HEALTH ENHANCING PROPERTIES OF WHEY PROTEINS AND WHEY FRACTIONS

By Rosemary L. Walzem, R.D., Ph.D.
Professor of Nutrition,
Texas A & M University, USA

U.S. whey products are now recognized for their high nutritional value and versatile functional properties in a multitude of food products. Evidence continues to accumulate that U.S. whey products contain a variety of factors and compounds capable of improving health and preventing disease. Moreover, U.S. whey and whey components are increasingly used in pharmaceutical, cosmetic and agricultural industries.

NEW AREAS OF DEVELOPMENT FOR WHEY PRODUCTS

The food industry continually strives to offer innovative new products to satisfy consumer needs. Consumer awareness of, and interest in, nutritious, healthy foods has driven much of the research into the healthful effects of whey and whey fractions. Evidence continues to accumulate that whey contains a variety of factors and compounds capable of improving health and preventing disease. In particular, newer information in the areas of probiotics, prebiotics and viral virulence indicate that there is good potential to produce healthful functional foods and cosmetics to reduce both infectious and chronic diseases. This brief review highlights some of the health-enhancing properties of whey and whey fractions, and points to exciting areas of development for whey products.

GENERAL NUTRITIONAL PROPERTIES

Whey is a reliable source for a number of high quality and biologically-active proteins, carbohydrates and minerals. Whey products can be classified into several categories (see Whey product descriptions and composition in Reference Manual for U.S. Whey Products). This review highlights some of the nutritional and health-enhancing properties of the major and minor whey proteins and whey components.

COMPOSITION OF MILK PROTEINS

The major whey proteins are β-lactoglobulin and α-lactalbumin; minor whey proteins include proteose-peptones, blood proteins and lactoferrin.

Whey proteins are easily digested and contain an amino acid profile that meets or exceeds all the essential amino acid requirements set by the Food and Agriculture Organization/World Health Organization (FAO/WHO). Long recognized as high quality protein sources, individual U.S. whey proteins also contain a variety of functional and nutritional attributes that should be considered by product developers. Immunomodulatory effects of individual whey proteins have become well documented with improvements in purification technologies. Animals fed diets containing whey as the protein source were much better able to resist chemically-induced cancer than those fed casein or soy. Nutritional and functional characteristics of whey proteins are related to the structure and biological functions of these proteins, and they possess a variety of characteristics.

β-Lactoglobulin is approximately 50% of the total whey protein content in bovine milk. It binds calcium and zinc and has partial sequence homology to retinol-binding proteins. β-Lactoglobulin has numerous binding sites for minerals, fat-soluble vitamins and lipids, and can be used to incorporate desirable lipophilic compounds such as tocopherol and vitamin A into lowfat products.

α-Lactalbumin represents about 25% of the total whey protein content in bovine milk. Seventy percent of protein in human milk is like whey protein, and 41% of that protein is α-lactalbumin. α-Lactalbumin accounts for 28% of the total protein in human milk. Addition of bovine α-lactalbumin is strongly advocated to "humanize" infant formulas and create other products for people with limited or restricted protein intakes. Serum albumin and immunoglobulins are blood proteins that become incorporated into milk and are recoverable as minor whey proteins. Serum albumin binds fatty acids as well as other small molecules. The immunoglobulins include IgG1, IgG2, IgA and IgM, and they provide passive immunity to infants and other consumers. Greater concentrations of immunoglobulins are found in whey derived from colostrum.

Two other proteins in whey are lactoferrin and lactoperoxidase. Lactoferrin is an iron-binding and transport protein that enhances iron absorption and does not cause constipation in infants, as do inorganic iron supplements. For these reasons, lactoferrin is widely used in Japan, Korea and other Asian countries in infant formulas. A number of other benefits exist, including antioxidant effects, enhancement of immunity and anticancer effects. Lactoferrin may also be an immunomodulator and it is the major non-specific disease resistance factor found in the mammary gland. Importantly, once lactoferrin has given up its iron for absorption, it can then bind free iron in the digestive tract. This iron-binding ability inhibits undesirable microflora and promotes desirable microflora in the intestinal tract by inhibiting the growth of enteropathic bacteria. The bacteriostatic activity of lactoferrin is being studied for possible uses as a preservative. Lactoferricin, a basic peptide derived from lactoferrin, is protective against intestinal pathogens. Lactoperoxidase is an enzyme that breaks down hydrogen peroxide. This nutraceutical component of milk and whey products is an enzyme with antibacterial properties. Lactoperoxidase has been studied as a means of controlling acidity development and pH shift during refrigerated storage of yogurt. Lactoperoxidase is being researched as a natural preservative. In combination with other preservatives, it is being used as a cavity-inhibiting ingredient for toothpaste.

