BIOACTIVE INGREDIENTS FROM MILK

Contents

INTRODUCTION

Milk has been a source of nutrition for centuries but recent advances in analytical and biochemical techniques have helped in identification of several bioactive components and verification of their bioactivities from milk. Although many of the bioactive components from milk remain unexploited as commercial ingredients, developments in new processing technologies such as membrane filtration and ion exchange chromatography have stimulated commercialisation of some of the biologically active components from milk. The growth in functional foods and nutraceuticals offer new opportuntites for bioactive ingredients from milk.

Milk contains bioactive components both in the lipid and in the skim part. Although the milk phospholipids have been commercially available from the lipid part for a while, other components are still under development. By far, skim milk and whey are the major bioactive-rich streams of milk and a range of bioactive ingredients have been commercialised. Further research and development, no doubt is likely to lead to commercialisation of more ingredients in future. This chapter highlights biological functionality and applications of major bioactive ingredients from milk and provides a list of frequently asked questions to help users of these ingredients.

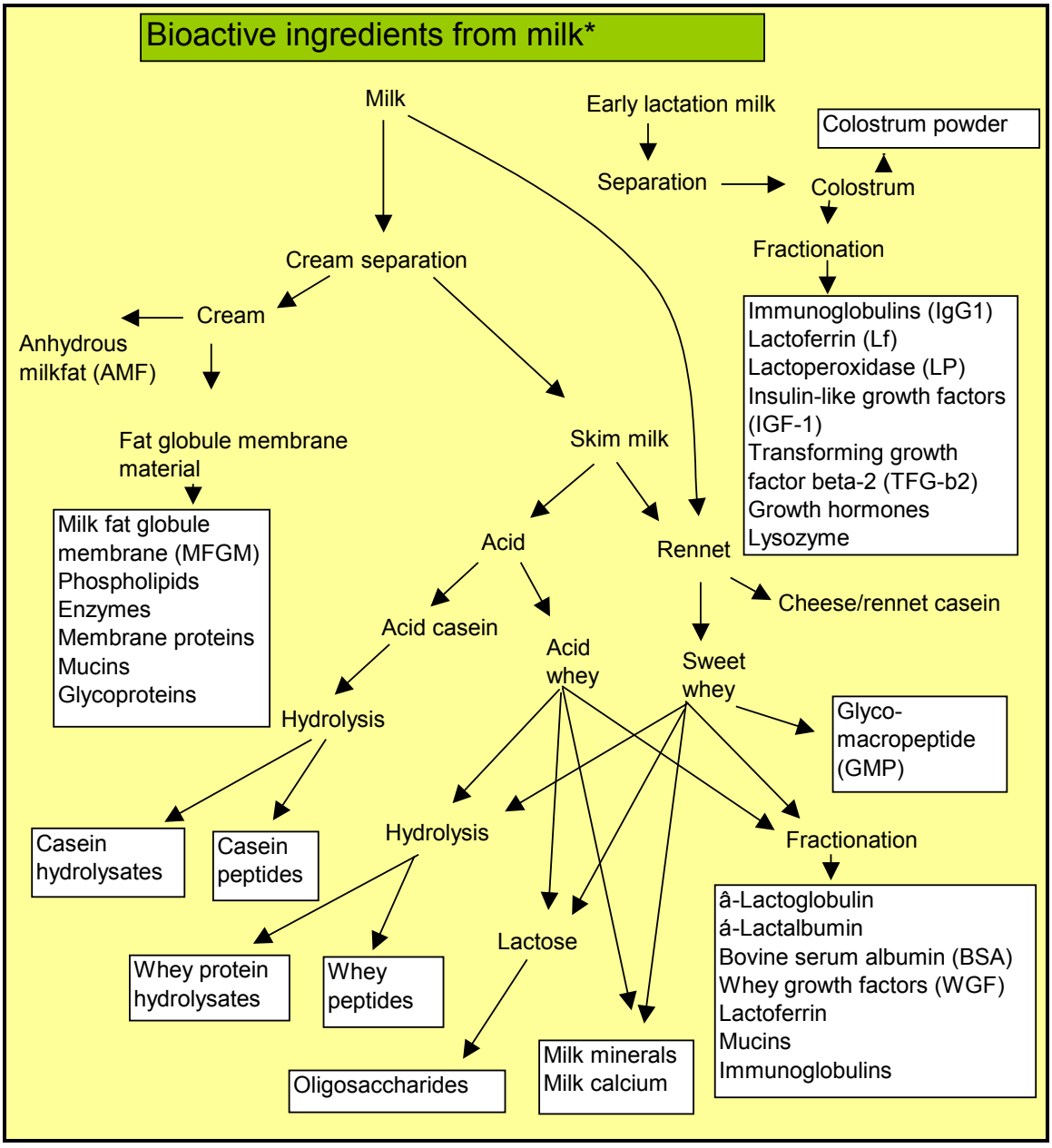


Figure 1: Bioactive ingredients from milk.
*Some of the ingredients may not yet be available commercially.

Figure 1 shows some of the bioactive components identified from milk and summarises general processing steps employed during fractionation of milk into bioactive ingredients. One of the foremost bioactive-rich **fluids** produced by the cow is the colostrum. Colostrum provides life-supporting immune and growth factors that ensure the health and vitality of the newborn. Colostrum is a rich source of several bioactive components including immunoglobulins, lactoferrin, lactoperoxidase, lysozyme and several growth factors.

In order to fractionate, isolate and concentrate bioactive components from milk, the first step is separation of fresh milk into cream and skim milk. The cream part contains almost all of the fat globules, and the associated fat globule membrane which is a rich source of several bioactive components (see Figure 1). Skim milk is usually treated with acid or rennet to separate casein from whey proteins; both casein and whey streams then become raw materials for isolation of bioactive components. Although many biologically active peptide segments have been identified in casein (identified as casokinins), **the** current commercial product range is limited to casein hydrolysates and phosphopeptides. During cheese manufacture, part of κ-casein released in whey during rennet action is commercially available as glycomacropeptide (GMP). In the dairy industry, whey available from cheese manufacture remains the most abundant source of whey-based bioactive ingredients. Several bioactive ingredients have been commercialised from whey as shown in Figure 1. Among these are ingredients enriched in whole proteins (*e.g.* β-lactoglobulin, α-lactalbumin, lactoferrin and lactoperoxidase), hydrolysed forms of proteins (*e.g.* whey protein hydrolysates with varying degrees of hydrolysis), and milk minerals. Dairy manufacturers are actively researching and developing new physiological or bioactive ingredients from milk that no doubt will be available in the future.

Table 1 summarises the main biological functions of bioactive ingredients from milk.

Bioactive ingredient	Potential biological function	Potential food applications
Colostrum	Immune factors, growth factors, anti-microbial	Sports formulation, calf feeding
Immunoglobulins	Antibacterial and immune enhancing	Infant formula
Lactoferrin	Iron binding ability responsible for many functions such as bacteriostatic effect, cell growth promotion, antioxidation and iron delivery and absorption	Infant formula, sports nutrition, meat preservation
Lactoperoxidase	Preservation effect. Bacteriostatic effect against Gram +ve bacteria and bactericidal effect against Gram –ve bacteria, e.g. pseudomonads, coliforms, salmonella, Listeria	Food preservation in general, meat products
Casein and whey protein hydrolysate	Reduced allergenicity, increased protein absorption, increased peptide bioactivity, lowering blood pressure	Infant and enteral formulation, geriatric products, sports beverages, weight control diets
Casein and whey peptides	Fast absorption Non-allergenic	Infant and enteral formulation, isotonic beverage, sports nutrition
Caseinophopeptide (CPP)	Mineral carrier, helps in re-mineralisation and mineral absorption, protection against dental caries, antibacterial	High mineral beverages, chewing gum, breakfast cereals
Glycomacropeptide (GMP) or Caseinomacropeptide (CMP)	Satiety, low phenylalanine	Phenylketonuric diets, sports nutrition
Milk minerals and milk calcium	Bone health and osteoporosis	Mineral fortification of beverages, breakfast cereals

Table 1 Biological function of bioactive ingredients from milk

Colostrum

Colostrum is the first milk produced by a cow after the birth of a calf. Colostrum is a rich source of antibodies, growth factors and nutrients for the suckling neonate and may provide passive immunity to the newborn against various infectious microorganisms, particularly those that affect the gastrointestinal tract. It may also have other health benefits.

