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Whey and Whey Proteins- A Puissant Catalyst for Future Foods and Better Health

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Abstract— **Whey, a liquid by-product, is widely accepted to contain many valuable constituents. It is one of the two proteins in cow's milk, making up about 20% of the total protein content. It is a complete, high quality protein with a rich amino acid (AA) profile, Whey proteins are well known for their high nutritional value and versatile functional properties in food products. Estimates of the worldwide production of whey indicate that about 700,000 tonnes of true whey proteins are available as valuable food ingredients. Nutritional and functional characteristics of whey proteins are related to the structure and biological functions of these proteins. Some functional characteristics of whey proteins are discussed in relation to their properties for application in food products.**

I. INTRODUCTION

In the past 20 years, whey protein has gone from being a waste product of cheese-making to a highly valued product rich in nutritional and functional properties [1].Whey is now used in various products – infant formulas, food supplements, sport bars and beverages – to meet a variety of health goals for people of all ages[1]. Knowledge and application of the health benefits of whey protein often lags behind the research.

Whey protein is a mixture of globular proteins isolated from whey, the liquid material created as a by-product of cheese production. Whey protein is commonly marketed as a dietary supplement, and various health claims have been attributed to it in the alternative medicine community[2]. Although whey proteins are responsible for some milk allergies, the major allergens in milk are the caseins[3].

Column1	Column2
water	87%
solid	13%

Figure 1: (a) Composition of whole milk

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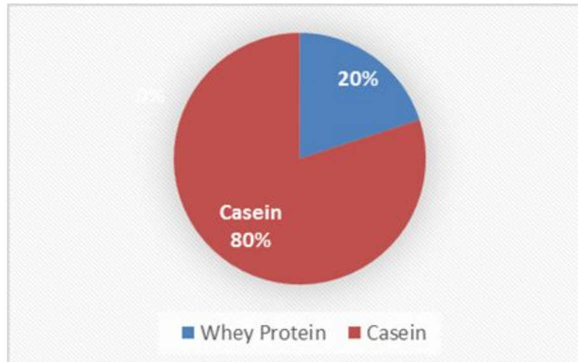

b) Composition of whey and casein protein in milk [47]

II. TYPES OF PROTEINS

In the latest two decades, the evolution of separation technologies, viz. those relying on selective, porous membranes have permitted a number of protein whey components to become widespread additives in food. Whey may indeed be subject to several treatments, thus originating whey products with specific qualitative and quantitative profile of proteins, minerals, lipids and sugars; the aforementioned membrane-based separation technologies include ultrafiltration (UF) to concentrate proteins, or diafiltration (DF) to remove most lactose, minerals and low molecular weight components – and thus produce whey protein concentrates (WPC). Depending on their concentration, there are WPC containing 35%, 50%, 65% and 80% (w/w) protein. When the threshold of 90% (w/w) protein is reached, a whey protein isolate (WPI) is accordingly obtained – which is a protein concentrate bearing high quality and purity. Both those products are used as vectors for the promotion of many biological properties upon addition to foods. If a thermal process is applied to whey then a-LA denatures easily, so it fraction can be separated via precipitation.

Most whey concentrates and isolates are available as intact proteins, but either can be also hydrolyzed. Hydrolysates have been partially broken down by exposing the protein to heat, acid or enzymes that break apart the bonds linking amino acids. This makes it taste more bitter, but also allows it to absorb more rapidly than a concentrate or isolate. Concentrates and isolates are already fast-digesting, so a hydrolysate, which digests minimally faster, may not be worth the taste tradeoff and extra cost for the small benefit[10].

Table-1 Types of Commercially Available Whey Proteins

Product Description	Protein Concentration	Fat, Lactose, and Mineral Content
Whey Protein Isolate	90-95%	Little if any

Whey Protein Concentrate	Ranges from 25-89% Most commonly available as 80%	Some fat, lactose, and minerals As protein concentration increases, fat, lactose, and mineral content decreases.
Undenatured Whey Concentrate	Variable Usually ranges from 25-89%	Some fat, lactose, and minerals As protein concentration increases, fat, lactose, and mineral content decreases. Processed to preserve native protein structures; typically have higher amounts of immunoglobulin and Lactoferrin.

