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## Review

## Whey and whey proteins—From ‘gutter-to-gold’

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## A B S T R A C T

Whey was discovered about 3000 years ago. Apart from being valued as a medicinal agent in the 17th and 18th centuries, whey has primarily been considered a waste by the dairy industry, and thus destined for the ‘cheapest gutter’. In the late 20th century, regulations prevented disposal of untreated whey. At the same time, recognition of the value of whey components accelerated. Modern science has unravelled the secrets of whey proteins and other components, and established a sound basis for their nutritional and functional value. In parallel, technology developments exploited this underpinning knowledge, manifested as advanced whey-processing regimes. These advances have continued through the early 21st century with the focus more on the biological functionality of whey components. Cost effectiveness has been a driver in recent whey-processing developments, manifested as novel separation techniques for a range of functional isolates. This paper traces the history of whey, and highlights milestones that have seen whey and whey proteins transformed from ‘gutter-to-gold’.

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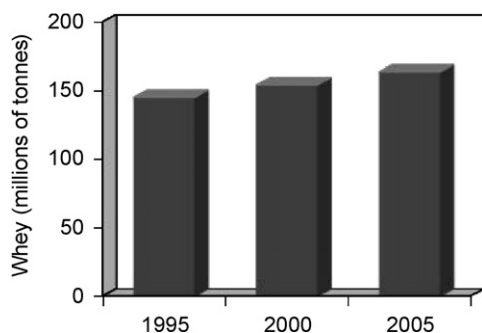
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## 1. Introduction and background

Whey is a co-product of cheese-making and casein manufacture in the dairy industry. After the casein curd separates from the

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**Fig. 1.** Annual volume of dairy whey produced globally (1995–2005). Volume increase over this period shows ~1–2% annual growth rate, approximately equivalent to the average annual growth rate in milk output over this same period (FAO, 2006).

**Table 1**  
Comparison of the proximate analysis of bovine milk and whey<sup>a</sup>

Component	Content (% w/v)	
	Milk	Whey
Casein protein	2.8	<0.1
Whey protein <sup>b</sup>	0.7	0.7
Fat	3.7	0.1
Ash	0.7	0.5
Lactose	4.9	4.9
Total solids	12.8	6.3

<sup>a</sup> Zadow (1994), Smithers et al. (1996), and references therein.

<sup>b</sup> Whey protein comprises ~50%  $\beta$ -lactoglobulin, ~20%  $\alpha$ -lactalbumin, ~15% glycomacropeptide (in renneted whey only), and ~15% minor protein/peptide components (e.g., immunoglobulins, lactoferrin, lactoperoxidase, serum albumin, lysozyme, and growth factors).

milk, following coagulation of the casein proteins through the action of chymosin (rennet) or mineral/organic acid, the remaining watery and thin liquid is called whey (Zadow, 1994). It has a yellow/green colour, or sometimes even a bluish tinge, but the colour depends on the quality and type of milk used. Whey can be made from any type of milk, with cows' milk being the most popular in western countries, while in some regions of the world, goats', sheeps', and even camels' milk can be used in the manufacture of dairy products that result in the generation of whey.

Whey was discovered some 3000 years ago when calves' stomachs were used to store and transport milk. Through the action of the naturally occurring enzyme chymosin (rennet) found in the calves' stomachs, the milk coagulated during storage and transport resulting in 'curds and whey',<sup>1</sup> and as such spawned the start of the cheese (and whey) industry.

Nowadays, whey is a major co-product of modern cheese and casein industries. On average across the world, volumes of whey are growing at about the same rate as milk volumes (>2% per year; FAO, 2006) (Fig. 1). This increased quantity of milk is being channelled into the production of larger volumes of cheese, casein/caseinate, and other dairy products, resulting in concomitant increases in the volume of whey (Fig. 1).

A comparison of the proximate analysis of bovine milk and whey is presented in Table 1. This analysis reveals that about 50% of the milk solids appear in the whey, together with essentially

100% of the lactose and some 20% of the protein. The lactose makes up a high proportion (>75%) of the total whey solids, and contributes in large part to dairy whey being considered one of the most polluting food by/co-product streams (biochemical oxygen demand (BOD) >35,000 ppm; chemical oxygen demand (COD) >60,000 ppm) (Bullerman & Berry, 1966; Siso, 1996). While the polluting power of whey is well known, this dairy stream also represents an excellent source of functional proteins and peptides, lipids, vitamins, minerals, and lactose (Table 1), that until relatively recently have been less well recognised. It is these latter components of whey, notably the proteins and peptides, and their properties that have helped transform whey from a waste material that has often been shunned, to a valuable dairy stream containing a multitude of components available for exploitation in the agri-food, biotechnology, medical, and related markets. This paper traces the history of this transformation, from 'gutter-to-gold', and highlights key advances and milestones that have occurred over the past approximately 50 years that have helped to shape the modern whey industry. In researching this topic of whey and whey protein utilisation, the reader is directed to a seminal publication by Sienkiewicz and Riedel (1990) as an excellent starting point.

## 2. Cheese-makers' problem—find the most economical gutter

Historically, whey has been considered a waste stream and nuisance by cheese-makers and casein manufacturers alike. In these circumstances, dairy companies have sought out the most economical management (read disposal) method (i.e., the 'cheapest gutter'). Such methods have included (i) spraying the whey onto fields, although the smell and salt often proved to be troublesome, (ii) discharging the whey into rivers, lakes, or the ocean, however the very high polluting power of whey eventually restricted this disposal approach, (iii) discharging the whey into the municipal sewage system, although the high BOD and COD of whey usually leads to an overload of the system and as such this approach is either very expensive or banned, and (iv) selling the whey for a low return as animal feed. All these approaches to the management of whey have been based on the premise that this dairy stream is a waste product with little value, and therefore needed to be disposed of in the most economical manner. In this circumstance, what changed to turn a so-called nuisance into a valuable and prized raw material?