A comparison of the composition of colostrum obtained during the first three milkings of a cow with normal cow milk is shown in Table 2. As seen in the Table 2, the composition of colostrum rapidly changes with the increase in number of milkings. The first milking has the highest amounts of protein and bioactive ingredients such as immunoglobulins, and is normally fed to the calf. This is particularly important for the defence of the newborn calf as a newborn calf is born without antibodies in the blood that are critical for the proper function of the immune system. Colostrum differs considerably from normal milk. Colostrum contains over 10 times the amount of immunoglobulins present in normal milk (Table 3). Immunoglobulins are very heat-sensitive proteins, which makes the processing of colostrum into an ingredient a difficult process.

Table 2 Composition of colostrum[1]

Component	Milking number after birth of calf			Normal milk
	1	2	3	
Specific gravity	1.056	1.040	1.035	1.032
Total solids, %	23.9	17.9	14.1	12.9
Protein, %	14.0	8.4	5.1	3.1
Casein, %	4.8	4.3	3.8	2.5
IgG, mg/mL	48	25	15	0.6
Fat, %	6.7	5.4	3.9	3.7
Lactose, %	2.7	3.9	4.4	5.0
Vitamin A, µg/L	2950	1900	1130	340
Vitamin D, IU, g fat	0.9-1.8			0.4
Riboflavin, µg/L	4.8	2.7	1.9	1.5
Choline, mg/mL	0.70	0.34	0.23	0.13

Table 3. Immunoglobulins found in bovine colostrum and normal milk[2]

Immunoglobulin	Colostrum (g/L)	Milk (g/L)
IgG1	52-87	0.31-0.4
IgG2	1.6-2.1	0.03-0.08
IgA	3.7-6.1	0.03-0.06
IgM	3.2-6.2	0.04-0.06

1 Foley & Otterby (1978)
2 Pakkanen & Aalto (1997)

Colostrum is also a rich source of growth factors as shown in Table 4. Growth factors are key regulators of a variety of cellular functions and are involved in the control of tissue growth and repair. Extensive research has identified a number of applications for their use in clinical medicine and biotechnology. The most important of these is likely to be a therapeutic potential in wound healing.

Table 4. Concentration of growth factors in colostrum and normal milk[2]

Growth factors	Colostrum (µ/L)	Milk (µg/L)
IGF-1	50-2000	<10
IGF-2	200-600	<10
TGF-1	n.d.	4.3
TGF-2	n.d.	n.d.
EGF	n.d.	<2

IGF – insulin-like growth factors, TGF – transforming growth factor, EGF – epidermal growth factor * n.d. – not determined

A general scheme for manufacture of colostrum ingredients is shown in Figure 2. Manufacture of colostrum powder or protein concentrate requires stringent quality control during collection, transport, processing, handling and storage so that the bioactivities of components are retained**. Colostrum obtained from the first few milkings is generally pooled, and either frozen or transported chilled to the dairy processing facility.** After separation of colostrum cream (which can be fed to the calf), the skim colostrum can be concentrated and dried into colostrum powder. In order to remove casein, skim colostrum is usually treated with rennet or acid, and the casein-free whey is used for development of colostrum whey powder or colostrum whey protein concentrate. Colostrum whey protein concentrate is particularly valuable as it is rich in bioactive protein components and contains low levels of lactose. Commercial drying of colostrum is either carried out by freeze-drying or by spray drying under mild temperatures.

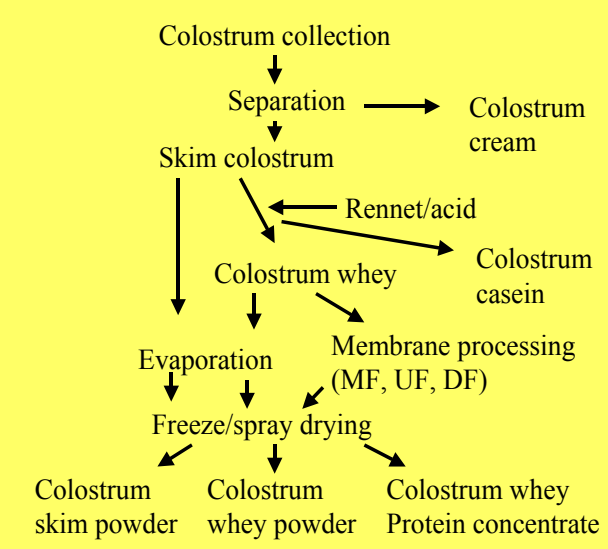


Figure 2 Processing schemes for manufacture of colostrum based ingredients

Bovine colostrum is marketed in several forms. Commercial colostrum products are available with immunoglobulin contents ranging from 16 to 50%. Composition of a colostrum product with 22% IgG is shown in Table 5.

Table 5. Composition of a commercial colostrum product with 22% IgG.

Component	Amount
Moisture (%)	5
Protein (%)	75
IgG (%)	22
Ash (%)	6
Lactose (%)	10
Fat (%)	2
Lactoferrin (%)	0.3
Calcium (%)	1.5

Colostrum: Functionality and applications

Colostrum products can provide a number of physiologically functional properties. The benefits of colostrum to the human body - from boosting the immune system to promoting cell repair are continually being researched and discovered. Major functionality and applications of colostrum include3:

- Source of growth factors
- Source of antimicrobial components
- Immune-enhancing properties and intestinal benefits
- Sports and performance applications

Source of growth factors

In addition to being a rich source of essential nutrients such as amino acids, carbohydrate, lipids and minerals, colostrum contains a range of growth factors that can stimulate healthy development of cells and tissues. Growth factors from colostrum and whey have been commercially available for some time. Growth factors present in colostrum include insulin-like growth factors-1 and 2 (IGF-1 and IGF-2), transforming growth factors – β1 and β2 (TGF-β1 and TGF-β2) and epidermal growth factor (EGF). Some of these factors are also present in regular milk but in very small amounts (100-1000 times less than colostrum). The IGFs stimulate the immune system, promote cell repair and growth, and influence how the body uses fat, protein and sugar. The IGFs acting as endocrine, autocrine and paracrine hormones enhance cellular glucose uptake stimulating synthesis of proteins, DNA, RNA and lipids. The amino acid sequence of bovine IGF-1 is identical to that of human IGF-1 and bovine IGF-2 differs from human IGF-2 by only three amino acid residues. Both IGF-1 and IGF-2 are heat-stable proteins that help in growth and differentiation of cells.

Antimicrobial components

Colostrum is a rich source of antimicrobial compounds that can help in protection against infections. Antimicrobial components present in colostrum include lactoferrin, lactoperoxidase, lysozyme and immunoglobulins. Each of these components is available commercially in purified forms and discussed in more details in the later part of this chapter. Antibodies from colostrum in oral immunotherapy have aided treatment of various human infections, including those caused by antibiotic resistant bacteria[4].

Immune-enhancing properties and intestinal benefits

One of the main reasons for hospital admission of infants and young children is infectious diarrhoea, usually caused by a rotavirus infection. Infants can also acquire rotavirus in hospital neonatal and paediatric wards; the infection can also be transmitted to adult members of the family. Colostrum has been successfully tried out in the protection against rotavirus and diarrhoea[5]. It has been suggested that infant formulas could be fortified with colostral immunoglobulins[6]. Colostrum may help protect the gastrointestinal tract against stomach cancers[7] and ulcers[8]. Colostrum has also been shown to prevent non-steroidal anti-inflammatory drug (NSAIDS)-induced gut damage. NSAIDS are given for the treatment of pain and are a common cause of gastritis.

