SYMPOSIUM: ADVANCES IN DAIRY FOODS PROCESSING AND ENGINEERING

New Opportunities from the Isolation and Utilization of Whey Proteins

GEOFFREY W. SMITHERS,[1] F. JOHN BALLARD,[2] ADAM D. COPELAND, KIRTHI J. DE SILVA, DAVID A. DIONYSIUS,[3] GEOFFREY L. FRANCIS,[2] CHRIS GODDARD,[2] PAUL A. GRIEVE,[3] GRAEME H. MCINTOSH,[4] IAN R. MITCHELL, R. JOHN PEARCE, and GEOFFREY O. REGESTER[5]
Division of Food Science and Technology-Melbourne Laboratory, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Highett, Victoria 3190, Australia

ABSTRACT

Management of dairy whey has often involved implementation of the most economical disposal methods, including discharge into waterways and onto fields or simple processing into low value commodity powders. These methods have been, and continue to be, restricted by environmental regulations and the cyclical variations in price associated with commodity products. In any modern regimen for whey management, the focus must therefore be on maximizing the value of available whey solids through greater and more varied utilization of the whey components. The whey protein constituents offer tremendous opportunities. Although whey represents a rich source of proteins with diverse food properties for nutritional, biological, and functional applications, commercial exploitation of these proteins has not been widespread because of a restricted applications base, a lack of viable industrial technologies for protein fractionation, and inconsistency in product quality. These shortcomings are being addressed through the development of novel and commercially relevant whey processing technologies, the preparation of new whey protein fractions, and the exploitation of the properties of these fractions in food and in nontraditional applications. Examples include the following developments: 1) whey proteins as physiologically functional food ingredients, 2) α-lactalbumin and β-lactoglobulin as nutritional and specialized physically functional food ingredients, and 3) minor protein components as specialized food ingredients and as important biotechnological reagents. Specific examples include the isolation and utilization of lactoferrin and the replacement of fetal bovine serum in tissue cell culture applications with a growth factor extract isolated from whey.
(**Key words**: whey proteins, functionality, dairy protein food ingredients, medical applications)

INTRODUCTION

Whey, the yellow-green liquid that separates from the curd during manufacture of cheese and casein, has long been considered by the dairy industry to be a waste by-product and, thus, a disposal problem. Volumes of whey continue to grow around the world and currently amount to >80 × 10^9 L/yr. Analysis of the gross composition of whey (Table 1) reveals two features of particular significance to its disposal. First, whey contains essentially 100% of the total milk carbohydrate (lactose), and, second, whey contains about 20% of the total milk protein. These components are responsible for the high putrescibility and biological oxygen demand of whey and have, for some time, precluded its disposal as untreated sewage. Thus, management of this waste stream has, in the main, involved implementation of the most economical alternative regimen for disposal. Such management has included simple discharge into waterways, into the ocean, and onto fields or incorporation into low cost animal feed. The advent of strict environmental regulations worldwide has encouraged the dairy industry to reappraise waste management in general and whey disposal more specifically. Re-

Received June 26, 1995.
Accepted December 27, 1995.
[1]To whom correspondence should be addressed.
[2]Cooperative Research Centre for Tissue Growth and Repair, Adelaide, South Australia 5000, Australia.
[3]Queensland Department of Primary Industries, Centre for Food Technology, Hamilton, Queensland 4007, Australia.
[4]CSIRO Division of Human Nutrition, Adelaide, South Australia 5000, Australia.
[5]Current address: Child Health Research Institute Inc., Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia.

TABLE 1. Typical composition of bovine milk and whey.

Component	Concentration	
	Milk	Whey
	(%, wt/vol)	
Casein protein	2.8	0.0
Whey protein	0.7	0.7
Fat	3.7	0.05
Ash	0.7	0.7
Lactose	4.9	4.9
Total solids	12.8	6.35

cently, relatively high prices for whey powder, together with pressure from environmental agencies, have caused much whey to be spray-dried into whey powder. Although the environmental issues remain, the price factor will probably continue to vary because dairy commodities normally exhibit cyclical price variations. In any modern regimen for whey management, the focus must therefore be on maximizing the value of the available whey solids.

In the search for alternative whey disposal methods by the dairy industry, the exciting opportunities and financial benefits offered by whey utilization, particularly of the whey protein components, have in the past often been underrated or overlooked. Although the compositional features of whey (Table 1) highlight reasons for the status of this dairy stream as "difficult to dispose", these features also reveal a number of untapped opportunities. Nowhere are these opportunities more apparent than in the protein constituents of whey.