## 3. Key milestones—'gutter-to-gold'

Transformation of whey from a nuisance into a valuable dairy raw material has been facilitated by key events over the recent past, some imposed on the industry, and some championed and exploited by the industry. Such milestones have included the following.

### 3.1. Environmental considerations

The very substantial polluting power of whey, with a BOD some 175-fold higher than typical sewage effluent, has in the recent past prompted governments and other regulatory authorities to restrict and/or ban the disposal of untreated whey. Community pressure, notably in regional areas, has also forced dairy factories to reconsider how they manage the large and growing volumes of whey generated from cheese and casein manufacture.

<sup>1</sup> Dr. Thomas Muffet (possibly Moffett or Moufet) (1553–1604), an entomologist, wrote the nursery rhyme 'Little Miss Muffet' that makes reference to 'curds and whey'. Miss Muffet is thought to depict his daughter (Patience), although this accreditation is considered doubtful by some.

### 3.2. Science knowledge underpinning

Application of modern scientific disciplines, approaches and techniques has helped advance a greater understanding at molecular level of the chemical, physical, biological, and nutritional characteristics of whey components, notably proteins and peptides. This understanding has extended to an enhanced knowledge of the interactions between whey components, and the influence of these interactions on component functionality.

### 3.3. Technology advances and sophistication

In parallel with advances in establishing a sound science foundation for whey components and their behaviour, similar advances have occurred in whey-processing technologies. These have included efficient and cost-effective unit processes for concentration, transformation, fractionation and dehydration of whey, together with the introduction of modern biochemical techniques, some from outside the dairy and food industries, for the manufacture of new whey-based ingredients with an expanded applications base for increasingly receptive markets.

### 3.4. Marketplace expansion and sophistication

In a truly global food industry, whey-based ingredients compete with many other ingredients for market share. Often, the trading of these ingredients is subject to the typical cyclical variation in world prices of commodities. Increasing sophistication in the modern food marketplace, to meet consumer demands for healthy, tasty, and convenient foods, has led to new and receptive markets (e.g., functional foods, nutraceuticals) and thereby opportunities for new high-value whey-based ingredients.

### 3.5. Modern knowledge catching up with ancient wisdom

There are historical references (Baricelli, 1623)<sup>2</sup> to the use of lactoserum (whey) for medicinal purposes, including sepsis, wound healing, and 'stomach disease' (Fig. 2). Indeed, so-called 'whey houses' thrived in Europe in the 17th and 18th centuries for the treatment of such ailments using whey (Hoffmann, 1961). Nowadays, the basis for this apparent bioactivity is better understood, and sound scientific substantiation of the nutritional and biological qualities of whey components, notably the proteins, is increasing as whey-based ingredients compete for a share of the burgeoning functional foods market.

## 4. From waste to utilisation ('gutter-to-good')

### 4.1. Environmental regulations and legislation

All the major dairy producing nations have strict controls over food industry by/co-product management and disposal. Legislative regulations governing effluent disposal (including whey) have been progressively introduced and strengthened over the past approximately 25 years. These regulations are strictly enforced and, in many localities, individuals can be held personally liable for any breaches. Useful starting points in researching the

<sup>2</sup> Baricelli, a physician and philosopher from the 17th century, authored a treatise on milk, whey, and butter (Fig. 2). In this treatise, Baricelli deals with the essence of milk, the production of milk by the mammary glands, and the different qualities of milk. Human and various animal milks are examined and their uses detailed, together with whey ('lactoserum') and butter.

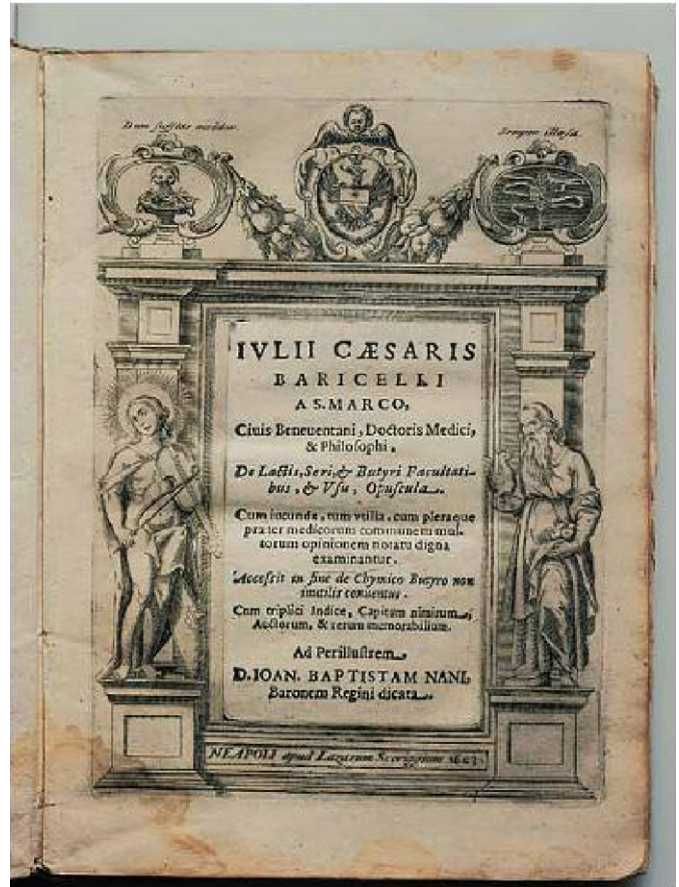


Fig. 2. Front cover of the treatise by Baricelli, a physician and philosopher from the 17th century, on milk, whey, and butter (Baricelli, 1623).

treatment of dairy wastes and the legislative scene governing disposal of whey and other dairy streams can be found in Marshall and Harper (1984), Sienkiewicz and Riedel (1990), Siso (1996), and Durham and Hourigan (2007).