Sports and performance applications

Colostrum appears to aid strength and speed in athletes, plus increase insulin, which has anabolic effects. Studies have shown that supplementation with bovine colostrum (20 g/d) in combination with exercise training for 8 weeks may increase bone-free lean body mass in active men and women[9].

Colostrum has been shown to enhance serum IGF-1, IgG, hormone and saliva IgA during athletes' training[10]. In clinical trials, IGF-1 is known to have strong anabolic effects on muscle tissue, as it is able to mimic most of the actions of growth hormones. IGF-I is of benefit to athletes, body builders and people concerned about weight because it can help burn fat and encourage lean muscle tissue.

[3] Pakkanen & Aalto (1997)
[4] Steven et al (1990)
[5] Davidson et al (1989)
[6] Seung et al (1995)
[7] Masuda et al (2000)
[8] Playford et al (2000)
[9] Antonio et al (2001)

Glycomacropeptide

Glycomacropeptide (GMP) is a hydrophilic peptide (amino acid residue 102 to 169) of κ-casein that provides stability to casein micelles in milk. When rennet acts on κ-casein during the manufacture of cheese, GMP is released into the whey. GMP makes up about 15% to 20% of the whey proteins. Recent advances in fractionation have allowed separation of GMP from cheese whey into commercial GMP-enriched ingredients. Due to the highly negative charge of GMP at low pH where whey proteins are positively charged, an ion exchange process can isolate GMP. When whey at pH 3 is contacted with a cation exchanger, the GMP is not adsorbed by the cation exchanger and may be concentrated and desalted by ultrafiltration. Alternatively, GMP from whey at pH less than 4 can be bound to an anion exchanger while the rest of the whey proteins are not bound. Pure GMP can then be eluted from the ion exchanger.

GMP is unique among some of the whey proteins in that it is a glycoprotein and, thus, has an oligosaccharide chain attached to it. It also is unique because it contains no phenylalanine, tryptophan or tyrosine. GMP also has high levels of the branched-chain amino acids, leucine, isoleucine and valine. This composition of GMP gives it some unique characteristics that can be utilized in a variety of interesting applications. A small population has phenylketonuria (PKU), meaning they are unable to digest phenylalanine. GMP is one of the few amino-acid sources PKU patients can tolerate because the pure GMP does not contain phenylalanine.

Composition of a commercial GMP is shown in Table 6.

Table 6. Composition of a commercial GMP

Component	Amount
Moisture, %	5
Protein, %	80
GMP (% of total protein)	90
Sialic acid, %	4

GMP: functionality and applications

Published research has linked GMP with many physiological functions, including: promotion of bifidobacterial growth; suppression of gastric secretions; inhibition of bacterial and viral adhesion; modulation of immune-system responses; and binding of cholera and *E. coli* enterotoxins. In simpler terms, GMP offers potential benefits to intestinal health, appetite control, reduced dental caries, enhanced immunity and protection against diarrhoea.

Some of the bioactive properties of GMP are:

- Anti-inflammatory[11]
- Toxin binding[12]
- Inhibition of bacterial and viral adhesion[13]
- Immune modulation and protection against diarrhea[14]
- Prebiotic effect[15]
- Source of amino acids for population suffering from phenylketoneuria (PKU)

Suggested applications of GMP are:

- Dental care products such as toothpaste and mouthwash for prevention of dental caries and remineralisation
- Supplements and diets for PKU sufferers
- Prebiotic for probiotic supplements and foods
- Sports nutrition products as source of branched chain amino acids
- High protein diets for weight control

Lactoferrin

Lactoferrin is an iron-binding glycoprotein present in colostrum, milk and whey. Lactoferrin exists as a single peptide chain with a molecular weight of 77,000. It is folded into two globular units with each unit able to bind 1.4 mg of iron per gram of protein. Bovine lactoferrin is somewhat similar in structure to the human form, having approximately 70% of the same amino acids. The iron-binding ability of lactoferrin is responsible for many biological functions such as bacteriostatic effect, growth-promoting effect on certain cell lines, and prevention of lipid peroxidation and promotion of iron absorption in the body. Lactoferrin is one of few proteins in whey that are positively charged at pH 7.0 (isoelectric point of approximately pH 7.9) while most other proteins are negatively charged. This feature of lactoferrin has been exploited in commercial isolation of lactoferrin. Using cation based resins and selective salt solutions, lactoferrin can be separated from other positively charged proteins attached to the resin. Further concentration of lactoferrin is carried out using ultrafiltration and spray drying. When reduced to its purest form, it is pink in colour. Commercially, lactoferrin is available in a range of protein concentrations. Due to the low amount present in milk and whey, the cost of separation is high and therefore, ingredient cost is high. Table 7 shows the composition of a commercial lactoferrin powder.

[11] Daddaoua *et al.* (2005)
[12] Kawasaki *et al.* (1992)
[13] Nesser *et al.* (1988)
[14] Otani *et al.* (1995)
[15] Azuma *et al.* (1985)

Table 7. Composition of a commercial lactoferrin product

Component	Amount
Moisture (%)	5
Protein (%)	95
Lactoferrin (% of total protein)	90
Iron (%)	13
Ash (%)	1
Fat (%)	<1
Lactose (%)	<1

Lactoferrin: functionality and applications

Lactoferrin can provide several physiological functional (bioactive) properties, which are mainly derived from its ability to bind iron. Each molecule of lactoferrin can bind two atoms of iron. The main bioactive properties of lactoferrin include antibacterial and antiviral properties, antioxidant properties, immune modulation, and ability to carry iron.

Antibacterial and anti-viral properties

Lactoferrin inhibits the growth of pathogenic bacteria and fungi, due to its ability to bind large quantities of iron. Lactoferrin binds iron very strongly, thus rendering this essential nutrient unavailable to support microbial growth. Lactoferrin also disrupts bacterial digestion of carbohydrates, further limiting their growth. In addition, the action of pepsin in the stomach converts lactoferrin into lactoferricin, which has broad-spectrum activity against pathogenic bacteria and yeast. Lactoferrin also has the ability to bind to parasites and the outer membrane of Gram-negative bacteria, making the cell wall more permeable and, thus improving the efficiency of antibiotics.

Additionally, segments of the lactoferrin molecule can exert a direct bactericidal effect on certain strains of bacteria and is also thought to inhibit the attachment of bacteria to the gut wall, therefore reducing the probability of infection. Anti-viral effects of bovine lactoferrin against several types of viruses have been reported; lactoferrin appears to achieve this effect by inhibiting virus absorption and its penetration into cells.

Antioxidant properties

Lactoferrin can be used as a natural antioxidant and may reduce the susceptibility to aging processes and disease. Lactoferrin provides protection against oxidative damage by scavenging excess iron, which catalyzes the undesired formation of free radicals from hydrogen peroxide produced as a result of microbial respiration, thus allowing the cell's own peroxidase to harmlessly break down the hydrogen peroxide.

Immune modulation

Lactoferrin contributes to the defence against pathogens by activation of cells involved in the anti-inflammatory response during the course of microbial infection, thus enhancing the self-immunity.

Iron transport and absorption

Lactoferrin is an excellent iron carrier and increases the bioavailability of iron.

Commercial lactoferrin is suitable for applications in health supplements, functional foods and drinks, infant formulas, cosmetics and oral care products, as well as for animal feed. Examples of potential markets for lactoferrin are supplements for the elderly or immune-compromised patients; supplements for recovery from gastrointestinal infections; products used to stimulate the body's immune system to help deal with toxic environments, disorders or treatments; and prophylactic products for travellers' diarrhoea. Lactoferrin can be used in a number of food applications such as

- Sports nutritional formulations
- Infant formula
- Yogurt
- Meat applications
- Chewing tablets or gums
- Antioxidant in cosmetics

Lactoferrin can be added in the range 10-100 mg per 100 g of product. This broad application range requires knowledge on effective incorporation of this bioactive component based on the prediction of its properties during processing, storage and consumer use.

