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Nonmeat Protein Alternatives as Meat Extenders and Meat Analogs

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Abstract: The direct consumption of vegetable proteins in food products has been increasing over the years because of animal diseases, global shortage of animal protein, strong demand for wholesome and religious (halal) food, and economic reasons. The increasing importance of legume and oilseed proteins in the manufacturing of various functional food products is due to their high-protein contents. However, the greatest obstacle to utilizing these legumes and oilseeds is the presence of antinutrients; but these antinutrients can be successfully removed or inactivated by employing certain processing methods. In contrast, the potential negative impact of the antinutrients is partially balanced by the fact that they may have a health-promoting role. Legumes and oilseeds provide well-balanced amino acid profiles when consumed with cereals. Soybean proteins, wheat gluten, cottonseed proteins, and other plant proteins have been used for texturization. Texturized vegetable proteins can extend meat products while providing an economical, functional, and high-protein food ingredient or can be consumed directly as a meat analog. Meat analogs are successful because of their healthy image (cholesterol free), meat-like texture, and low cost. Mycoprotein is fungal in origin and is used as a high-protein, low-fat, health-promoting food ingredient. Mycoprotein has a good taste and texture. Texturized vegetable proteins and a number of mycoprotein products are accepted as halal foods. This article summarizes information regarding the molecular, nutritional, and functional properties of alternative protein sources to meat and presents current knowledge to encourage further research to optimize the beneficial effects of alternative protein sources.

Introduction

Meat is considered the highest quality protein source not only due to its nutritional characteristics but also for its appreciated taste. The role of meat proteins is two-fold. On the one hand, meat proteins contain all the essential amino acids closely resembling the human body (Xiong 2004) making them highly nutritious. On the other hand, meat proteins greatly contribute to the food industry by imparting specific functionalities. The overall properties of meat and meat products, including appearance, texture, and mouthfeel, are dependent on protein functionality. The latter role is of particular importance because all the functional properties exhibited by meat proteins cannot be reproduced by any other food protein (Xiong 2004).

The principal protein functionalities in processed meats are gelation and related properties (for example, meat particle binding and adhesion), emulsification, and water- holding. Among commercial proteins used in the food industry, gelatin has been regarded as both special and unique serving multiple functions with a wide range of applications in various industries (Karim and Bhat 2008). Gelatin is a product obtained by the partial hydrolysis of collagen derived from animals (Morrison and others 1999). Marinated, processed (salted) meats, restructured meat rolls and loaves, luncheon meats, boneless ham, sausage, frankfurters, and bologna are examples of food with functional properties of gelatin proteins in meat and meat products.

The food industry does not only use the muscle meat but also other sections of the animal. This is for the manufacturing of a variety of marketable products, such as sausages, hams, and bologna (Pearson and Gillett 1996), and these products have a high level of fat. For example, frankfurters and bologna may have as much as 30% fat, and fresh pork sausages may contain up to 50% fat (Roth and others 1997; Belloque and others 2002).

The nutritional value of meat products is mainly due to their high biological values of proteins and their vitamins and minerals. However, from a health point of view, an excessive intake of meat products cannot be recommended, especially for certain population groups because of their significant fat content (Muguerza and others 2004; Cengiz and Gokoglu 2005). It is well known that meat contains cholesterol and a higher proportion of saturated fatty acids than polyunsaturated fatty acids (PUFAs) (Muguerza and others 2004). Increased ratios of n-3 PUFAs exert suppressive effects on the pathogenesis of many diseases including cardiovascular disease (CVD), cancer, and inflammatory and autoimmune diseases (Simopoulos 2002). Of the n-3 PUFAs, α-linolenic acid (ALNA, C18:3) is present in large quantities in plant products (Jimenez-Colmenero 2007) but not so much in animal products. For prevention of CVD, the World Health Organization (WHO)

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has the following nutritional recommendations: fat should provide between 15% and 30% of the calories in the diet, saturated fat should provide less than 10% of these calories, and cholesterol intake must be limited to 300 mg/d (WHO 2003).

Rationales for Developing Nonmeat Alternatives

The issue of meat protein replacement has existed for many years for halal and kosher markets. However, meat protein replacement has gained interest in the last decade, particularly within Europe, with the emergence of bovine spongiform encephalopathy ("mad cow disease") in the 1980s (Morrison and others 1999). Since then, there has been much concern about using meat protein from potentially infected animals.

Religious lifestyle choices may prohibit certain consumer groups from eating meat and meat products. Meat from pigs is not acceptable for the Islamic faith, and meat from cattle is acceptable only if it has been prepared according to religious requirements. Islam is one of the world's fastest growing religions, and the development of meat protein alternatives is highly desirable to food processors as the global market for certified halal foods is rapidly growing. Global trade in halal food products has been estimated to be around 80 billion U.S. dollars or 12% of total trade in agri-food products (Anonymous 2007). With expected increases in both population and incomes of halal consumers, this percentage is certain to increase. Furthermore, halal food may easily account for 20% of world trade in food production in 2025 with Muslims projected to account for 30% of the world's population by 2025 (Anonymous 2007). Meat protein alternatives would also cater to other consumer groups such as Jews and Hindus.