Glycomacropeptide (GMP), the glycosylated portion of caseinomacropeptide (CMP), is present in sweet whey formed following κ-casein cleavage and casein precipitation by rennin. This protein is absent from acid whey formed when caseins are precipitated by lowering the pH to 4.6. Glycomacropeptide can suppress appetite via stimulation of the pancreatic hormone cholecystokinin (CCK) release, it alters pigment production in melanocytes, acts as a prebiotic and has immunomodulatory actions. Physiologic activity of GMP depends upon its glycosylation.

Composition of Milk Proteins.
Milk contains two broad protein classes: caseins and whey proteins

Protein	Concentration (g/l)	Approximate % Total Protein
Caseins	24–28	80
• *alpha*-casein	15–19	42
• *beta*-casein	9–11	25
• *delta*-casein	3–4	9
• *gamma*-casein	1–2	4
Whey proteins	5–7	20
• *beta*-lactoglobulin	2–4	
• *alpha*-lactalbumin	1–1.5	
• protease-peptones	0.6–1.8	
• blood proteins	1.4–1.6	
serum albumin	0.1–0.4	
immunoglobulins	0.6–1.0	
		100

*Fennema, O. 1965. *Food Chemistry*. 2nd Edition. Marcel Dekker Inc., New York, NY, p. 796.

BIOACTIVE WHEY PROTEINS AND PEPTIDES

Several countries have pioneered the use of whey as a resource for many medically-active components. Several physiological roles have been either defined or suggested for minor whey proteins or peptides. These components can provide passive protection against infection; modulate digestive and metabolic processes; and act as growth factors for different cell types, tissues and organs.

Whey components with potential for commercial application include α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins, lactoferrin and lactoperoxidase. Many of these components exhibit biological activity that is valuable in nutraceuticals or antimicrobials, and specific digestion products of milk proteins identified as bioactive peptides include exorphins (casomorphins), phosphopeptides and immunopeptides. The bioactive peptide sequences are in an inactive state inside the polypeptide chain of the intact whey protein. These peptides, released during intestinal digestion of whey proteins, may be involved in regulation of nutrient entry and influence the postprandial metabolism via stimulation of the secretion of hormones.

Therapeutic benefits of whey proteins can also result from bioactive peptide production during fermentation. In a study where milk proteins were separated and fermented, α-lactalbumin inhibited division of cells in culture, whereas peptides from fermented casein did not. A decrease in cell proliferation by such bioactive peptides may be linked to the association of the reduced incidence of colon cancer and yogurt consumption. In another study, cell growth assays identified fibroblast growth factors in partially-purified whey fractions. Another bioactive component identified in whey is the neurotrophic, or nerve-growth factor, prosaposin. More work will be needed to prove many of the effects demonstrated in vitro. However, the fact that in vitro effects are readily demonstrated underscores the potential for product development within agricultural and biotechnology markets. Growth supplements and metabolically-active peptides can be used to control or alter culture growth or production qualities. Thus, the healthful effects of whey and whey components should also be considered for non-human consumers such as cell-lines or fermentative cultures. Bioactive peptides are proving capable of conferring health benefits in the form of non-food products such as cosmetics or pharmaceuticals.

APPETITE SUPPRESSION

Glycomacropeptide is a powerful stimulator of CCK, which is an appetite-suppressing hormone that plays many essential roles relating to gastrointestinal function, including the regulation of food intake. In addition to being a regulator of food intake, CCK stimulates gall bladder contraction and bowel motility, regulates gastric emptying and stimulates the release of enzymes from the pancreas. In animals, a rise in CCK is followed by a large reduction in food intake. High protein intakes lead to increases in CCK release until pancreatic protease release (primarily trypsin) matches protein intake. Ingestion of whey and casein in six healthy volunteers resulted in a large increase in CCK release. Casein has a higher concentration of GMP than whey, but whey does appear to have an effect on CCK release. Research on the effects of GMP and CCK could lead to the use of certain milk proteins as an appetite suppressant and/or diet aid.

BONE PHYSIOLOGY

In addition to containing minerals that enhance bone growth, whey protein was recently reported to contain an active fraction that stimulated the proliferation and differentiation of cultured osteoblasts (bone-forming cells). If the intestine absorbs these active components, they may play an important role in bone formation in humans.