Lactoperoxidase

Lactoperoxidase [EC 1.11.1.7] is an enzyme present in colostrum and milk, with a molecular weight of approximately 77.5 kDa. Bovine colostrum and milk contain about 11-45 mg/L and 13-30 mg/L lactoperoxidase respectively16. In whey, lactoperoxidase constitutes approximately 0.5% of whey proteins17. The biological significance of lactoperoxidase is its involvement in the natural host defence system against invading microorganisms. Separation of lactoperoxidase from whey is based on the same principle as used for isolation of lactoferrin. Lactoperoxidase is positively charged at the normal pH of whey (isoelectric point in the pH range 9.0-10.0) and can be bound to cation exchange resins and fractionated from the rest of the whey proteins.

Lactoperoxidase inactivates or kills a wide spectrum of microorganisms through an enzymatic action. This reaction involves two cofactors, hydrogen peroxide and thiocyanate

ions, which together with lactoperoxidase constitute the lactoperoxidase system (LP system). Activation of the enzyme results in the formation of hypothiocyanite ions, which are responsible for the antimicrobial action. The mechanism of the LP system can be described by the following reaction

$$Glucose + H_2O + O_2 \xrightarrow{Glucose\ oxidase} H_2O_2 + Glucuronate \xrightarrow{P^- + SCN^-} HOSCN / OSCN^-$$

The reaction of LP system relies on the production of short-lived intermediary oxidation products of the thiocyanate ion ($OSCN^-$) that reacts with bacterial cytoplasmic membranes, as well as impairs the function of metabolic enzymes. As addition of H_2O_2 is not permitted in certain countries, *in situ* development of H_2O_2 is carried out by the addition of glucose oxidase (a permitted additive).**The** source of thiocyanate ion can be either naturally present (as in the case of animal tissues and plant), or added as sodium or potassium thiocyanate.

Commercially, lactoperoxidase is isolated from either skim milk or whey using an ion-exchange process similar to that used for isolation of lactoferrin. The basic principle underlying the process is the fact that lactoperoxidase has an isoelectric pH in the alkaline range (9.0-9.5) which means that it is positively charged at the normal pH of cheese whey (6.0 – 6.6) while rest of the proteins are negatively charged. This difference in pH is used to adsorb lactoperoxidase to an anion exchange column that is subsequently separated from other proteins. Gross composition of a commercial lactoperoxidase is shown in Table 8.

Table 8. Composition of a commercial lactoperoxidase

Component	Amount
Moisture (%)	6.8
Protein (%)	91
LP (% of protein)	83
LP activity (ABTS method) U/mg protein	270
Ash (%)	2

Lactoperoxidase: functionality and applications

Lactoperoxidase when used in the form of LP system has a broad spectrum of antibacterial activity, having a bacteriostatic effect against Gram-positive bacteria and a bactericidal effect against Gram-negative microorganisms, e.g. pseudomonads, coliform, salmonella and Listeria18. The following are the examples of potential applications of lactoperoxidase:

- Use of LP system to improve yield of aquaculture by bactericidal effect on fish pathogens[19]
- Application of lactoperoxidase together with LP system activating ingredients (thiocyanate and hydrogen peroxide) in toothpaste formulations to protect against oral streptococci[20]. Activation of salivary peroxidase antimicrobial system in toothpaste and mouth rinse reduces acid formation by oral microorganisms and clinical studies have shown that plaque accumulation, gingivitis and early carious lesions and aphthous lesions may all be reduced by appropriate applications of the applied enzyme preparations[21].
- Promising results have been obtained when activating components are included in calf feed with the aim of activating the LP system in the intestinal tract[22]
- Using LP system to protect contamination of *Campylobacter jejuni* in poultry during slaughter[23]
- Lactoperoxidase can be used improve the shelf life in meat products by creating conditions that allow activation of the LP system
- Lactoperoxidase can be used successfully for controlling lactose fermentation and acidity development during storage of yogurt[24]
- Application of the LP system for preservation of cosmetics showed a broad-spectrum antimicrobial activity against bacteria yeasts and moulds[25]

Casein and whey protein hydrolysates

The enzymatic hydrolysis process produces protein ingredients designed for nutritional, dietetic and medical foods. Hydrolysed milk protein is a highly purified ingredient, hydrolysed under controlled conditions to obtain unique functional and nutritional properties. Both casein **and** whey proteins can be hydrolysed to produce protein hydrolysates with variations in the degree of hydrolysis. During enzymatic hydrolysis, casein and whey proteins are broken down into peptides of different sizes, and free amino acids. Specific enzymes are used that allow a good control over the size and functionality of peptides formed during hydrolysis. Enzymatic protein hydrolysates containing short chain peptides with characteristic amino acid profiles and defined molecular size are used in specific formulations such as those used for feeding hospitalised patients.

16 Korhonen (1977)
17 de Wit & van Hooydonk (1996)
18 Reiter & Harnulv (1984)
19 Kussendrager & van Hooydonk (2000)
21 Hoogendoorn (1985)
22 Reiter (1981)
23 Borch et al (1989)
24 Nakada et al (1996)
25 Guthrie (1992)

A general process for manufacture of protein hydrolysates is shown in Figure 3.

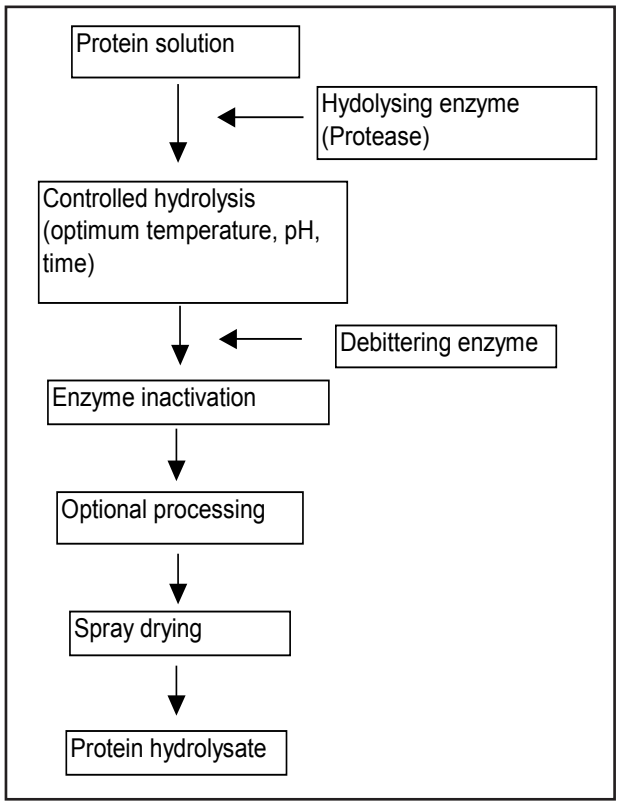


Figure 3. A general process for manufacture of milk protein hydrolysate

For manufacture of protein hydrolysates, milk proteins (casein, caseinate, milk protein concentrate, lactalbumin, whey protein concentrate, whey protein isolate) are first dispersed and solubilized in water and the pH and temperature are adjusted to the desired levels (generally to the optimum temperature for the enzyme). An appropriate enzyme is then added to the protein solution (substrate) at a certain enzyme: substrate ratio that optimises the enzymatic reaction. Under controlled conditions, the enzyme cleaves the peptides bonds and produces the desired level of protein hydrolysis. The hydrolysed protein is optionally processed through steps such as clarification, flavour reduction, concentration, and subsequently is spray dried.

