost effectiveness for industrial use is questionable. A process for LF which is in commercial operation uses alginate or carrageenan beads [26]. The main characteristic of the process is the use of polysaccharide beads with low protein permeability so that adsorption is limited to the surface. Either a batch or column process may be employed and proteins are adsorbed directly from skimmed milk or sweet whey without pH adjustment and desorbed with aqueous CaCl₂ yielding a mixture of LF and LP with excellent recovery.

Several methods have been reported for separation of whey proteins by selective precipitation using various precipitating agents [27] for a recent review). None are known to be in current commercial use. Since the presence of precipitant in these products has posed a major problem, a recent approach to selective precipitation, not requiring additives, has attracted considerable attention. This is based
on the aggregation to a fine floc of α-lactalbumin in acid media under controlled conditions of ionic strength and temperature [28, 29]. Both products of the process, purified α-lactalbumin and α-lactalbumin–depleted whey protein, have potential as nutritional or functional food ingredients. Scale-up of floc separation presents some difficulties that have yet to be resolved.

The most widely used molecular separation technology in the dairy industry is membrane filtration. Within the past 20 years, use of this technology for production of whey protein concentrates or for concentration of milk has become widespread, which have greatly enhanced membrane performance [30].

To date, however, application is confined predominantly to the permeate stream of small molecules such as water and lactose, and early aspirations to apply the technology to protein–protein separations have not been realised. A major characteristic of the operation of membranes with complex materials such as milk is the occurrence of ‘concentration polarization’, the build-up of rejected particles/molecules (mainly protein, fat) at the surface of the membrane, leading to reduced membrane flux and gradual blockage of the membrane pores (fouling). This alters permeation characteristics in a way that can reduce greatly the molecular exclusion limits of the membrane and thereby impair resolution of protein mixtures. It has been suggested that a solution to this problem may be in sight with the advent of a new generation of membranes of ceramic composition (ZrO2, Al2O3, CsI) [31, 32]. A feature of these membrane systems is pore homogeneity and high tangential flow velocities. This impairs concentration polarization and maintains pore fidelity during continuous operation. Currently, these membranes are manufactured with pore sizes of 0.08–5 μm, for operation in the microfiltration range, and have been used for clarification of fine suspensions or separation of bacteria and large proteins such as immunoglobulins [31–34]. More widespread application to protein–protein separations can be expected, in particular where a substantial differential in protein size exists. Separations which exploit the aggregation behaviour of caseins can be anticipated, leading to a new approach to whey protein–casein separation or to production of individual caseins such as β-casein [31].

In conclusion, technology for new protein–protein separations in the dairy industry is in its infancy, but recent advances in technology and the potential for enhancing the value of milk proteins through separation, should lead to the emergence of new milk protein products in the not-too-distant future.

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Manufacture of diagnostic enzymes

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Diagnostic enzymes are extensively used in the analysis of clinical samples for the diagnosis of disease. Enzymes are utilized in various methodologies [1–4] including spectrophotometric assays, enzyme-linked immunoassays, multilayer films, dry reagent strips and the emerging technology of biosensors. The enzymes utilized in these methodologies are selected to exhibit high specificity for the target analyte and purified to achieve low background rates and extended reagent shelf-life. The competitive market pressure on product price and quality requires that new manufacturing processes are developed with elevated yields and greater product purity. These pressures ensure that diagnostic