THERAPEUTIC VALUE IN INFANTS AND THE ELDERLY

Whey proteins have found considerable usage in infant's nutrition as whey-predominant formulas as well as whey protein hydrolysates for infants with an intolerance to cow's milk protein. An active area of research is the formation of biologically-active peptide sequences during digestion and resultant effects on secretion of entero hormones as well as immune enhancement. A number of other biologically-active molecules, such as the pluripotent polypeptides called cytokines that possess autocrine/paracrine actions, are present in milk. A whey-predominant protein was found preferable to casein-predominant protein in the diet of very-low-birth-weight neonates possibly because it may lessen the risk of metabolic acidosis and its potential adverse effects. Another study showed that whey hydrolysate formula was an acceptable alternative to soy or casein hydrolysate formulas in most infants less than 6 months of age with gastrointestinal symptoms of cow milk and/or soy protein-based infant formula intolerance.

Restoration or stimulation of the intestinal system is often needed in the elderly. When a Lactobacillus-GG fermented whey drink was administered to elderly nursing home residents complaining of difficulties in defecation, the consistency of the subject's stools appeared to normalize, and there were no significant changes in the fecal frequency, weight and pH.

IMMUNE SYSTEM STIMULATION

The immune system plays a central role in protection against bacterial, viral, parasitic and fungal infections, and also cancers. Deficiencies in any aspect of the immune system can predispose an individual to a greater risk of infection and may enhance the severity of disease. The immune system employs both non-specific and specific immune responses to confer protection against the disease. Non-specific components of the host defense include physicochemical barriers such as skin, mucus, lysozyme, complement and interferons, as well as natural killer cells and from phagocytic cells (cellular immunity) such as neutrophils and monocytes/macrophages. Specific immune responses are mediated by antibodies (IgA, IgG, IgM, IgD and IgE) produced by B-lymphocytes (humoral immunity), while T-lymphocytes give rise to T-helper, T-suppressor and cytotoxic lymphocytes (cell mediated immunity).

There is inadequate knowledge about the effect of bovine milk or its components on the human immune system, although immunocompetent cells of humans have receptors for milk proteins and peptides. A seemingly important feature of whey proteins is their high concentration of cysteine, which is thought to be rate-limiting for GSH synthesis. The GSH-modulating effects of whey proteins is believed to underlie both immuno-enhancing and some antioxidant actions of whey proteins. In animals, whey protein concentrate feeding enhanced immune responsiveness and GSH concentrations in the spleen, but these effects were decreased following treatment with an inhibitor of GSH. Animal studies have also shown that dietary whey protein concentrate elevates humoral and cell-mediated immune responses.

ANTIOXIDANT ACTIONS

The ability of whey components to improve the host antioxidant defenses and lower oxidant burden is emerging as a premier contribution to population health. Viral virulence is now linked to passage of non-virulent forms through hosts with compromised antioxidant status. Research has repeatedly demonstrated that selenium and vitamin E will prevent viruses from converting to a virulent genotype. This effect is non-specific in that it appears to be related to increased oxidant tone or poor ability to defend against oxidants, so that factors that act as antioxidants or that reduce biological oxidant generation are protective. Nutritionally, whey products provide active lactoferrin/metal-binding activities.

Although indirect, passive immunity against infection in the intestinal lumen is afforded by immunoglobulins, enzymes (lysozyme, lactoperoxidase), lactoferrin, casocidin, isracidin or caseinomacropeptides found in whey could also reduce oxidant burdens imposed by inflammation. Immunoglobulins are involved in the passive protection of the young and they resist, in part, degradation in the intestinal lumen. Lactoferricin, a peptide cleaved from lactoferrin by pepsin, exerts in vitro antibacterial effects against both bacteria and yeast, an effect that is linked to iron-binding activity. Lactoferricin also exhibits an antimicrobial activity that originates from a direct interaction with the surface of bacteria. Lactoferrin binds iron and provides the means for both stable iron delivery and scavenging of free iron that could catalyze oxidative reactions. Whey protein, and lactoferrin are all good candidates for dietary inhibitors of oxidative stress in a rat model, even when dietary Vitamin E is low.

ANTICARCINOGENIC ACTIVITIES

Epidemiological studies indicate that humans who consume milk are less likely to develop cancer of the colon and rectum than those who do not consume milk. Calcium and vitamin D, mainly from milk, were identified as protective against colorectal cancer. Recently, 30 grams of whey protein concentrate was fed daily for six days to seven patients with carcinoma of the breast, pancreas or liver. Normal and cancerous tissues had different responses in GSH status to whey protein concentrate feeding. The initial high concentrations of GSH in blood lymphocytes, which reflected high tumor GSH levels, normalized in two of the patients who exhibited signs of tumor regression. These results indicate that whey protein concentrate might deplete tumor cells of GSH and render them more vulnerable to chemotherapy. Epidemiological and experimental studies suggest that dietary milk products may exert an inhibitory effect on the development of several types of tumors. Experiments in rodents indicate that the antitumor activity of the dairy products is in the protein fraction and more specifically in the whey protein component of milk.