The United States, Canada, Australia, New Zealand, and the European Union countries have all introduced strict environment protection legislation, and such regulation has forced a re-think within the dairy industry in regard to whey disposal. Such a legislative scene, coupled with recent community pressure against disposal of whey into the environment and in favour of recycling wherever possible, encouraged the dairy industry to explore other approaches to and opportunities for management of this dairy stream. However, such alternatives needed to be founded on a sound science understanding of whey components.

### 4.2. Scientific and technical advances, new applications

Legislative restrictions on whey disposal encouraged a deeper exploration of the widely recognised but less well understood physical, chemical, nutritional, and biological properties of whey components, notably the proteins and peptides. While the nutritional quality (energy source, amino acid profile), and physical (e.g., gelling, foaming, water binding, etc.) and physiological functionality of whey proteins have been recognised for some time, several factors have limited the more widespread use of whey (protein)-based ingredients. These factors have included (i) limited underpinning science and technology understanding of whey component properties, (ii) restricted applications base in which whey and whey proteins have been viewed solely as low-value products, (iii) poor promotion of whey protein qualities



versus competitor products (e.g., soy), (iv) inconsistent and unreliable performance in food systems, notably in the area of physical functionality, and (v) lack of viable industrial technologies for cost-effective isolation and manufacture.

Science and technology advances have addressed many of the limitations outlined above, and have helped set a strong foundation for a modern whey industry, and the widespread use of whey protein and other (e.g., lactose) ingredients in the food and related industries. Several of these advances are outlined below.

#### 4.2.1. Lactose—the first ‘nugget’ from whey

The valorisation of whey arguably commenced with approaches to utilisation of the primary whey solid—lactose. Techniques for enhanced crystallisation, isolation and purification, hydrolysis, and transformation (into various valuable derivatives) of this dairy sugar, all based on a sound understanding of lactose chemistry, have been developed and implemented around the world. In parallel, food, pharmaceutical and industrial applications for lactose and its derivatives have been successfully developed and commercialised. These and other advances and milestones in the utilisation of lactose have been addressed in a complementary paper by Gaenzle, Haase, and Jelen (2008).

#### 4.2.2. Nutritional value of whey proteins

**Biological value (BV):** Whey protein has an exceptional BV that exceeds that of egg protein by about 15%, the former benchmark, and a range of other common edible proteins (Fig. 3A). BV is a measure of the percentage of a given nutrient (e.g., protein) that is utilised by the body (Mitchell, 1924). In essence, BV refers to how well and how quickly the body can utilise the protein consumed. In this regard, whey protein excels and is the protein of choice for body builders, elite athletes, and those whose health is compromised (Buckley, Abbott, Martin, Brinkworth, & Whyte, 1998; Carey, Larsen, Rowney, & Cameron-Smith, 2006; Coombes, Conacher, Austen, & Marshall, 2000; Ha & Zemel, 2003; Playford et al., 1999; Rankin & Darragh, 2006).

**Essential amino acids:** Whey protein is a rich source of the essential amino acids when compared with other typical food proteins (Fig. 3B), and is also rich in the branched chain amino acids (leucine, isoleucine, and valine) (>20%, w/w). These latter amino acids are thought to play a role as metabolic regulators in protein and glucose homeostasis, and in lipid metabolism, and as such may play a role in weight control (Smilowitz, Dillard, & German, 2005; Zemel, 2004).

**Sulphur amino acids:** Whey protein is a rich and balanced source of the sulphur amino acids (methionine, cysteine) (Fig. 3C). These amino acids serve a critical role as anti-oxidants, as precursors to the potent intracellular anti-oxidant glutathione, and in one-carbon metabolism (Shoveller, Stoll, Ball, & Burrin, 2005).

#### 4.2.3. Physical functionality—understanding and controlling whey protein performance

Over the past 25 years, one of the greatest restrictions to the widespread use of whey protein ingredients has been inconsistency and unreliability of their performance in food systems. The challenge has been to better understand the molecular basis for this functional inconsistency and unreliability through sound application of modern science and technology, and to then exploit this knowledge. Dairy research facilities around the world—United States, Australia, New Zealand, Europe—have devoted their attention to better understanding: (i) the effects of heat and shear processing on whey protein functionality; (ii) variations in the major and minor whey components, their interactions, and the