III. WHEY PROTEIN MANUFACTURING

Protein from bovine whole milk consists of approximately 20-percent whey protein. When casein is removed from whole milk, liquid whey remains, having a protein concentration of about 65 percent. The following is a summary of the Ohio State University method of manufacturing whey protein powder. Milk is high-temperature, short-time pasteurized (163 degrees F for 30 seconds) and held overnight at 40 degrees C. The following morning the mixture is cooled to 30 degrees C, inoculated with a lactic acid culture, and incubated for 30 minutes. Rennet extract is added and the mixture is stirred, resulting in coagulation of curd. Rennet is derived from the abomasum (fourth stomach) of newly born calves. Chymosin, the active enzyme ingredient of rennet, aids in the coagulation of milk by separating it into curds and whey. In a newly born calf, chymosin aides in the digestion and absorption of milk. Adult cows do not have this enzyme. The liquid whey is drained through a stainless steel screen and the remaining curd is cut and cooked at 30 degrees C. Whey liquid is then filtered at 45 degrees C and brought to a pH of 3 by adding citric acid. The liquid is filtered to one fifth its original volume, resulting in whey concentrate that is approximately 80-percent protein. This can be additionally micro-filtered to increase protein concentration to as high as 95 percent. The final whey protein concentrate is warmed and spray-dried to achieve whey protein powder. Whey protein concentrates can then be put through an ion-exchange process to remove fat and lactose. In addition, some manufacturers hydrolyze (cleaving peptide bonds via enzymes or heat) the whey to provide more peptides and free amino acids in the final product[5]. The commercial success of whey protein has led to the development of high quality whey protein supplements manufactured as primary products and not as a byproduct of cheese manufacturing. Manufacturers take special care to preserve the biological activity, native protein structure, and protein-bound fats in the finished product. Proteins are processed under low temperatures and not exposed to fluctuating pH changes to avoid denaturing the native structures. In addition, the source of milk and the health of the milking cows is thought to contribute to immune-enhancing activity of whey products [6][7].

IV. LACTOFERRIN

Lactoferrin, an iron-binding glycoprotein, is a non-enzymatic antioxidant found in the whey fraction of milk as well as in colostrums. The lactoferrin component of whey consists of approximately 689 amino acid residues, while human lactoferrin consists of 691 residues. Whey lactoferrin is composed of a single polypeptide chain with two binding sites for ferric ions. Before processing, bovine lactoferrin is only 15-20 percent saturated with iron. Iron-depleted lactoferrin, defined as containing less than five percent iron, is referred to as apolactoferrin. Human breast milk contains apolactoferrin. The concentration of lactoferrin in human milk and colostrums is approximately 2 mg/mL and 7 mg/ mL, respectively, while in bovine milk and colostrums it is approximately 0.2 mg/mL and 1.5 mg/ mL, respectively[11].Lactoferrin is a dominant component of whey protein in human breast milk; however, the concentration in most commercial whey protein powders is only 0.35-2.0 percent of total proteins. Table 2 illustrates the difference in amino acid profiles between bovine and human Lactoferrin.

V. IMMUNOGLOBULIN

An immunoglobulin (Ig) is an antibody or gamma-globulin. There are five classes of antibodies – IgA, IgD, IgE, IgG, and IgM, IgG constitutes approximately 75percent of the antibodies in an adult. IgG is transferred from mother to child in utero via cord blood and by breast-feeding, and serves as a child's first line of immune defense – referred to as "passive immunity." IgA is secreted in breast milk and ultimately transferred to the digestive tract in the newborn infant, providing better immunity than a bottle-fed child[12]. Colostrums contain significantly greater concentrations of immunoglobulin than mature milk. Immunoglobulin reach maximum concentration the first 24-48 hours post-parturition and decline in a time-dependent manner following peak concentration[13].Similarly, the whey fraction of milk appears to contain a significant amount of immunoglobulins, approximately 10-15 percent of total whey proteins. An in vitro study demonstrated bovine milk-derived IgG suppresses human lymphocyte proliferative response to T cells at levels as low as 0.3 mg/mL of IgG. The authors further conclude bovine milk IgG typically ranges between 0.6-0.9 mg/mL and is therefore likely to confer immunity that could be carried to humans[14]. Studies show raw milk from non-immunized cows contain specific antibodies to human rotavirus, as well as antibodies to bacteria such as E. coli, Salmonella enteriditis, S. typhimurium, and Shigella flexneri [15],[16].