Enzymatic hydrolysis of protein causes several changes in protein structure that affect the functional properties of protein. These changes include:

- Decrease in molecular weight due to breakdown of long polypeptides into smaller peptides, and increase in the number of peptides
- Decrease in the pH when hydrolysis is carried out at neutral to basic pH due to the release of H^+
- Increase in pH when hydrolysis is carried out at acidic pH due to the consumption of H^+
- Increase in the solubility due to increase in NH_3^+ and COO^- contents of proteins
- Increase in the number of hydrophobic residues due to destruction of the aggregated (globular) structure of protein

There are a number of variables that need to be considered and controlled during the manufacture of high quality protein hydrolysate. The first consideration is the selection of appropriate substrate, i.e. the protein, and most commonly, either whey protein concentrate or whey protein isolate is used, as these result in hydrolysates which are of high quality and have bland flavour. Casein is also used as a substrate for specific applications. Selection of an appropriate enzyme is the next consideration and is one of the most critical steps in the development of high quality protein hydrolysate. Considerable efforts go into selecting an approved, food-grade enzyme, and also in the desired amount (enzyme/substrate ratio) required for hydrolysis. Hydrolysis of protein results in a decrease in the pH of the protein solution and therefore regular pH adjustment is needed during manufacture. If a low-sodium hydrolysate is desired, potassium hydroxide is used for pH adjustment, otherwise sodium hydroxide is used. Throughout the process, pH, time and temperature are monitored and controlled which helps in producing high quality, nutritional and functional protein hydrolysates.

A common way of differentiating between protein hydrolysates is the assessment of the degree of hydrolysis (DH) where the higher value reflects higher level of hydrolysis. Users of protein hydrolysates need an understanding of the desired attributes, and selection should be based on flavour, degree of hydrolysis, required bioactivity, and nutritional composition.

The bitter taste of protein hydrolysates is a major barrier to their use in food and health care products. The intensity of the bitterness is proportional to the number of hydrophobic amino acids in the hydrolysate. The presence of a proline residue in the centre of the peptide also contributes to the bitterness. The peptidases that can cleave hydrophobic amino acids and proline are valuable in debittering protein hydrolysates. Aminopeptidases from lactic acid bacteria are available under the trade name Debitrase. Carboxypeptidase A has a high specificity for hydrophobic amino acids and hence has a great potential for debittering. A careful combination of an endo-protease for the primary hydrolysis and an aminopeptidase for the secondary hydrolysis is required for the production of a functional hydrolysate with reduced bitterness.

Commercial milk protein hydrolysates are available in a range of degrees of hydrolysis and molecular weight profiles. Table 9 shows approximate composition and functional properties of commercial hydrolysates.

	Whey protein or casein hydrolysate			
Degree of hydrolysis (%)	<5	6-10	11-20	>20
Protein (%)	80-92	80-92	80-92	80-92
Amino nitrogen (%)	1-2	1-3	1-4	3-10
pH, 5% solids	6.0-7.6	6.0-7.6	6.0-7.6	6.0-7.6
Fat (%)	0.1-3.5	0.1-3.5	0.1-1.0	0.1-1.0
Lactose (%)	0.1-3.0	0.1-3.0	0.1-1.0	0.1-1.0
Ash (%)	2.0-4.0	3.0-4.0	3.0-4.0	3.0-5.0
Major differences	Improved physical functionality (solubility, emulsification, foaming, etc.)	High levels of medium chain peptides, high solubility and heat stability	High levels of short to medium chain peptides, reduced protein allergy, high heat stability, low lactose and low fat, reduced allergenicity	High levels of di- and tri-peptides and free amino acids, high heat stability, low lactose and low fat, reduced allergenicity
Potential food applications	Dry and liquid beverages, infant formula, sports nutritional products	High protein beverage powders, powdered diet supplements, infant, sports and enteral nutritional formulations	Hypoallergenic infant, sports and enteral formulations, high protein formulations	Medical and clinical nutritional formulations, hypoallergenic infant and sports nutritional formulations, lactose-free formulations

Table 9. Approximate composition and potential functionality and applications of milk protein hydrolysates

Protein hydrolysates: functionality and applications

Milk protein hydrolysates can be used for the following bioactive or physiologically functional properties

- Reduced allergenicity and antigenicity
- Increased protein absorption
- Release of bioactive peptides

Reduced allergenicity

Due to the differences in the protein composition of human and cow milk, feeding of cow milk to newborn babies can cause allergic reactions. Hydrolysis of cow milk proteins into smaller peptides reduces the risk of allergenicity and allows the use of hydrolysate as a substitute for human milk protein in infant formula. Milk protein hydrolysates are also suitable for replacement of intact proteins in adult nutritional formulations where reduced allergenicity is needed. The antigenicity of a protein, *i.e.* its ability to induce an allergic reaction, is related to the size of protein, amino acid sequence, and presence of secondary and tertiary structures. The antigenicity of hydrolysates can be measured by an enzyme-linked immunosorbent (ELISA) inhibition assay that measures the amount of immunologically active protein. Intact casein shows a high value for immunologically active casein (IAC), at 10^6 µg/g protein equivalent (Figure 3). Increasing the degree of hydrolysis can decrease this value from 10^6 to 10^3 µg/g or below, as seen for casein hydrolysate in Figure 4.

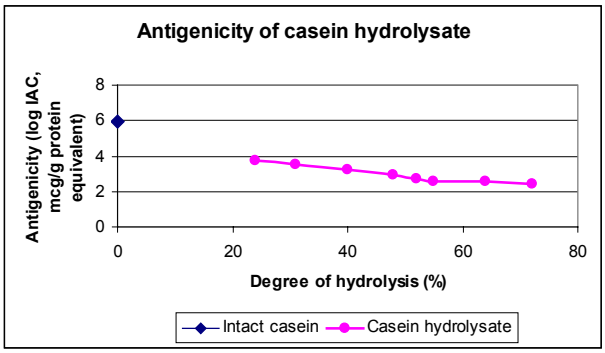


Figure 4. Allergenicity of casein and casein hydrolysate[26]

Food applications that can benefit from reduced allergenicity are infant formula, adult nutritional formulations, isotonic sports, enteral formulations and medical nutritional formulations

Increased protein absorption

Depending on the degree of hydrolysis, type of enzyme and conditions during hydrolysis, a range of peptides can be obtained during hydrolysis. The decrease in the peptide size generally leads to an increase in absorption of peptides. Milk protein hydrolysates containing mostly di- and tripeptides are absorbed more rapidly than free form amino acids and much more rapidly than intact proteins. This is desirable for athletes who want to maximise the amino acid delivery to muscles, and to the patients with impaired absorption system.

[26] Mahmoud *et al* (1992)

Release of bioactive peptides

Hydrolysis of milk proteins may produce biologically active peptides that are usually buried inside the aggregated structures of protein molecules. Milk protein hydrolysates for example, contain several biologically active peptides such as antihypertensive peptides. The antihypertensive effect of several peptides has been related to the inhibition of the angiotensin-converting enzyme (ACE). ACE activity results in blood pressure increase via conversion of angiotensin I to angiotensin II, which is a vasoconstrictive peptide, and via degradation of bradykinin, which is a vasodilatative peptide. Inhibition of ACE, e.g. by peptides in milk protein hydrolysates, results in a decrease in blood pressure.

Milk minerals

Calcium and phosphorus are the major minerals required for the growth and development of bones and teeth. Calcium deficiency is far too common in diet, and awareness of this deficiency has led to calcium fortification of a range of food products including breakfast cereals and fruit juices. Although consumers consider milk and dairy products to be the richest sources of calcium, many have limited their consumption to reduce fat in their diets, or because of their intolerance to lactose. Milk minerals are a rich source of calcium used for calcium fortification of food and beverage products. Commercial milk mineral complex is obtained from cheese whey after removal of proteins, which are converted into protein concentrates, and lactose, which is dried into lactose powder. A typical composition of a commercial milk mineral product (milk calcium) with 24% calcium is shown in Table 10.