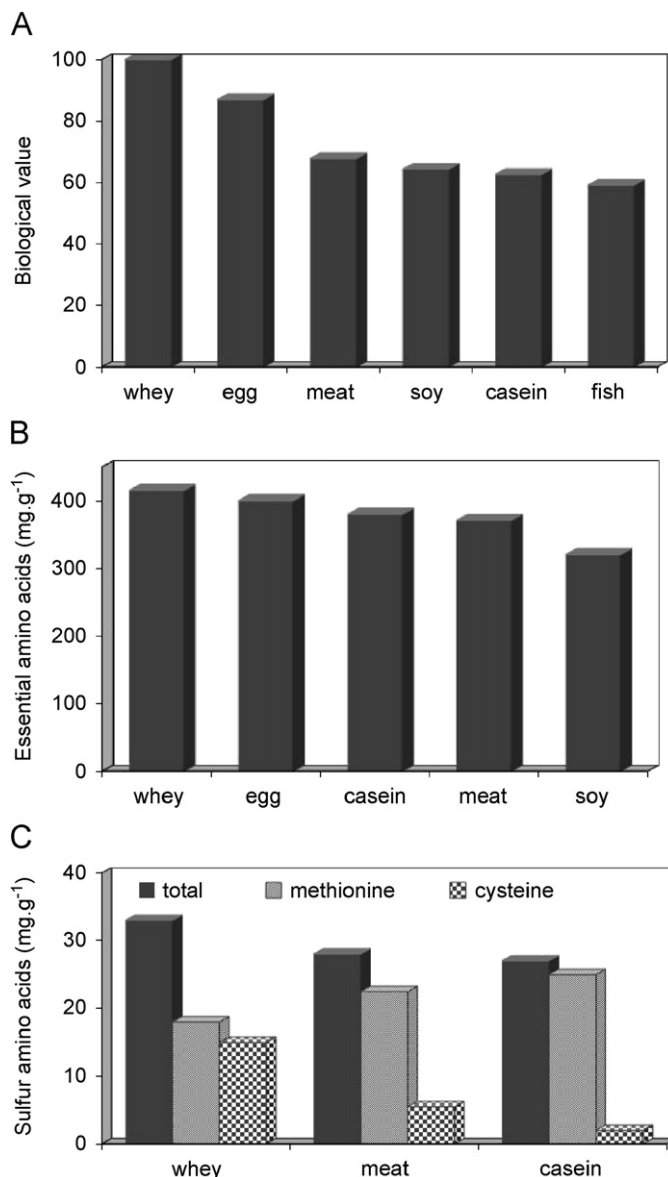


Fig. 3. Nutritional value of whey protein when compared with other common edible proteins, as measured by biological value (A), essential (B), and sulphur (C) amino acid content.

effects of seasonality and processing on functionality; and (iii) physical and chemical behaviour of key functional components (proteins, lipids, and minerals) in whey (see, for example, Bernal & Jelen, 1985; Foegeding, Davis, Doucet, & McGuffey, 2002; Harper, 1984; Huffman, 1996; Jelen & Rattray, 1995; Ju, Hettiarachchy, & Kilara, 1999; Kinsella & Whitehead, 1989; Mangino, Kim, Dunkerley, & Zadow, 1987; Marshall & Harper, 1988; Patel, Kilara, Huffman, Hewitt, & Houlihan, 1990; Regester, Pearce, Lee, & Mangino, 1992; Regester & Smithers, 1991; Zadow, 1994). The work of these researchers and others has laid the foundation for consistency and reliability in whey-based products that nowadays is the norm, and has also formed a sound science base for new applications and new processing technologies (De Silva, Stockmann, & Smithers, 2003; Etzel, 2004; Huffman & Harper, 1999).

#### 4.2.4. Processing technologies—advances through the 1980s and 1990s

Complementing a deeper science understanding of whey component traits, particularly whey proteins, have been advances

in technologies for processing whey, built upon a sound understanding of the behaviour of whey solids during concentration, fractionation and dehydration. In particular, chromatographic, electrodialysis, and membrane techniques (e.g., nanofiltration) for demineralisation of whey have formed the technical base for modern whey powders with applications in various specialised infant and clinical formulations (Guengerich & Hutson, 1996; Houldsworth, 1980; Jeantet, Rodríguez, & Garem, 2000; Kelly, Horton, & Burling, 1992). Modern membrane processing, including industrial applications of microfiltration, ultra/diafiltration, have helped to pioneer the development of high-protein and low-fat functional whey ingredients, such as whey protein concentrates (WPCs) (35%, 75%, and 80% protein) and first-generation whey protein isolates (WPIs) (~85–90% protein), that have expanded the applications base for whey protein ingredients (Clark, 2005; Jost & Jelen, 1997; Kelly et al., 2000; Maubois, 1980; Saboya & Maubois, 2000; Zadow, 1992, 1994). Keeping pace with developments in liquid whey processing have been those in the dehydration of whey, notably in the area of spray drying. Engineering developments, particularly in the areas of liquid and powder handling, have led to a number of innovations and improvements in the drying of whey, and whey concentrates and permeates, manifested in tall-form, dual/multi-stage, and filter-mat dryers (Písecký, 2005). Key dryer developments have included (i) fluid bed improvements and enhanced approaches to agglomeration leading to highly dispersible, soluble and free-flowing powders, (ii) energy efficiencies leading to more cost-effective operation, and (iii) novel approaches to the drying of challenging fluids resulting from the processing of whey (e.g., permeate) (Písecký, 2005).