Isolation of whey proteins as spray-dried whey powder and, in more limited quantities, as whey protein concentrate has realized only a small portion of the commercial potential of these proteins. Indeed, whey protein concentrate, once heralded as a value-added outlet for whey solids, is now considered to be a commodity item. Clearly, expanded utilization of whey proteins relies primarily on the isolation of individual whey proteins as products with increased nutritional, functional, and biological value and, thus, increased commercial value to the dairy industry.

Whey represents a rich and heterogeneous mixture of secreted proteins with wide ranging functional attributes for nutritional, biological, and food purposes. The main constituents are β-lactoglobulin and α-lactalbumin, two small globular proteins that account for approximately 70 to 80% of total whey protein. Minor protein components include the glycomacropeptide (rennet whey), bovine serum albumin, lactoferrin, immunoglobulins, phospholipoproteins, and numerous bioactive factors and enzymes. With such a myriad of proteins, all with varied and valuable properties, why has utilization of whey protein not been more widespread?

The answer is most likely threefold. First, whey protein products have usually been viewed solely as low value food ingredients for a limited number of specific applications, rather than also having application in nontraditional markets such as the biotechnology, health care, and veterinary industries. In addition, with some notable exceptions, the dairy industry has not excelled at promoting the qualities of whey proteins. Second, viable industrial technologies are lacking for the isolation of individual whey protein species. Such technologies are an essential prerequisite if the unique properties of each protein constituent are to be exploited. Finally, whey protein has an unfortunate record of inconsistent and unreliable physical functional performance in food systems. These shortcomings are being addressed through an expansion of the applications base for whey proteins into the area of physiologically functional foods, through the development of novel fractionation technologies, and through the exploitation of properties of the resultant whey protein fractions in food and in potentially lucrative nontraditional applications for dairy proteins.

WHEY PROTEIN AS A PHYSIOLOGICALLY FUNCTIONAL FOOD INGREDIENT

A physiologically functional food, sometimes simply referred to as a functional food, is one that offers a health benefit when consumed. A more rigorous definition is that used by the Japanese Ministry of Health and Welfare, which indicates that such a food must be derived from natural substances, be intended for consumption as part of the daily diet, and have a targeted physiological function when ingested.

The functional food market is in a phase of rapid growth, particularly in Asia and in North America. The worldwide market size for this food sector is predicted to reach $50 billion by the turn of the century and thus represents a lucrative opportunity for the dairy industry. This burgeoning interest, by both consumers and the food industry, in foods that offer a health benefit can be traced to the social and economic cost of poor or inappropriate diet. Coronary heart disease, cancer, stroke, and diabetes, all linked to diet, account for more than 55% of deaths in a number of western countries, including the US and Australia (Figure 1). Moreover, the costs of health care associated with these disease states are growing steadily and currently amount to nearly 0.5% of gross

TABLE 2. Processing history of whey protein concentrate and its influence on the physical, chemical, and biological functionality of the proteins in the final powder.

Property	Whey protein concentrate 1	2	3
Severity of processing[1]	High	Medium	Low
Solubility, %	75.1	85.3	98.3
Lactoferrin, % recovery from whey	12	55	62
Lactoperoxidase, % recovery from whey	0	8	33
Lactoperoxidase, % recovery of activity from whey	0	0.1	11
Biological activity,[2] arbitrary units	5	30	155

[1]Relative measure of the severity of processing (e.g., heat treatment and shear) used in the manufacture of the whey protein concentrate powders.

[2]Relative ability of the protein, when included in the diet, to illicit an immune response to a particular antigen. The larger the number, the more elevated was the immune response.

domestic product in Australia. Whey proteins can play a role in reducing these social and economic costs.

Dietary whey proteins have a number of putative biological effects when ingested, including an anticancer action. In the current study, we chose to focus on colon cancer because this disease state is clearly associated with diet and lifestyle, because colon cancer is a serious problem for western industrialized societies (1 person in every 20 to 30 will suffer from the disease, and 1 in every 3000 to 4000 will die from the disease), and because the impact of dietary protein on the incidence of the disease has not been well studied, in sharp contrast to other food components, notably fat and fiber.

This study compared the ability of several common dietary protein sources, including whey, casein, meat, and soybean, to prevent the development of colon cancer in a well-established rat model system (6). The effect of dietary protein source was quite dramatic. The rats fed whey protein displayed the lowest incidence of colon cancer of the four dietary groups studied; only 30% of animals developed tumors in the colon compared with 55 and 60%, respectively, for animals receiving the protein diets containing meat or soy (6). The rats fed whey protein also showed a statistically significant ($P < 0.005$) reduction in the total number of tumors within the group compared with those animals receiving the diets with meat or soy. In addition, tumors that did develop in the rats fed whey protein were, on average, smaller than those found in rats receiving the other diets (6).