Table 10 Typical compositions of milk minerals with 24 % calcium

Component	Amount per 100 g
Moisture (free & bound) (g)	10.0
Protein (g)	5.0
Fat (g)	1.0
Lactose (g)	5.0
Ash (g)	78.0
Sodium (g)	0.5
Potassium (g)	0.15
Calcium (g)	24.0
Magnesium (g)	0.8
Phosphorus (g)	13.7
Phosphorus as phosphates (g)	39.0
Chloride (g)	0.20
Iron (mg)	11.0
Copper (mg)	0.1
Manganese (mg)	1.0
Zinc (mg)	48
Iodine (ug)	20

Milk minerals: functionality and applications

Milk minerals are a natural milk calcium complex product manufactured from milk and whey. Calcium in milk minerals is a highly bioavailable calcium phosphate which is a natural milk calcium complex. Dietary calcium has been linked to osteoporosis and bodily functions such as regulation of cell function, nerve conduction, muscle contraction and blood coagulation. Milk minerals are rich in calcium and milk calcium has been suggested to have the following bioactive functional properties

- Prevention of osteoporosis and growth of healthy bones and teeth[27],[28]
- Blood pressure and cardiovascular disease control[29], [30]
- Lower effect on hypertension[31]
- Prevention of colon cancer[32]
- Control of weight gain and obesity[33],[34]

Suggested applications of milk minerals include

- Dairy products such as recombined milk, flavoured milk, yogurt and cheese
- Nutritional and functional foods such as sports and adult nutritional beverages, weight loss products and sports bars
- Bakery products such as breads and cakes
- Confectionery products
- Breakfast cereals
- Convenience foods such as soups, sauces and frozen desserts
- Food supplements such as capsules and tablets

27 Cadogan *et al.* (1997)
28 Murphy *et al.* (1994)
29 McCarron & Reusser (1999)
30 Miller *et al.* (2000)
31 Hatton & McCarron (1994)
32 Holt (1999)
33 Zemel *et al.* (2000)
34 Davies *et al.* (2000)

Frequently Asked Questions

Colostrum

What is colostrums and how is it different from normal milk?
Colostrum is the first milk produced by the cow during the first 24-36 hours after calving and serves as the first natural food for a newborn calf. Colostrum is not only a source of nutrients such as fat, protein, carbohydrate, vitamins and minerals, it also contains several biologically active componets that are present in minute quantities in normal milk. The most important bioactive components of colostrum are growth factors and antimicrobial factors that help in providing prevention from infections.

Which calves could benefit from supplementation of diet with colostrum?
Colostrum supplementation is advised for calves whose mother dies during birth or would not allow immediate nursing. Calves that are too weak to stand and suckle immediately after the birth could benefit from high amounts of immunoglobulins and other growth factors from colostrum.

Glycomacropeptides (GMPs)

Why is GMP promoted as a "weight control" ingredient?
Research has shown that GMP stimulates synthesis and release of the hormone cholecystokinin (CCK) in the duodenum. The two important physical events triggered by CCK during digestion are the release of the pancreatic enzymes and the contraction and emptying of the gall bladder/hepatic bile duct. The pancreatic enzymes are critical for the complete digestion of fats, proteins and carbohydrates and therefore the full nutritional realization of food. CCK has the effect of slowing the overall digestive process by slowing intestinal contractions, thus giving the digestive enzymes more time to work on their respective substrates resulting in more complete absorption of a given digestive loading. In fact, there is interest in GMP isolated by itself as an appetite suppressant for inclusion with other foods, because by slowing digestion one perceives the "full" feeling longer (satiety effect) following a meal, possibly discouraging between- meal snacking. This process is likely to have an effect as a "weight control" agent in diet.

What is PKU and why is GMP suitable for PKU patients?
Phenylketonuria (PKU) is a rare, metabolic disorder that is inherited from ancestors. People with PKU cannot utilize the essential amino acid phenylalanine and its derivatives due to the absence of the enzyme needed for utilization of phenylalanine. Consequently, a **phenylketonuric** person consuming a normal diet would accumulate high levels of phenylalanine, which may cause toxicity to the central nervous system and possible brain damage. Such persons are recommended special low-phenylalanine diets that provide adequate protein. High quality glycomacropeptide (GMP) is an ideal ingredient for phenylketonurics, as it contains negligible levels of phenylalanine.

Lactoferrin

How much lactoferrin can be added in food formulations?

The addition of lactoferrin to food products may be based on the amount of lactoferrin or the desired level of iron. The desired level of iron could vary from as little as 1 mg/100 mL for infant formula to as high as 7-mg/100 mL for a sports formulation.

How can lactoferrin be used in improving shelf life of meat products?
Recently, the USDA approved the use of activated lactoferrin on fresh beef. Activated lactoferrin, or lactoferricin, is a pepsin hydrolysate of lactoferrin. Lactoferricin has enhanced antimicrobial action in comparison to lactoferrin. This activated form has been shown to protect fresh beef against E. coli 0157:H7, Salmonella, Campylobacter and more than 30 types of other pathogenic bacteria. The activated lactoferrin prevents pathogenic bacteria from attaching to the surface of the meat and also prevents their growth.

What is the best method for lactoferrin addition to acidified beverages such as drinking yogurt and sports drinks?
One of the important properties of lactoferrin is its suitability for low pH environment. Lactoferrin is stable at low pH and can be added to drinking yogurt after preheat treatment, after fermentation, or through mixing with the fruit preparation. In fruit yogurt, lactoferrin can be added after the heat treatment of milk together with the starter or with the fruit preparation.

What are the main bioactive properties of lactoferrin?

The main bioactive properties of lactoferrin are derived from its ability to bind iron. These properties include

Improved bioavailability of iron: lactoferrin carries iron efficiently and helps in absorption of iron in the body

Antioxidant properties: lactoferrin can be used as a natural antioxidant that can prevent oxidative damage to body tissues by controlling the production of free radicals

Antibacterial property: This property is an effect of the depletion of iron in the environment, which limits the growth of bacteria. Action of pepsin in stomach converts lactoferrin into lactoferricin that has broad applications against pathogenic bacteria and yeasts. Also there are reports suggesting that lactoferrin interferes directly with bacterial cell surface thereby killing sensitive organisms.

Antiviral properties: Potentially, lactoferrin can inhibit the absorption of viruses to mammalian cells thereby preventing viral infection of cells

Immunomodulatory properties: During the course of microbial infection, lactoferrin provides anti-inflammatory

What are the major applications of lactoferrin?
Potential food applications of Australian lactoferrin powders include functional foods, beverages, sports and infant formulations, health supplements and animal feed. In food applications, lactoferrin can provide several health

benefits such as antiviral and antibacterial effects, immune enhancement and antioxidation properties. Lactoferrin powder can also be used in cosmetics and oral care products, exploiting the antibacterial and antiviral benefits.

- Sports and infant formulations
- Chewing gum
- Mouthwash and toothpaste
- Veterinary and feed specialties
- Natural preservative in foods

Lactoferrin can be added in the range 10-100 mg per 100 g of product. This broad application range requires knowledge on effective incorporation of this bioactive component based on the prediction of its properties during processing, storage and consumer use.

How does heat treatment such as pasteurization affect the activity of lactoferrin?
Like other whey proteins, lactoferrin is sensitive to high temperature heat treatment. However, formulation containing lactoferrin can be pasteurised at normal pasteurization temperature i.e. 72°C for 16 s with less than 5% loss of bioactivity. Although pasteurisation causes minimal reduction in the activity, excessive heat treatment during processing of food products can reduce the activity of lactoferrin.