Processing advances facilitating the manufacture of whey powders and WPC/WPI have realised only a portion of the potential of whey proteins. Indeed, WPC, once heralded as a high-value outlet for whey solids, is now considered a commodity. With increasing knowledge of the physico-chemical properties of the individual whey proteins, development of techniques for the cost-effective fractionation of these proteins became feasible (Etzel, 2004; Huffman & Harper, 1999; Smithers et al., 1996). Such isolates would allow expanded utilisation of whey proteins based on their increased nutritional and functional value. Several procedures for isolation of the major whey proteins have been available for some time (Conti, Napolitano, Cantisani, Davoli, & Dall'Olivo, 1988; Korhonen, Pihlanto-Leppälä, Rantamäki, & Tupasela, 1998; Pearce, 1983). These techniques all exploit one, or a combination of, selected physico-chemical properties of the major whey protein molecules. For example, selective precipitation of  $\beta$ -lactoglobulin (and  $\alpha$ -lactalbumin) through the addition of  $\text{FeCl}_3$  to whey at a specified pH formed the basis of one of the earliest fractionation technologies (Kuwata, Pham, Ma, & Nakai, 1985). Unfortunately, these bench-scale procedures are not readily amenable to commercial scale-up, and they can also compromise the functional properties of the isolated proteins. To address these shortcomings, Bounous and Gold (1990, 1991), Bounous, Turgeon, and Aurouze (1994), and Bounous (1996) have described processes, primarily based on micro/ultrafiltration used standalone or in combination, for production of 'undenatured' WPC (enriched in  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin) that has a variety of proposed biological functions (Bounous, Baruchel, Falutz, & Gold, 1993; Bounous, Batist, & Gold, 1991). Further, procedures have been developed for industrial-scale manufacture of  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin isolates. The most promising of these include (i) selective precipitation of  $\alpha$ -lactalbumin from sweet whey concentrated by ultrafiltration, under specified conditions of pH and temperature, leaving  $\beta$ -lactoglobulin in solution and unaffected by the pH/temperature treatment (Bramaud, Aymar, & Daufin, 1997; Maubois, Pierre, Fauquant, & Piot, 1987; Pearce,

1983, 1987, 1988, 1995; Pierre & Fauquant, 1986) and (ii) liquid chromatography using both organic and inorganic resins (Ayers, Elgar, Palmano, Pritchard, & Bhaskar, 2002; Ayers & Petersen, 1985; De Silva et al., 2003; Skudder, 1985).

#### 4.2.5. Expanded applications base

Sound science understanding of the characteristics of whey proteins and their interactions with other components in complex food systems, together with efficient technology for whey processing, concentration, fractionation, and dehydration has formed the foundation for an ever expanding applications base for whey solids and whey-based ingredients (Foegeding et al., 2002; Jelen, 1992; Playne, Bennett, & Smithers, 2003).

A greater understanding of the behaviour of whey proteins and their interactions with other components, both in sweet and acid whey, in response to physical (e.g., temperature) and chemical (e.g., pH) changes, has facilitated the development of value-added outlets for whey solids in the form of beverages and whey-based cheeses (Jelen, 1992). Whey cheeses are prepared using a process that involves application of heat ( $>90^\circ\text{C}$ ), and often the addition of organic acid (e.g., acetic, citric) and/or mineral salt (e.g., calcium), to the whey. Milk is sometimes added to the source whey to facilitate rapid formation of a firmer curd, and the whey is also occasionally pre-concentrated by ultrafiltration prior to the heat treatment (Pintado, Macedo, Malcata, Macedo, & Malcata, 2001). Typical yield is only ~6%; however the addition of milk, calcium salts, and/or pre-concentration of the source whey can improve the yield. A well-known example of a whey cheese is the Italian Ricotta variety. This cheese is prepared from Mozzarella or Provolone whey, is white in colour, and has a delicate and grainy texture. Ricotta has a short shelf-life and is intended to be consumed soon after manufacture. Other short shelf-life whey cheeses include Requeijão and Manouri, whereas those with a longer shelf-life include the Scandinavian varieties Gjetost and Mysost, prepared from a highly concentrated lactose and whey protein mixture (Jelen & Buchheim, 1976). Whey cheese varieties differ considerably in their chemical composition and sensory properties, due mainly to variations in the source and type of whey, as well as the processing regimen employed. The typically high-moisture content and pH of whey cheeses favour microbiological growth and the rapid development of flavour notes (Jelen, 1992; Pintado et al., 2001). The nutritional value of whey solids has also encouraged their use in a variety of drinks (Jelen, 1992). High whey protein beverages remain a sensory challenge due often to astringency and an unpleasant after-taste following consumption. Processing and flavour-masking have both been usefully applied in addressing this and other challenges in the manufacture of drinks containing a high content of whey protein (Beecher, Drake, & Foegeding, 2006; Drake, 2006; Johnson, Jelen, Mitchell, Regester, & Smithers, 1996). The most popular and well-known commercial whey-based beverage is the Swiss 'Rivella' available in several European countries. It is marketed in a number of varieties including the traditional version, a low-joule product, and a variety supplemented with catechins from green tea.

In the manufacture of a range of modern formulated foods, whey protein ingredients are now sought after for their consistent and reliable nutritional and functional properties, the latter including solubility, gelation, aeration, water-binding, and emulsification (Foegeding et al., 2002; Huffman, 1996; Kinsella & Whitehead, 1989; Korhonen et al., 1998; Smithers et al., 1996).

Increased understanding of the molecular functionality of the whey proteins has led to new and interesting applications that complement the more traditional areas noted above. One such example has been the development of whey proteins and peptides

as effective encapsulant or co-encapsulant materials for the protection and delivery of sensitive functional and bioactive components, including anhydrous milk fat and essential oils (Rosenberg, 1997), omega-3 oils (Augustin, Sanguansri, & Bode, 2006), and probiotic organisms (Crittenden, Weerakkody, Sanguansri, & Augustin, 2006; Picot & Lacroix, 2004), into a range of functional foods.