The processing history of the whey protein used in the experiments described appears to influence dramatically the physiological functionality of the final protein powder. Our studies have shown a clear, inverse relationship between a number of key processing indices, including powder solubility, the content and activity of the labile proteins lactoferrin and lactoperoxidase, and the severity of whey processing used in the manufacture of the whey protein powder (Table 2). This relationship extended to the biological activity of the whey protein, as measured by the ability of the dietary protein powder to illicit an elevated immune response to a particular antigen. The product with the lowest biological functionality had been subjected to the most severe processing, including high heat treatment and mechanical damage; by contrast, the most efficacious product had received relatively mild treatment (Table 2). Biological activity of whey proteins appears to be particularly dependent upon the processing history of the proteins and ultimately their native conformation.

These results indicate that native whey protein can have a positive impact on gastrointestinal health

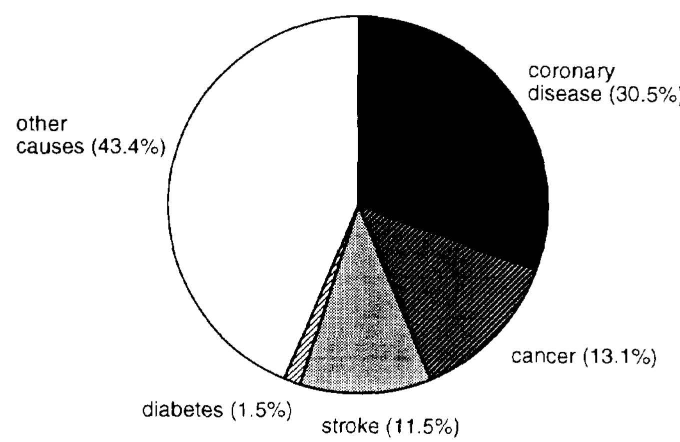

Figure 1. Causes of death in Australia in 1994. Figures quoted are for diseases related to diet (coronary heart disease, cancer, stroke, and diabetes) and other causes.

when consumed in the diet and thus has great potential as a physiologically functional food ingredient. These findings provide clear messages and promotional opportunities for the dairy industry, both in the processing and the marketing of whey proteins as valuable ingredients and in promoting the quality of current products containing whey proteins. Perhaps of greatest significance, the work provides a rational basis for the development of functional foods based on whey protein that have substantiated health benefits.

α-LACTALBUMIN AND β-LACTOGLOBULIN: NOVEL FOOD INGREDIENTS

An industrially feasible process for isolation from bovine cheese whey of the two major proteins, α-lactalbumin and β-lactoglobulin, has been developed. The process, thermal fractionation of whey protein, results in products enriched with one of the two proteins. The products are termed the α-fraction and the β-fraction (9). Both products should have wide application in the food industry, the former for its nutritional qualities and the latter for its physically functional properties.

The principal protein constituent of human milk is α-lactalbumin, which represents approximately 30% of the total protein in this milk. Moreover, human milk contains negligible quantities of β-lactoglobulin. Under these circumstances, the α-fraction (highly enriched in α-lactalbumin and depleted in β-lactoglobulin) from bovine cheese whey should find immediate application as the primary protein constituent of the next generation of infant formulas with protein compositions that are more similar to that of human milk (10, 11). Bovine and human α-lactalbumins also show excellent amino acid and structural homology (4), providing further support for use of the former protein in enhanced formulas for infants.

The β-fraction, highly enriched in β-lactoglobulin, has excellent heat-set gelation properties (Figure 2) and should find immediate application in manufactured meats, fish products, and a variety of formulated foods. The β-fraction also shows excellent whippability and provides a superior and cost-effective replacement for egg albumin in some food applications. The the β-fraction shows high solubility over a broad pH range, in particular, at low pH (>97%, pH 3), and is stable to UHT treatment under these conditions. These properties of the β-fraction should allow its use as the active agent in protein-fortified acidic beverages, such as fruit juices and sports drinks, and in varieties of these beverages with long shelf-life (10).