Lactoperoxidase

How do I use lactoperoxidase for preservation of meat?
Lactoperoxidase provides an anti-bacterial, preservative effect when used in combination with thiocyanate ion (SCN^-) and hydrogen peroxide (H_2O_2). In combination, three ingredients form a system called lactoperoxidase system (LP System). The resultant product of the oxidation reaction is hypothiocyanite ion ($OSCN^-$) that inhibits bacterial metabolism via the oxidation of essential sulphydryl groups in proteins. The preservative effect of the LP System involves use of three ingredients:

- Lactoperoxidase powder at enzyme concentration in the range 1-20 mg/kg of meat
- Sodium or potassium thiocyanate ion at a concentration in the range 5-40 mg/kg meat (as thiocyanate ion)
- A source of hydrogen peroxide (H_2O_2) (*in situ* production using glucose oxidase – glucose oxidase is an approved processing aid in some countries) at a concentration in the range 5-50 mg/kg meat

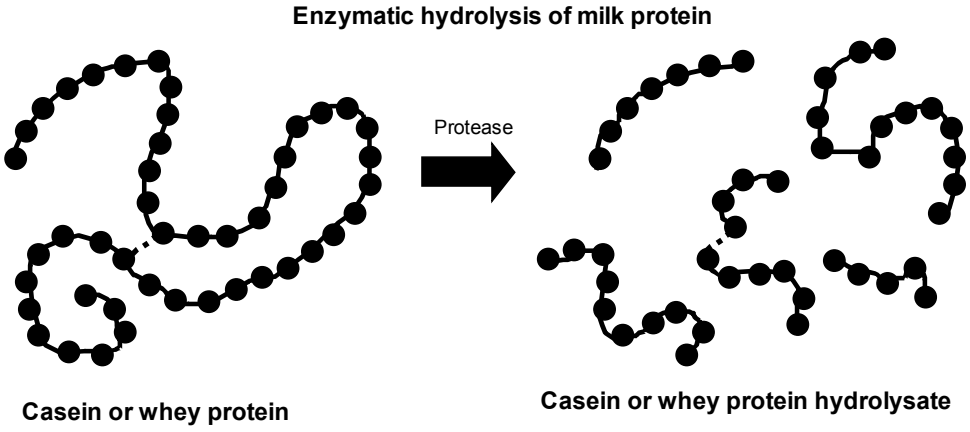
Protein hydrolysates

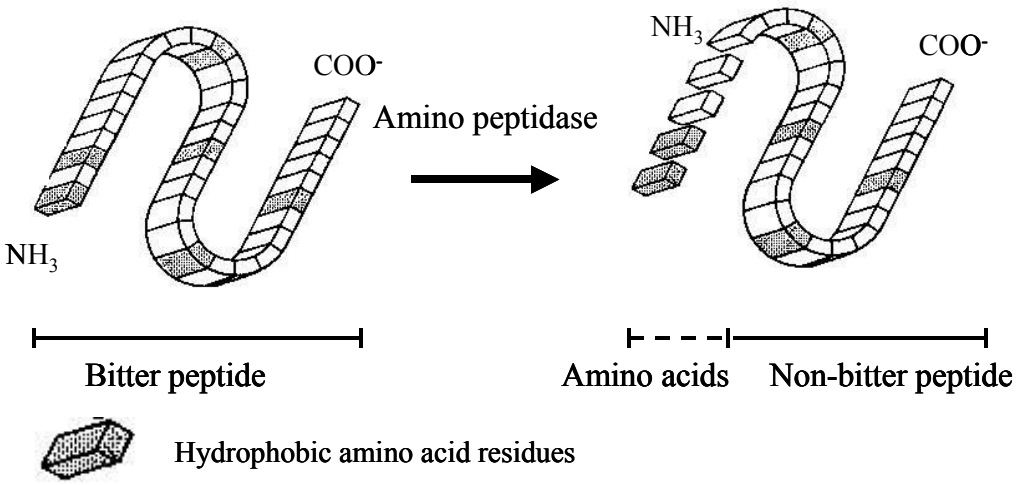
What is enzymatic hydrolysis of milk proteins and how is it carried out?
Enzymatic hydrolysis involves breakdown of protein molecules into smaller peptides and amino acids through the action of a protease or a peptidase as seen in the diagram below.

For manufacture of whey protein hydrolysate, a dispersion of whey protein concentrate or isolate is adjusted to temperature and pH that are optimum to the hydrolyzing enzyme. The enzyme is then added and allowed to react with protein for a specified time period. Sometimes more than one enzyme is added to optimize the hydrolysis for flavour and peptide size. After completion of hydrolysis the enzyme is deactivated, usually via heat treatment, and the milk protein hydrolysate is pasteurized. Subsequent treatments of the hydrolysate may include filtration, clarification, concentration and spray drying.

What are the benefits of protein hydrolysate and peptides over whole protein?
Protein hydrolysate and peptides are produced by enzymatic hydrolysis of casein or whey protein. The hydrolysis breaks the peptide bonds at specific locations and produces low molecular weight fragments with altered functionality and health benefits. There are three main benefits of using milk protein hydrolysates and peptides over whole protein:

- The digestibility of proteins is improved which helps people with impaired digestive functions in providing essential amino acids
- The allergenicity associated with whole protein is reduced, which is particularly beneficial for infants who consume infant formula, or adults who are allergic to proteins.
- The overall absorption of amino acids is improved, as the amino acids are already digested in hydrolysates and peptides. This is of particular interest to sports people who are at risk of negative nitrogen balance, and need to get fast-absorbed amino acids





Why do some protein hydrolysates taste bitter, and how is bitterness reduced in commercial hydrolysates?
A major barrier in the wide acceptance of protein hydrolysates is the unpleasant bitter flavour of some products. Bitterness results from the content of oligopeptides, which are formed by endoproteinases during the hydrolysis of native protein. The bitter taste only develops from a certain degree of hydrolysis onwards, when the peptides have molecular weight between 1,000 and 5,000 Daltons and high content of hydrophobic amino acids (leucine, isoleucine, proline, valine, phenylalanine, tyrosine and tryptophan). Almost all peptides containing these amino acids tend to be bitter, with intensity proportional to the number of hydrophobic amino acids and the size of the peptide. Depending on their share in the total protein, one can predict the tendency to bitter peptide formation during hydrolysis. As caseins contain more hydrophobic amino acids than whey proteins, hydrolysates from casein result in bitterness even at a low degree of hydrolysis.

Commercially, protein hydrolysates with little or no bitterness have been manufactured. Methods tried for removing bitterness include adsorption to active carbon, binding to ion exchange resins, plastein reaction or masking, but most have been discarded for technical or economic reasons. More success has been achieved with exopeptidases that attack the protein molecule only at the N- (aminopeptidases) or C-terminal end (carboxypeptidase), and split off small peptide fragments or amino acids. Aminopeptidases such as DebitraseTM, remove single or pairs of amino acids from the N-terminal of a peptide chain rendering the peptides free from bitterness (see the Figure above).

During hydrolysis, letting proteinase and aminopeptidase act at the same time can reduce bitterness. In this case, the process is conducted beyond the degree of hydrolysis at which the bitter point would have been reached with proteinase alone. In addition, a two-step process can be used to reduce the bitterness in the hydrolysate. In the two-step process, the conventional proteinase is allowed to act in the first step at which the bitter point is exceeded. In the second step, the aminopeptidases are given the opportunity to breakdown the bitter peptides. Although proteinases with natural exopeptidase activities already shift the point of unpleasant bitterness very noticeably, the pure aminopeptidases will shift that point much further.

What can be done to improve the emulsifying properties of protein hydrolysate?
As the process of hydrolysis breaks down the large macromolecules of protein into smaller polypeptides, the amphiphilicity of protein is reduced thereby reducing the ability to emulsify fat. Stable emulsions such as infant and adult nutritional formulations with protein hydrolysate, can be manufactured by appropriate manipulation of emulsifying conditions and by the use of appropriate emulsifiers. Often a combination of the two is required to make stable emulsions that can withstand high temperature treatment.

What is ACE-I activity and how can protein hydrolysate and peptides help in hypertension?
Angiotensin converting enzyme (ACE) is a key enzyme involved in the regulation of blood pressure. The ACE inhibitors (ACE-I) work by blocking (inhibiting) the enzyme that converts the inactive form of angiotensin (angiotensin I) in the blood to its active form (angiotensin II). Angiotensin-II is very potent vasoconstrictor and leads to high blood pressure (also called hypertension). Hypertension is considered to be the most important cause of human deaths in affluent countries. Hypertension affects over 80 per cent of diabetics and is one of the primary risk markers for metabolic syndrome. Protein ingredients, such as hydrolysates and peptides with ACE-I activities, help in lowering hypertension and risk of related diseases.