## 5. From utilisation to value-added ('good-to-gold')

### 5.1. Functional foods 'revolution'

Functional foods are those that provide a specific health benefit to the consumer over and above their nutritional value. Functional foods are relatively recent developments that meet a strengthening consumer demand for foods that enhance health and wellbeing. The global market size has already reached \$73.5 billion from a modest base just 10 years ago (Just-food.com, 2006). The United States market dominates (>30% of the total global market) and is showing a sustained growth of ~14% per year. Other significant markets include the European Union and Japan. Growth in the functional foods market across the world is currently ~8% per year, and at this rate the market will be valued at >\$100 billion by 2012. In this large and burgeoning marketplace, the food industry is demanding economical, high-quality, novel, and substantiated ingredients. In such a setting, whey (protein)-based ingredients provide the industry with an excellent choice, and these ingredients start from a firm traditional foundation. Hippocrates, the father of modern medicine, is thought to have praised the health benefits of whey, and there are historical references to the widespread use of whey for medicinal purposes in Europe during the 17th and 18th centuries (Baricelli, 1623; Hoffmann, 1961). While our forebears appeared to have wisdom regarding the biological properties of whey, this bioactivity had to be both recognised and proven before whey ingredients would be seriously considered for widespread use in the functional foods and related products of the 21st century (McIntosh et al., 1998). Modern science and technology has again helped meet this challenge and capture this opportunity for the whey industry (Playne et al., 2003).

### 5.2. Scientific and technical advances, new applications

#### 5.2.1. Bioactivity of whey proteins and peptides

Whey contains a multitude of biologically active proteins and peptides, and these and other components have formed the basis for the use of whey in medicinal applications during the 17th and 18th centuries (Hoffmann, 1961). Apart from the major whey proteins— $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, and glycomacropeptide—whey contains a number of other proteins with potent bioactivity (Table 2). All the whey proteins have at the very least been implicated in a variety of nutritional and physiological effects, including (i) physical performance, recovery after exercise, and prevention of muscular atrophy (Carey et al., 2006; Farnfield, Carey, & Cameron-Smith, 2005; Ha & Zemel, 2003; Ohr, 2004; Rankin & Darragh, 2006; Tipton et al., 2004), (ii) satiety and weight management (Ohr, 2004; Tome, 2006; Zemel, 2004), (iii) cardiovascular health (Ohr, 2004; Pins, Harminder, & Keenan, 2006), (iv) anti-cancer effects (Bounous et al., 1991; Gill & Cross, 2000; McIntosh, Regester, Le Leu, Royle, & Smithers, 1995), (v) wound care and repair (Regester, Belford, West, & Goddard, 2003; Smithers, 2004), (vi) management of infections (Bounous et al., 1993; Playford et al., 1999; Regester & Belford, 1999), (vii) infant nutrition (Chatterton, 2006; Heine, Klein, & Reeds, 1991), and (viii)

**Table 2**

Content of minor bioactive proteins in cheese whey

Protein	Content (mg L <sup>-1</sup> )
Lactoferrin	50–70
Lactoperoxidase	8–20
Immunoglobulins	300–600
Growth factors <sup>a</sup>	< 0.06
IGF-I	< 0.001
IGF-II	< 0.001
PDGF	< 0.0002
TGF- $\beta$	< 0.01
FGF	< 0.0001
Betacellulin	< 0.002

<sup>a</sup> IGF, insulin-like growth factor, PDGF, platelet-derived growth factor, TGF, transforming growth factor, and FGF, fibroblast growth factor.

**Table 3**

Summary of literature reports linking whey proteins and peptides to putative anti-cancer effects<sup>a</sup>

Cancer	WPC	Lactoferrin and lactoperoxidase	Serum albumin	Peptides
Colon	+	+	+	+
Breast	+		+	+
Skin		+		
Prostate	+			+

<sup>a</sup> '+' indicates the existence of literature report(s) linking the nominated protein/peptide to anti-cancer effects against the nominated cancer (adapted from Bounous et al., 1991; Gill & Cross, 2000; and references therein).

healthy aging (Smilowitz et al., 2005). While some of these effects remain putative, a number have received considerable scientific scrutiny and have been substantiated in several laboratories around the world.

A comprehensive review of milk-derived bioactive factors, including those from whey, has recently been published by Elsevier as a special issue of the International Dairy Journal (Korhonen, 2006). This special issue explores technological and health aspects of bioactive components of milk, and as such covers all the major and minor whey proteins, peptides, and other whey components with biological functionality. Rather than repeat aspects of this thorough review, selected key milestones in the establishment of whey proteins and peptides as modern bio-functional ingredients and agents have been highlighted below.

**Anti-cancer effects:** Evidence is growing that specific whey proteins and peptides have potential anti-cancer effects against certain tumours (Table 3) (Bounous et al., 1991; Gill & Cross, 2000). Most evidence to date has been based on in vitro cell culture, in vivo animal studies, and some epidemiological investigations. For example, in work reported by McIntosh et al. (1995) and Hakkak et al. (2001), whey protein diets were shown to be more effective than other dietary proteins (casein, meat, and soy) in reducing the incidence and burden of colon tumours in a rat animal model of the disease.

**Growth factor activity:** Pioneering work over the past approximately 15 years has laid the foundation for exploitation of the remarkable cell growth promotional activity of an extract from cheese whey containing a plurality of growth factors (Table 2) (Belford et al., 1995; Francis, Regester, Webb, & Ballard, 1995; Pouliot & Gauthier, 2006; Rayner et al., 2000; Regester & Belford, 1999; Rowney, Hobman, Read, & Denichilo, 2005; Smithers, 2004). This whey growth factor extract has been shown to have potent mammalian cell growth activity, notably for fibroblast cell lines



(Belford et al., 1995), and remarkable wound healing effects (Rayner et al., 2000; Regester & Belford, 1999; Regester et al., 2003). This extract, shown to be efficacious in delaying the onset of mouth ulcers in an animal model of oral mucositis<sup>3</sup> (Clarke, Butler, Howarth, Read, & Regester, 2002), is currently in human clinical trials as a natural prophylactic and potential therapeutic agent against mucositis in cancer patients (Personal communication, L. Read—TGR Biosciences Pty Ltd., Australia).