Table-2 Components Found in Whey Protein

Whey Components	% of whey protein	Benefits
beta-Lacto globulin	50-55 %	Source of essential and branched chain amino acids

alpha-Lactalbumin	20-25 %	Primary protein found in human breast milk Source of essential and branched chain amino acids
Immunoglobulin	10-15 %	Primary protein found in colostrums Immune modulating benefits
Lactoferrin	1-2%	Antioxidant Antibacterial, antiviral , and antifungal Promotes growth of beneficial bacteria Naturally occurs in breast milk, tears, saliva, bile, blood, and mucus
Lactoperoxidase	0.50%	Inhibits growth of bacteria
Bovine Serum Albumin	5-10%	Source of essential amino acids Large protein
Glycomacropeptides	10-15 %	Source of branched chain amino acids Lacks the aromatic amino acids phenylalanine, tryptophan, and tyrosine

VI. BETA-LACTOGLOBULIN

Beta-Lactoglobulin represents approximately half of the total protein in bovine whey, while human milk contains no beta-lactoglobulin. Besides being a source of essential and branched chain amino acids, a retinol-binding protein has been identified within the beta-lactoglobulin structure. This protein, a carrier of small hydrophobic molecules including retinoic acid, has the potential to modulate lymphatic responses [17].

VII. ALPHA-LACTALBUMIN

Alpha-Lactalbumin is one of the main proteins found in human and bovine milk. It comprises approximately 20-25 percent of whey proteins and contains a wide variety of amino acids, including a readily available supply of essential and branched chain amino acids. Purified alpha-lactalbumin is most readily used in infant formula manufacturing, as it has the most structurally similar protein profile compared to breast milk. However, due to cost effective measures, most dairy-based infant formulas contain ingredients such as demineralized whey with higher levels of beta-lactoglobulin, making them less similar to human milk. In a murine study, alpha-lactalbumin, in both the native and hydrolyzed state, enhanced antibody response to systematic antigen stimulation[18]. The same group proved alpha-lactalbumin has a direct effect on B-lymphocyte function, as well as suppressing T cell-dependent and -independent responses[19].

VIII. LACTOPEROXIDASE

Whey contains many types of enzymes, including hydrolases, transferases, lyases, proteases, and lipases. Lactoperoxidase, an important enzyme in the whey fraction of milk, is the most abundant enzyme and the majority of it ends up in whey following the curding process. Lactoperoxidase accounts for 0.25-0.5 percent of total protein found in whey. It has the ability to catalyze certain molecules, including the reduction of hydrogen peroxide[20]. This enzyme system catalyzes peroxidation of thiocyanate and some halides (such as iodine and bromium), which ultimately generates products that inhibit and/or kill a range of bacterial species[21]. During the pasteurization process, Lactoperoxidase is not inactivated, suggesting its stability as a preservative.

IX. GLYCOMACROPEPTIDES

Glycomacropeptide (GMP) is also referred to as casein macro peptide. GMP is a protein present in whey at 10-15 percent, due to the action of chymosin on casein during the cheesemaking process. GMP is only present when chymosin is used during processing; therefore, cheeses such as cottage cheese not made with chymosin do not produce GMP in the curding process[22]. GMP is high in branched chain amino acids and lacks the aromatic amino acids including phenylalanine, tryptophan, and tyrosine. It is one of the few naturally occurring proteins that lacks phenylalanine, making it safe for individuals with phenylketonuria (PKU).

X. BOVINE SERUM ALBUMIN

Bovine serum albumin (BSA) is a large protein that makes up approximately 10-15 percent of total whey protein. BSA is a source of essential amino acids, but there is very little available information regarding its potential therapeutic activity. Table 2 summarizes the components found in whey.

XI. MECHANISMS OF ACTION

Antioxidant Effects

Whey has potent antioxidant activity, likely by contributing cysteine-rich proteins that aid in the synthesis of glutathione (GSH), a potent intra cellular anricaidant. As an antioxidant, glutathione is most effective in its reduced form. As a result of whets glutathione/antioxidant component, it is being investigated as an anti-aging agent[23].

Detoxification

Practitioners use whey protein as a source of cysteine to increase intracellular glutathione levels. Glutathione peroxidase (GSHPx) activity in cow's milk, and presumably whey, is the same as in human millk. As a detoxifying agent, GSHPx, which is derived from selenium and cysteine, is an endogenous antioxidant enzyme that converts lipid peroxides into less harmful hydroxy acids. In addition to the above -mentioned properties, the alpha-lactalbumin component of whey chelates heavy metals" and reduces oxidative stress because of its ironchelating properties. [23].