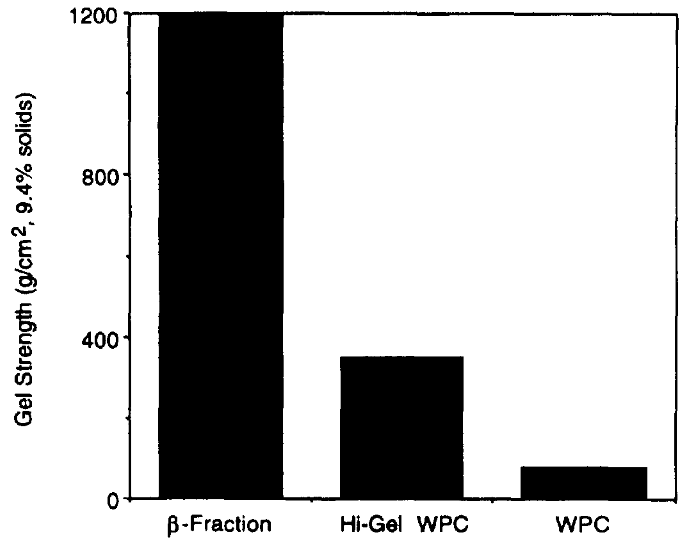

Figure 2. Comparison of the heat-induced gel strengths of commercial whey protein concentrate (WPC) (WPC-80; United Milk Tasmania Ltd., Devonport, Tasmania, Australia), high gel WPC (Hi-Gel) (Alacen 132; New Zealand Milk Products, Wellington, New Zealand), and β-fraction (enriched in β-LG).

MINOR WHEY PROTEIN COMPONENTS

Cheese whey contains a multitude of minor proteins and enzymes that have often been overlooked for industrial-scale isolation and commercial exploitation. Many of these constituents have the potential to enter new markets for dairy proteins as high value products and thus have a significant impact on whey management and on efforts to maximize the financial return from whey solids.

LACTOFERRIN: SPECIALTY FOOD INGREDIENT AND ANTIMICROBIAL AGENT

Among the many minor protein constituents of whey are several that display antimicrobial activity. Lactoferrin, present at low concentrations in whey (50 to 150 mg/L), exhibits both bacteriostatic and bactericidal activity against a range of microorganisms, including those responsible for gastroenteric infections, food poisoning, listeriosis, and mastitis (2). Increased demand for natural antibiotics and for components to supplement infant formulas and other specialty foods has stimulated much interest in the isolation of lactoferrin from whey and its effective utilization. Lactoferrin is a cationic protein (pI > 9); this property has been used to advantage in procedures for isolation of the protein from whey, because the major, and many of the minor, protein constituents are acidic (pI < 7). Thus, cation-exchange column chromatography at neutral pH is the most commonly reported method for the extraction of lactoferrin from whey (5).

Membrane adsorbers, which immobilize the exchange functional groups on a microporous membrane, offer an alternative and perhaps superior process for ion exchange. In contrast to chromatography, in which solute binding is limited by diffusion, the membrane-based system is suited to very high processing flow rates, making membrane adsorption the method of choice when large volumes of a dilute solution such as whey are treated. In addition, high flow rates can be maintained without sacrificing selectivity, and methods for cleaning and sterilization are simple and diverse. Work from our research group has demonstrated that lactoferrin can be effectively extracted from whey using a strongly acidic membrane adsorber (7, 8). Areas of application for this protein include specialty dietary formulations, natural preservatives, pharmaceuticals, and personal health items, such as antibacterial toothpaste and mouthwash.

CELL GROWTH FACTORS: SPECIALTY BIOTECHNOLOGY REAGENT

Whey contains proteins that serve as potent growth stimulants for a number of mammalian cell lines in culture. These growth factors have a dramatic impact on cell growth by promoting synthesis of DNA and protein and by inhibiting degradation of protein. A process for the extraction and purification of these factors from cheese whey, using membrane and chromatographic techniques, has been developed and recently patented (1). The isolation process results in the elimination of about 97% of other whey proteins that remain essentially unaltered by the process and that thus are available for further processing (e.g., manufacture of whey protein concentrate). Growth factor activities present in the whey extract include IGF-I, IGF-II, platelet-derived growth factor, acidic and basic fibroblast growth factors, and transforming growth factor-β (3, 12, 13).

Mammalian cells are grown in culture to provide a number of important pharmaceutical, diagnostic, and veterinary products, such as vaccines, hormones, drugs, and monoclonal antibodies. Tissue growth in culture, particularly that of human skin, is a rapidly expanding activity with applications in the treatment of burns, ulcers, and lacerations. Growth of these cells and tissues is absolutely dependent upon a source of growth factors, and this requirement has traditionally been met through the use of fetal bovine serum. However, the supply of fetal bovine serum is limited, unreliable, costly, and often highly variable in quality. Trials with a number of research and commercial cell lines in culture have shown that growth factors isolated from whey serve as a cost-effective and reliable replacement or as a supplement for fetal bovine serum in these applications (3) (Table 3). These whey-derived growth factors thus have the potential to have an impact on the biotechnological and pharmaceutical industries.