**Physiological activity of lactoferrin:** Ready availability of large quantities of purified lactoferrin has allowed intensive study of the biological effects of this protein in vitro and in vivo (Brock, 2002; Vorland, 1999; Wakabayashi, Yamauchi, & Takase, 2006). The mechanisms of the well known anti-microbial effects of lactoferrin have been established (Farnaud & Evans, 2003), and nowadays form a sound basis for the application of this protein in enhancing the safety of meat (Naidu et al., 2003). In elegant work reported by Cornish et al. (2004), lactoferrin has been shown to have potent bone growth enhancement properties manifested through stimulation of the growth of osteoblasts and inhibition of osteoclasts. This research is helping form a strong science foundation for the use of lactoferrin as a complement to various strategies in the prevention and treatment of osteoporosis.

**Anti-microbial activity of lactoperoxidase:** The natural anti-microbial action of lactoperoxidase is being exploited in a range of oral healthcare products (Boots & Floris, 2006), and is finding application in such products directed toward the prevention and treatment of xerostomia (dry mouth) (Tenovuo, 2002; van Steenberghe, Van den Eynde, Jacobs, & Quirynen, 1994). The lactoperoxidase containing products have been clinically proven to inhibit harmful microorganisms associated with gingivitis and oral irritation, to promote the healing of bleeding gums and reduce inflammation, and to combat both the causes and effects of halitosis (bad breath) (Tenovuo, 2002).

**Physiological action of immunoglobulins:** Immunoglobulins represent the most abundant of the established bioactive proteins found in whey (Table 2). While colostrum represents the preferred choice of raw materials for the manufacture of immunoglobulin-enriched milk products (Huffman & Harper, 1999), cheese whey has been used for the preparation of similar ingredients (Ayers, Elgar, & Pritchard, 2003). These immunoglobulin-rich isolates all impart passive immunity to the consumer, and evidence is building that they combat infections, improve athletic performance and recovery times, assist those who may be immunocompromised, and enhance gut health (Buckley et al., 1998; Coombes et al., 2000; Mehra, Marnila, & Korhonen, 2006; Mero et al., 1997; Playford et al., 1999).

**Bioactive peptides:** Apart from the inherent bioactivity of many of the whey proteins, their primary amino acid sequences contain peptides with bioactivity, additional and often varied from that of the parent molecule (Meisel, 2001, 2005). A number of these bioactive whey peptides, together with the parent protein source, and proposed biological functionality are shown in Table 4. Commercially, the most promising of these peptides is the potent anti-microbial lactoferricin and closely related peptides, derived by peptic or chymosin digestion of lactoferrin (Bellamy, Takase, Wakabayashi, Kawase, & Tomita, 1992; Hoek, Milne, Grieve, Dionysius, & Smith, 1997).

**Milk fat globule membrane (MFGM) proteins:** Considerable attention is being paid to the proteins derived from the MFGM and their biological functionality and role (Mather, 2000; Peterson, Scallan, Ceriani, & Hamosh, 2001). MFGM proteins like

**Table 4**  
Bioactive whey-derived peptides<sup>a</sup>

Protein source	Peptide	Bioactivity (some putative)
$\alpha$ -Lactalbumin	$\alpha$ -Lactophorin	ACE <sup>b</sup> inhibitor
$\beta$ -Lactoglobulin	$\beta$ -Lactophorin	Ileum stimulation
	$\beta$ -Lactotensin	Ileum contraction
Serum albumin	Albutensin	ACE <sup>b</sup> inhibitor, ileum contraction
	Serophorin	Opioid activity
Glycomacropeptide	108–110; 106–116	Anti-thrombotic
Lactoferrin	Lactoferricin	Anti-microbial

<sup>a</sup> Adapted from Meisel (2001, 2005) and Korhonen (2006).

<sup>b</sup> ACE, angiotensin-converting enzyme.

mucin, xanthine oxidase, butyrophilin, and adipophilin appear to have anti-microbial and anti-infectivity functionality, and may also play a role in receptor function (Peterson et al., 2001).

### 5.2.2. Processing technologies—advances through the 1990s and 2000s

Whey-processing developments over the past approximately 15 years have focused on advanced liquid handling techniques, maximisation of quality and safety, and the introduction of sophisticated separation and fractionation methods. Emphasis has been on cost effectiveness and the retention, wherever possible, of native protein functionality, notably bioactivity. In this regard, chromatographic techniques, including traditional fixed bed and continuous approaches, have helped pioneer cost-effective processes for whey protein isolation and fractionation at commercial-scale (De Silva et al., 2003; Etzel, 2004; Huffman & Harper, 1999). During this period, the dairy industry became receptive to new and novel processing technologies that would allow cost-effective manufacture of whey ingredients destined for lucrative functional food applications. One such technique was Continuous SEparation (CSEP) chromatographic technology, a system approach to continuous simulated moving bed chromatography (De Silva et al., 2003). This technology captured the benefits of conventional chromatography (specificity, reproducibility, and mildness) but also addressed the shortcomings (cost, throughput, flexibility, productivity, and complexity), and has facilitated world-first developments in the manufacture of unique WPIs (enriched in  $\beta$ -lactoglobulin and/or glycomacropeptide), lactoferrin, and bioactive factors from whey (De Silva et al., 2003; Rowney et al., 2005). Membrane processing has also become more sophisticated with the development and application of membrane adsorbers (e.g., ion exchange) for the isolation and fractionation of whey proteins (Chiu & Etzel, 1997; Kim, Choi, & Row, 2003). These and other advanced processing techniques have facilitated the manufacture of a wide range of modern whey-based food ingredients, including specialised second-generation WPI offerings, high protein/peptide isolates, and fractionated/purified bioactive proteins (e.g., immunoglobulins, lactoferrin, lactoperoxidase, and growth factors) for specific high-value applications (Chatterton, Smithers, Roupas, & Brodtkorb, 2006; De Silva et al., 2003; Dionysius, Herse, & Grieve, 1991; Etzel, 2004; Francis et al., 1995; Korhonen & Pihlanto, 2003; Korhonen et al., 1998; Smithers, 2004; Rowney et al., 2005).