HIV

Whey protein isolate is rated so highly by the medical profession that it is used to treat HIV patients. Whey elevates deficient levels of GSH and so provides an extremely important antioxidant involved in the maintenance of functional and structural integrity of muscular tissue undergoing oxidative damage during exercise and aging. HIV has an antagonistic relationship to GSH, that is, low cellular GSH allows HIV to multiply and high GSH dramatically slows viral replication. In cells with an improved GSH status after the ingestion of whey protein concentrate, there was a substantial reduction in virus activity and increased survival expectancy.

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CALCIUM AND OTHER MINERALS IN WHEY

Whey and mineral concentrates from whey are high quality sources of calcium, magnesium and phosphorus. For example, calcium, magnesium and phosphorus content in mg per 100 grams of sweet whey powder is, respectively, 796, 176 and 932, and the content in acid powder is 2,054, 199 and 1,348. Lactose-reduced whey is also a good source of calcium, with concentrations in excess of 800 mg/100 g.

For comparison, the WHO recommends a daily intake by an adult male of 500 mg calcium and 300 mg magnesium. Commercially, U.S. whey products can be incorporated into fortified products, thus enhancing the mineral nutrients of the products.

Dairy foods are a good source of bioavailable minerals and they support normal growth; however, interaction with other nutrients is an important factor. "Bioavailability" is the term used to distinguish between the nutrient content of a food and how much the body can actually extract of that content. Although milk and calcium supplements decrease iron absorption, body iron stores are not compromised by high calcium intakes.

Calcium is widely recognized as important not only for bone health, but also has been implicated in disorders such as hypertension, preeclampsia, premenstrual syndrome and colon cancer.

HYPERTENSION

While sodium restriction has historically been associated with control of hypertension, evidence suggests that an adequate intake of minerals other than sodium should be the focus of population-wide dietary recommendations. The absence of calcium, potassium and magnesium, due to low intake of dairy products, fruits and vegetables, may be a better predictor of hypertension than sodium intake. In fact, the recent Dietary Approaches to Stop Hypertension (DASH) trial found that blood pressure in persons with dairy product intakes below currently recommended levels decreased when the recommended amounts were introduced into their diets.

PROBIOTICS AND PREBIOTICS

Novel healthful products have been developed that combine a variety of elements such as probiotics, immunoglobulins and prebiotics to achieve desired actions. Other probiotic products are reported to be effective cholesterol-lowering agents.

Fermented foods are an important part of most healthy diets and provide a common entry route for microbes that take up residence in the intestine. The generally accepted definition of a probiotic as applied to humans is a live microbial food supplement that beneficially affects the human host by improving its intestinal microbial balance. A prebiotic affects the host beneficially by selectively stimulating the growth and/or activity of one or a limited number of naturally present or introduced bacterial species in the colon, also leading to a claimed improvement in host health. Increasingly, probiotics and prebiotics are used in combination to achieve what is being termed a *synbiotic* effect. Until recently, much of the innovation in the use of probiotics and prebiotics has been in dairy products. However, prebiotics are beginning to find increasing application outside the dairy sector, particularly in baked goods. Several recent and extensive reviews on the topics of probiotics, prebiotics and functional food development are available and should be consulted for specific information. In particular, the review by Naidu et al. covers a diverse range of topics from gut microbial ecology to numerous biological effects (antimicrobial, physiological, supplementary, immunomodulatory, antitumor) to clinical and product applications, to culture production and safety effects. This review also identified the type of research data needed to make health claims and to bring a product successfully to market. The large number of lists of culture strains, features, uses and 490 references make this an authoritative information source.

The most common probiotics utilized in dairy products are lactobacilli and bifidobacteria, and it is their impact on the composition of the intestinal microflora that forms the basis for the probiotic concept. Potential health benefits arising through consumption of probiotics is impressive, and includes: increased resistance to infectious diseases, particularly of the intestine; decreased duration of diarrhea; reduction in blood pressure; reduction in serum cholesterol concentration; reduction in allergy; stimulation of phagocytosis by peripheral blood leukocytes; modulation of cytokine gene expression; adjuvant effects; regression of tumors; and reduction in carcinogen or cocarcinogen production.