Antihypertensive/ Hypolipiclemic Activity

Antihypertensive peptides have been isolated in bovine betalactoglobulin, suggesting whey reduces blood pressure. These peptides provide whey with significant angiotensin I converting enzyme (ACE) inhibitory activity, which blocks the conversion of angiotensin I to angiotensin II, a highly potent vasoconstrictor molecule. beta-Lactoglobulin has been described by Nagaokaet al as a cholesterol –lowering agent. In animal studies, beta-lactoglobulin inhibited cholesterol absorption by changing micellar cholesterol solubility in the intestine [23].

Functionality

Whey protein is used in many food applications because of its functionality and nutritive value. Whey protein foams well, meaning that it creates and stabilizes air bubbles in a liquid

Whey protein also remains soluble from pH 2 to 10 and stabilizes emulsions by forming interfacial films between hydrophobic and hydrophilic food components Ice creams, soufflé's, frothed drinks, and other food foams and emulsions are stabilized by surface-active agents for which whey protein products are frequently chosen. Processed cheeses, which are also emulsions, contain whey protein to improve melting, slicing, and spreading Acid whey powder improves the crust colour and enhances flavour in bread, biscuits, crackers, and snack foods, providing a golden surface on baking Whey proteins unfold and aggregate on heating, and are capable of binding large amounts of water depending on pH, ionic strength, and thermal conditions. Addition of WPI to muscle protein, for instance, improves moisture retention. WPC has been used to increase solids and protein content in milk and cheese and to replace fat in low-fat dairy products. Protein gelation may be defined as a balance of protein–protein and protein–water interactions, enabling the formation of a three-dimensional network. β-LG, the primary protein in whey, does not heat-denature until about 78◦C, providing good thermally induced gelation properties. β-LG also has a high solubility at low pH, making it an ideal active ingredient in fortified acidic beverages. Lactoferrin has potential as a natural antimicrobial agent in such products as personal health items, pharmaceuticals, and specialty dietary formulations. Whey proteins can also be extruded and added to other foods to improve nutritional value and texture. Variations in whey functionality depend on the variety of cheeses and the processes used in manufacturing them, the severity of heat treatment required to isolate the whey protein, and other factors[9].

Applications

Whey protein concentrate (WPC) and Whey protein isolate (WPI) are used in baked goods and baking mixes; cakes and pastries; candy, chocolate, and fudge; coffee whiteners; crackers and snack foods; diet supplements; fruit beverages; gravies and sauces; infant formulas and baby food; mayonnaise; pasta; pie fillings; processed dairy. Products including ice creams; processed fruits and vegetables; salad dressings; soups, meats, and sausage; and sports drinks WPC with lower protein content tends to be used more in the lower-value food products, such as dairy and bakery items, and higher concentrated WPC is generally used in higher-value products, such as meat and seafood. Medicinal and nutritional products are obtained by fractionation of whey by membrane filtration into its various proteins[9] .

Whey proteins are unique in their ability to optimize a number of aspects of the immune system, primarily by boosting glutathione (GSH) levels in various tissues[8]. GSH, the centrepiece of the body's antioxidant defence system, protects cells against free radical damage, pollution, toxins, infection and UV exposure. GSH levels are typically depressed in individuals with cancer, HIV, chronic fatigue syndrome and other immune-compromising conditions. GSH also decreases with age and may be partially responsible for diseases such as Alzheimer's disease, cataracts, Parkinson's disease and arteriosclerosis. Thus, incorporating whey proteins into the diet may protect the health of not just those with a compromised immune system but those of all ages[8].Specific components in whey thought to play a role in enhancing the immune system include:

Cistern – an amino acid found in high levels in whey proteins, cistern is involved in the intracellular production of GSH[8].

Lactoferrin- has been shown to exhibit immune-modulating activity through both antimicrobial and antitoxin activity; it may also provide protection against viruses such as hepatitis, cytomegalovirus and influenza[8].

Immunoglobulin – may confer disease protection to infants through passive immunity, and to adults by promoting the activity level of the immune system[8].

BCAAs – are metabolized in the muscle to manufacture glutamine, a precursor to GSH and another important component of the immune system[8].