Almost without exception, whey-processing technologies result in a waste by-product; usually a lactose and mineral-rich permeate in large volumes. Science and technology advances, particularly over the past 10 years, have led to a greater understanding of lactose behaviour, simple and efficient approaches to its hydrolysis or its isolation at commercial-scale, and improved overall cost effectiveness in the processing of whey solids (Durham, Sleight, & Hourigan, 2004; Gaenzle et al., 2008;

<sup>3</sup> An indication in cancer patients receiving chemo and/or radiation therapy presenting as severe ulceration of the mouth and upper gastrointestinal tract. In severe cases, the patient can no longer eat or drink and must be fed intravenously.

Jelen & Tossavainen, 2003). It should be noted that no whey utilisation strategy will succeed without suitable attention being paid to lactose, as this component represents >75% of whey solids (Table 1) (Durham & Hourigan, 2007; Durham, Sleight, & Hourigan, 2003; Gaenzle et al., 2008). Further, in a world increasingly focused on conservation and sustainability, notably in regard to water resources in places like Australia, perhaps the final piece of the puzzle in completing the valorisation of whey from 'gutter-to-gold' will be total and widespread utilisation of the so-called 'cow water' that remains following isolation and/or removal of all the whey solids (Jelen, 2003). This reclaimed water following the processing of whey to remove all fat and solids non-fat is considered potable and thus safe to drink. However, at present 'cow water' is not approved for drinking or other uses.

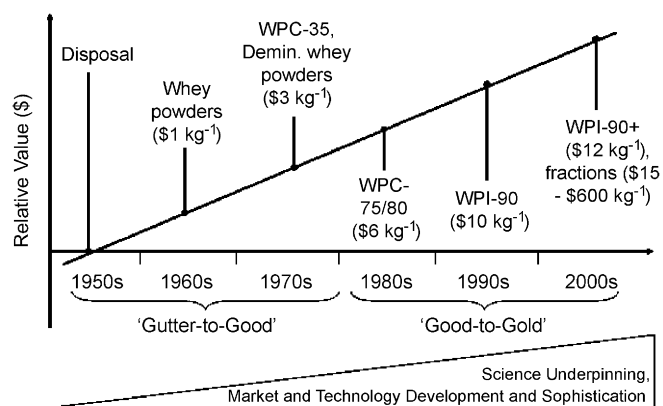
## 6. Concluding remarks—what's on and over the horizon ('gold-to-platinum')?

The transformation of whey from 'gutter-to-gold' over the past approximately 50 years, founded on advances in science and technology, has resulted in increasingly sophisticated products. Concomitant increases in the value of these products in an increasingly sophisticated marketplace have resulted in enhanced wealth to dairy manufacturers and the communities that rely on them. An illustration of this transformation, highlighting whey protein products and ingredients, through the periods 'gutter-to-good' and 'good-to-gold', is depicted in Fig. 4.

Now that whey and whey proteins are prized by the dairy industry, what does the future hold for these once maligned co-products of cheese and casein manufacture? While the future is yet to be written, some potential developments, both on and slightly over the horizon, have been noted below. These predictions, perhaps taking whey to 'platinum status', build on the science and technology advances that have taken place over the recent past.

Whey components, particularly the proteins and peptides, will increasingly be preferred as ingredients for functional foods and nutraceuticals, and as active medicinal agents, built upon the strong consumer trend for health and wellbeing, and continuing discovery and substantiation of the biological functionality of whey constituents.

Emerging technologies, including high pressure processing, high-power ultrasound, pulsed electric field, and microfluidisa-



**Fig. 4.** Schematic representation of the relative increase in value of whey protein/peptide products with increasing underpinning scientific knowledge of whey solids, and advances in technology and marketplace sophistication over the past approximately 50 years. The dollar values indicated for products and the chronology are not intended to be prescriptive, rather to provide a guide to the types of products developed, their relative value, and approximate developmental timeframes for the period indicated.

tion (e.g., Devlieghere, Vermeiren, & Debevere, 2004), will provide for new non-thermal approaches to the processing and preservation of whey, new avenues for modulation of protein functionality, and a continuing expansion of the applications base.

Application of a complex science approach will lead to a greater understanding of how whey components are structured and interact, and how they can be manipulated and exploited in supramolecular assemblies of whey biopolymers, leading to new and better understood food microstructures and architectures ('molecular-to-micro-to-macro').

Novel separation technologies, both standalone and in combination, will be developed, refined and introduced to whey processing for the cost-effective manufacture of whey-based products. Possibilities include vibratory shear enhanced processing, further enhancements to CSEP in combination with high-power ultrasound and other technologies, ion exclusion, and molecular recognition-based isolation techniques.

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