One of the most fundamental probiotic effects of fermented dairy products is an increased tolerance to lactose through introduction of lactose utilizing strains. More recently identified effects include suppression of H*elicobacter pylori* infection and so possible antiulcer and cancer effects. While the benefits of specific probiotic strains are well established through numerous clinical trials, the molecular mechanisms underlying probiotic properties remains controversial. Such mechanisms must be established to support valid health claims that appear on packaging. The features deemed desirable for probiotic strains include: 1) human origin; 2) normal intestinal biota; 3) resistance to digestive processes and factors such as pH, digestive enzymes, peristalsis (adherence), bile salts, diet shifts and local immune responses; 4) stability during product manufacture and storage; and 5) possession of health-promoting effects. It is the latter feature that confers the title of "probiotic" to the strain.

Culture features must be carefully considered for each application as some probiotic effects are only achieved when bioactive intracellular components are released following disruption of the microbial membrane by bile salts. When the microbial membrane is disrupted, beneficial intracellular components are released and available to the intestinal lumen. Thus different strategies can be developed to achieve different effects, and combinations of microbes with differing features may be the best approach in many settings. Moreover, different processing steps could alter culture metabolism and production of desirable (or undesirable) factors. In some situations, the best effects might be achieved by adding cultures after certain processing steps such as heating. Each of these considerations underscore the practical need for detailed information on the mechanisms for health promotion in order to develop quantifiable production targets and quality control programs.

PREBIOTICS

The prebiotic approach dictates that non-viable food components are specifically fermented in the colon by indigenous bacteria thought to promote health. Any food ingredient that enters the large intestine is a candidate prebiotic. However, to be effective, selectivity of the fermentation is essential. Most current attention and success has been derived using nondigestible oligosaccharides. For example, various data have shown that fructooligosaccaharides (FOS) and galactooligosaccharides, although resistant to digestion, are specifically fermented by bifidobacteria. During controlled feeding studies, ingestion of these prebiotics causes bifidobacteria to become numerically dominant in feces. Recent studies have indicated that a FOS dose of 4 grams per day is prebiotic. Lactose from whey is an important precursor for prebiotic products. For example, galactooligosaccharides can be produced through a transgalactosylation reaction when lactose is enzymatically hydrolyzed. These oligosaccharides are typically Generally Recognized As Safe (GRAS) as they are constituents of milk and can be produced from ingested lactose by resident intestinal bacteria. Currently, seven kinds of oligosaccharides have been licensed as Food for Specified Health Use (FOSHU) by the Ministry of Health and Welfare, Japan, including fructooligosaccharides, galactooligosaccharides, lactosucrose, xylooligosaccharides, soybean oligosaccharides, raffinose and isomaltooligosaccharides. Of 69 items licensed as FOSHU, 40 are products containing these oligosaccharides. In addition to the bifidobacteria growth-promoting factors, functional effects of indigestible oligosaccharides on calcium absorption have been reported in animal models. The potential use of combinations of FOS with B. *longum* or galactooligosaccharides with B. *breve* is now focused on the reduction of the risk of colon cancer in animal models.

Whey carbohydrates are currently the best understood prebiotics, however, prebiotic actions for whey proteins and peptides are known. Whey proteins may be particularly effective in situations where host nutrition or intestinal competence is compromised such as during cancer treatment.

Novel healthful products have been developed that combine a variety of elements such as probiotics, immunoglobulins and prebiotics to achieve desired actions. Other probiotic products are reported to be effective cholesterol-lowering agents. The effects of a probiotic mixture of B*acillus*, L*actobacillus*, S*treptococcus*, C*lostridium*, S*accharomyces* and C*andida* on lipid metabolism was compared with that of L. *acidophilus* and that of S. *faecalis*. Similarly, rats fed a cholesterol-containing diet in combination with whole milk, or standard yogurt, or standard yogurt fortified with whey products or similarly supplemented bifidus-containing yogurts demonstrated that bifidus yogurts and standard yogurts fortified with whey proteins can reduce total and LDL-cholesterol. In this trial, yogurt fortifiers were skim milk powder, condensed whey or lactose-hydrolyzed condensed whey. When L. *acidophilus* or S. *faecalis* organisms were fed in combination with rice bran as part of a fat and cholesterol-enriched diet, liver cholesterol synthesis decreased and intestinal sterol loss increased after four weeks of feeding, with the net effect of reducing blood cholesterol levels. L*actobacillus reuteri* contains an active bile salt hydrolase and can significantly decrease total and LDL-cholesterol concentrations in treated pigs.

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