Clinical indications Owing to the high content of bioactive compounds in whey, including lactoferrin, immunoglobulin, glutamine, and lactalbumin, whey protein has been associated with a lower risk of metabolic disorders and other diseases[8].

Cancer

A number of animal studies have examined the anti-cancer potential of whey, believed to be primarily associated with the antioxidizing, detoxifying, and immune-enhancing effects of GSH and lactoferrin [2]. In the presence of lactoferrin, colon cancer in rats demonstrated reduced tumor expression while the metastasis of primary tumors in mice was inhibited [24][25]. Results of an in vitro study have also been encouraging, demonstrating the inhibition of some of the important steps in breast cancer development when treated with the protein bovine serum albumin, although the mechanisms were not fully understood [26]. A few clinical trials have been undertaken, proposing that high levels of GSH in tumor cells confer resistance to chemotherapeutic agents. One of these studies showed that 20 patients with stage IV malignancies were treated daily with 40 g whey in combination with supplements such as ascorbic acid and a multi-vitamin/mineral formulation [27]. The 16 survivors demonstrated increased levels of natural killer cell function, GSH, hemoglobin, and hematocrit 6 months later. An aggressive combination of immunoactive nutraceuticals was effective in significantly increasing natural killer function, other immune parameters, and plasma hemoglobin in patients with late stage cancers.

Hepatitis B

The results of trials for the hepatitis B virus have been positive, particularly those from an open study that included 8 patients administered 12 g whey/day [28]. The patients demonstrated improved liver function markers, decreased serum lipid peroxidase levels, and increased interleukin-2 and natural killer cell activity. Regarding hepatitis C, several trials have proved inconclusive, although an initial in vitro study found that bovine lactoferrin prevented the hepatitis C virus in a human hepatocyte line [29].

HIV

Since patients with HIV commonly have low levels of GSH, several studies have sought to address this by testing if whey protein could induce beneficial effects on the GSH levels in HIV-positive patients. In one instance, 18 participants were randomized to receivewell recognized to be the most critical nutritional factor to achieve optimal peak bone mass; milk protein is also important for preventing osteoporosis. A number of clinical trials support milk protein's positive effects in both men and women, the latter ranging in age from young to postmenopausal. Daily doses of 40 mg MBP (equivalent to 400–800 mL milk) appear to be sufficient to significantly increase bone mineral density and reduce bone resorption [30-32].

Hypertension

Hypertension is a major global public health issue, and its specific treatment will likely reduce the risk of cardiovascular diseases. Various investigators have hypothesized that certain bioactive peptides formed through the hydrolysis of food proteins have the ability to inhibit ACE, and this subject has been comprehensively reviewed in a number of studies [33, 34, 35, 36-41] [42]. In general, it has been claimed that a diet rich in foods containing antihypertensive peptides is effective for the prevention and treatment of hypertension. ACEinhibitory peptides may be obtained from precursor food proteins via enzymatic hydrolysis, the use of viable or lysed microorganisms, or specific proteases [43] [44] [45]. However, studies relating to whey peptides with ACE inhibitory activities are more limited; this may be due to the rigid structure of beta-lactoglobulin, which makes it particularly resistant to digestive enzymes. ACE inhibitory peptides can reduce blood pressure in a process regulated, in part, by the reninangiotensin system; renin is a protease, which is secreted in response to various physiological stimuli that cleaves the protein angiotensinogen to produce the inactive decapeptide angiotensin I. In addition, ACE acts on the kallikrein-kinin system, catalyzing the degradation of the nonapeptide bradykinin, which is a vasodilator [46]. and ACE inhibitory peptides exert a hypotensive effect by preventing angiotensin II formation and the degradation of bradykinin.

Cardiovascular disease

According to the results of a number of studies, intake of milk and milk products can lower blood pressure and reduce the risk of hypertension [2]. Kawase et al. performed an 8-week trial in which 20 healthy men were given a combination of fermented milk and whey protein concentrate and examined the effect on serum lipids and blood pressure [35]. After the 8 weeks, the fermented milk group demonstrated comparatively higher high-density lipoproteins, lower triglycerides, and lower systolic blood pressure. Pal et al. published a systematic review on the effects of whey protein on cardiometabolic risk factors in which many of the reviewed studies demonstrated a beneficial effect of whey on cardiovascular disease [34]. The improvement of obesity by whey intake might contribute mostly to lower blood pressure.

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