[35] Pawlett D and Bruce G (1996)

What is the importance of branched chain amino acids (BCAAs), and which protein products provide high amounts of BCAAs?
The branched chain amino acids (BCAAs) are leucine, isoleucine and valine. BCAAs are considered essential amino acids because human beings cannot survive unless these amino acids are present in the diet. These amino acids are particularly useful for atheletes and sports people in general. BCAAs are needed for the maintenance of muscle tissue and appear to preserve muscle stores of glycogen (a storage form of carbohydrate that can be converted into energy). BCAAs also help prevent muscle protein breakdown during exercise.

During sustained exercise, muscle BCAAs are used for energy and NH3 production. The subsequent increase of free tryptophan to BCAA ratio is thought to increase the tryptophan availability for serotonin synthesis. This can cause sleep and could increase the mental effort necessary to maintain athletic activity. BCAA supplementation before and during exercise may therefore delay fatigue and improve athletic performance. Research suggests that regular supplementation with branched chain amino acids can prevent central fatigue by preventing tryptophan from entering the brain. However, more research is needed to support such findings further. Whey protein products originating from acid whey contain higher amounts of BCAAs than those from cheese whey unless cheese whey is largely free from GMP.

Milk minerals

What are the advantages of using milk calcium over commercial calcium salts such as calcium carbonate and calcium phosphate?
There are several sources of calcium available in the market including those from non-dairy sources. Milk calcium is a 100% natural source of calcium derived from milk. In addition to calcium, milk calcium may contain protein and other nutrients such as other minerals that are not available from non-dairy calcium salts. The flavour of milk calcium is superior to other calcium salts. The absorption of dairy calcium is considered superior to other sources since it is present with other minerals such as phosphorus, which are essential for bone metabolism. Milk calcium contains a calcium: phosphorus ratio of approximately 1.7 that is considered optimal for bone absorption (optimal range 0.2-2.0).

Is the milk calcium more bioavailable than other calcium salts?
Several clinical studies have been carried out on **bioavailability** of dietary milk calcium. **While milk calcium is bioavailable, studies have not found it significantly higher than the bioavailability of calcium from other sources**. Bioavailability of calcium is influenced by the several factors including the level of calcium intake, vitamin D status, phytates, oxalates, caffeine, lipids, phosphopeptides, proteins, lactose and **phosphorus**. A review on calcium and bone health showed a positive effect of dairy calcium on bone health and the prevention of osteoporosis[35]. The flavour of milk calcium is generally superior to other calcium salts.

How can we avoid sedimentation of milk minerals in liquid beverages such as a nutritional beverage?
Two strategies can be used to optimise the use of milk minerals in liquid beverages:

- Using micronised milk minerals and controlling viscosity. The sedimentation of particles in a beverage is governed by the movement of particles and can be roughly predicted by the Stokes Law, according to which the rate of movement of particles is directly proportional to the diameter of particles and the density difference between the particle and the surrounding medium, and inversely proportional to the viscosity of the liquid. Thus by reducing size (by micronisation) and increasing viscosity (using a stabiliser), the rate of separation of milk minerals can be slowed down
- Using appropriate hydrocolloid stabilisers that create a weak three-dimensional network and a yield stress in the formulation. **The yield stress needs to be just sufficient enough to hold the minerals, as too much yield stress may impart an undesirable appearance (gel-like structure) to the beverage**

[36] Kun *et al.* (2001)

Glossary

ACE - I
Angiotensin converting enzyme (ACE) is a key enzyme involved in the regulation of blood pressure. The ACE inhibitors (ACE-I) work by blocking (inhibiting) the enzyme that converts the inactive form of angiotensin (angiotensin I) in the blood to its active form (angiotensin II). Angiotensin-II is very potent vasoconstrictor and leads to high blood pressure (also called hypertension). Hypertension is considered to be the most important cause of human deaths in affluent countries

Antimicrobial
Antimicrobials are biologically active components that protect against bacterial infections and enhance immunity. Antimicrobial components of milk include growth factors, lactoferrin, lactoperoxidase and lysozyme

Antioxidant
In biological systems, the normal processes of oxidation (plus a minor contribution from ionizing radiation) produce highly reactive free radicals. These can readily react with and damage other molecules and body cells. An antioxidant is a chemical that prevents the oxidation of other chemicals. Many of the vitamins act as antioxidants

Bioactivity
The physiological functional role of a food component is called bioactivity. Examples of bioactivity include anti-hypertensive activity of peptides from milk, and antibacterial and immune enhancing properties of lactoferrin.

Casein hydrolysate
Peptides of casein obtained by enzymatic hydrolysis of casein or caseinate

Colostrum
Colostrum is the first milk produced by a cow after the birth of a calf. Colostrum is a rich source of antibodies, growth factors and nutrients for the suckling neonate, and may provide passive immunity to the newborn against various infectious microorganisms, particularly those that affect the gastrointestinal tract.

ELISA
ELISA, enzyme-linked immunosorbent assay is a sensitive laboratory method used to detect the presence of antigens or antibodies of interest in a wide variety of biological samples. An ELIZA method can be used to measure the antigenicity of milk proteins and hydrolysates

Functional food
Functional foods refer to foods and food components that provide health benefits beyond basic nutrition. Functional foods may be naturally present (e.g. broccoli), dietary supplements (e.g. vitamins and minerals) or fortified (e.g. health drinks)

Growth factors
Growth factors are bioactive proteins present in colostrum, milk and whey. Growth factors are key regulators of a variety of cellular functions and are involved in the control of tissue growth and repair. Extensive research has identified a number of applications for their use in clinical medicine and biotechnology. Commonly identified growth factors are insulin-like growth factors-1 and 2 (IGF-1 and IGF-2), transforming growth factors – β1 and β2 (TGF-β1 and TGF-β2) and epidermal growth factor (EGF)

Hydrolysis
Enzymatic hydrolysis involves breakdown of protein molecules into smaller peptides and amino acids through the action of a protease or a peptidase. Hydrolysis of protein improves its functional properties and releases bioactive peptides

Lactoferrin
Lactoferrin is an iron-binding glycoprotein present in colostrum, milk and whey. The iron-binding ability of lactoferrin is responsible for many biological functions such as bacteriostatic effect, growth-promoting effect on certain cell lines, and prevention of lipid peroxidation and promotion of iron absorption in the body

Lactoperoxidase
Lactoperoxidase [EC 1.11.1.7] is an enzyme present in colostrum and milk, with a molecular weight of approximately 77.5 kDa. Bovine colostrum and milk contain about 11-45 mg/L and 13-30 mg/L lactoperoxidase respectively. The biological significance of lactoperoxidase is its involvement in the natural host defence system against invading microorganisms

Milk minerals
Milk minerals are obtained from cheese whey after removal of proteins that is converted into protein concentrates and lactose, which is dried into lactose powder. Milk minerals are a rich source of calcium used for calcium fortification of food and beverage products

Peptide
Peptides refer to segments present in protein molecules formed by joining amino acid residues. The link between one amino acid residue and the next is an amide bond, and is sometimes referred to as a peptide bond

PKU
Phenylketonuria (PKU) is a rare, metabolic disorder that is inherited from ancestors. People with PKU cannot utilize the essential amino acid phenylalanine and its derivatives due to the absence of the enzyme needed for utilization of phenylalanine. Consequently, a phenylketonuric person consuming a normal diet would accumulate high levels of phenylalanine, which may cause toxicity to the central nervous system and possible brain damage.

Prebiotic
Prebiotics refer to ingredients that promote the growth of probiotic bacteria. Examples of prebiotics are oligosaccharides such as fructo-oligosaccharides or inulin

Probiotic
Probiotic refers to a substance containing beneficial live microorganisms that claims to be beneficial to humans and animals, e.g. by restoring the balance of microflora in the digestive tract. Examples of probiotic bacteria are acidophilus and Bifidobacteria

Whey protein hydrolysate
Peptides of whey proteins obtained by enzymatic hydrolysis of whey proteins

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