TABLE 3. Comparative growth-promoting activity of a growth factor preparation derived from whey versus fetal bovine serum.

	Whey growth factor required to replace	
Cell line	5% Serum	10% Serum
	(mg/ml)	
Human skin fibroblasts	0.1	0.2
Balb/3T3 fibroblasts	0.3	0.5
L6 Myoblasts	1.3	2.5

CONCLUSIONS

Although the volume and composition of whey have historically categorized this dairy stream as a disposal problem, the modern regimen for whey management must not focus merely on the most economical method to discard the fluid but also on maximizing the financial return available through the utilization of whey solids. In this respect, the greatest financial returns will flow through the isolation of high value whey protein products and the utilization of these proteins in the food industry and in lucrative nontraditional dairy protein markets, particularly biotechnology. Just as skim milk emerged from its image as a by-product of the butter industry during the mid-1900s, it is now time for whey to be viewed not as a by-product, but as a coproduct of cheese and casein manufacture and as a valuable feedstock for the production of value-added protein products. Whey should now be more often viewed as a portfolio of opportunities instead of as a problem.

ACKNOWLEDGMENTS

The authors acknowledge the expert technical assistance of support staff at the Commonwealth Scientific and Industrial Research Organisation, Queensland Department of Primary Industries, and Cooperative Research Centre for Tissue Growth and Repair. The authors thank the Dairy Research and

Development Corporation of Australia for financial support.

REFERENCES

1 Ballard, F. J., G. F. Francis, and G. O. Regester, inventors. 1991. Growth-promoting agent. Int. Pat. Appl. PCT/AU91/00303. GroPep Pty. Ltd., assignee.

2 Dionysius, D. A., P. A. Grieve, and J. M. Milne. 1993. Forms of lactoferrin: their antibacterial effect on enterotoxigenic *Escherichia coli*. J. Dairy Sci. 76:2597.

3 Francis, G. L., H. A. Webb, I. K. Priebe, C. S. Chandler, P. E. Walton, F. J. Ballard, G. O. Regester, K. J. De Silva, I. R. Mitchell, and G. W. Smithers. 1994. Protein extract from cheese whey stimulates the growth of mammalian cells in culture. J. Dairy Sci. 77(Suppl. 1):9.(Abstr.)

4 Heine, W. E., P. D. Klein, and P. J. Reeds. 1991. The importance of α-lactalbumin in infant nutrition. J. Nutr. 121:277.

5 Law, B. A., and B. Reiter. 1977. The isolation and bacteriostatic properties of lactoferrin from bovine cheese milk whey. J. Dairy Res. 44:595.

6 McIntosh, G. H., G. O. Regester, R. K. Le Leu, P. J. Royle, and G. W. Smithers. 1995. Dairy proteins protect against dimethylhydrazine-induced intestinal cancers in rats. J. Nutr. 125:809.

7 Mitchell, I. R., G. W. Smithers, D. A. Dionysius, P. A. Grieve, G. O. Regester, and E. A. James, inventors. 1992. Isolation of charged particles from fluids. Int. Pat. Appl. PCT/AU92/00381. Commonwealth Scientific and Industrial Research Organisation, Dairy Research and Development Corporation, and State of Queensland as represented by the Department of Primary Industries, assignee.

8 Mitchell, I. R., G. W. Smithers, D. A. Dionysius, P. A. Grieve, G O. Regester, and E. A. James. 1994. Extraction of lactoperoxidase and lactoferrin from cheese whey using membrane cation exchangers. Page 89 *in* IDF Seminar: Indigenous Antimicrobial Agents of Milk—Recent Developments. Int. Dairy Fed., Brussels, Belgium.

9 Pearce, R. J. 1987. Fractionation of whey proteins. Aust. J. Dairy Technol. 42:75.

10 Pearce, R. J. 1991. Applications for cheese whey protein fractions. Food Res. Q. 51:74.

11 Pearce, R. J., J. A. Dunkerley, S. C. Marshall, G. O. Regester, and R. M. Shanley. 1991. New dairy science and technology leads to novel milk protein products. Food Res. Q. 51:137.

12 Rogers, M.-L., D. A. Belford, G. L. Francis, and F. J. Ballard. 1995. Identification of fibroblast growth factors in bovine cheese whey. J. Dairy Res. 62:501.

13 Rogers, M.-L., D. A. Belford, C. Goddard, and F. J. Ballard. 1995. Transforming growth factor-β activity in bovine milk. J. Endocrinol. 144(Suppl.):51.