Vegetable proteins have a lower price than muscle proteins and, consequently, can reduce the cost of the meat product. High meat prices have prompted the food industry to produce nonmeat proteins. An important reason for the increased acceptance of vegetable proteins, such as texturized soy protein (TSP), is their low cost (Singh and others 2008). Furthermore, animal proteins are scarce in many undeveloped countries. According to a World Bank report (De Haan and others 2001), the total global demand for meat is expected to grow by 56% between 1997 and 2020. In the last few years, concern has grown regarding adequate supplies of food for the current (and growing) world population of nearly 7 billion (Boye and others 2010). It has been estimated that 800 million malnourished people exist in the least-developed countries (Myers 2002). Providing safe, nutritious, and wholesome food for poor and undernourished populations has been a major challenge for the developing world. More specifically, protein-energy malnutrition is among the most serious problems faced by developing countries today (Bhat and Karim 2009; Boye and others 2010).

Due to animal diseases, such as mad cow disease, global shortage of animal protein, strong demand for "healthy" (cholesterol free and low in saturated fat), and religious (halal) food, and for economic reasons, there is a pressure for the direct consumption of vegetable proteins in food products. Some even see the potential for a quite rapid end to the meat economy because of rising vegetarianism and the influence of the animal rights movement (Maurer 2002; Franklin 1999). Protein with a vegetable origin is an alternative to animal protein for food applications due to the widespread variety of sources, such as legumes, oilseeds, cereals, and fungi. In this article, we have summarized information about the molecular, nutritional, and functional properties of alternative protein sources to meat. We also present ideas and challenges for nonmeat proteins to encourage further research to optimize their beneficial effects.

Nonmeat protein

Soy protein. Soybeans (*Glycine max*) are leguminous plants related to clover, peas, and alfalfa. The utilization of soy food around the world varies widely. Asia utilizes soybeans primarily as traditional foods, such as soymilk (Fukushima 1994), tofu (Fukushima 1981), and fermented products (Fukushima 1981, 1985). In contrast, Western nations consume more soybeans in the form of refined soy protein ingredients that are used in food processing instead of tofu or soymilk. In Western countries, soybeans now have attracted people's attention as an economic and high-quality vegetable protein source for humans. Therefore, new soy protein products were developed, such as defatted soy flour, soy protein concentrates, and soy protein isolates (Fukushima 2004).

Soybeans in food applications became popular after the U.S. Food and Drug Administration (FDA) approved the "Soy Protein Health Claim" on October 26, 1999. The FDA confirmed that 25 g of soy protein a day, may lower cholesterol and reduce the risk of coronary heart disease. The market is highly responsive to this health claim. Therefore, taking advantage of this opportunity, soy foods rapidly infiltrated into Western cultures and diets. The consumption of soy foods in Western countries suddenly increased, with 1999 as the turning point. The overall soy food industry has grown dramatically from $1.2 billion in 1996 to an estimated $4.0 billion in 2004 (Golbitz and Jordan 2006). In contrast, an American Heart Ass. (AHA) scientific advisory assessed the work published in 1998 to 2005 on soy protein and its component isoflavones (Sacks and others 2006). In the majority of 22 randomized trials, isolated soy protein with isoflavones, as compared with milk or other proteins, decreased low-density lipoprotein (LDL) cholesterol concentrations; the average effect was ≈3%. This reduction is very small relative to the large amount of soy protein tested in these studies, averaging 50 g, about half the usual total daily protein intake (Sacks and others 2006). Soy products such as tofu, soy butter, soy nuts, or some soy burgers should be beneficial to cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat (Krauss and others 2000). Using these and other soy foods to replace foods high in animal protein that contain saturated fat and cholesterol may confer benefits to cardiovascular health (Sacks and others 2006).

Presently, 220.9 million metric tons of soybeans are grown worldwide. The United States produces 33% of the total soybean production in the world followed by Brazil (28%), Argentina (21%), China (6%), India (4%), Paraguay (3%), Canada (1%), and all other countries (4%) (ASA 2009). In 2008, 157.5 million metric tons of soy proteins were available for consumption (ASA 2009). Soybean proteins are the single largest source for the manufacturing of texturized protein products worldwide. This is because of the simple economic law of supply and demand (Riaz 2004). Worldwide soybean supply is plentiful, and overall it is cheap and a relatively easily managed source of protein. From whole soybeans, a number of raw materials can be made for use in extrusion texturization.

In comparison to many of today's major food sources, soybeans are a nutritional superpower. Soybeans vary widely in nutrient content based on the specific variety and growing conditions, but they typically contain 35% to 40% protein, 15% to 20% fat, 30% carbohydrate, and 10% to 30% moisture (Golbitz and Jordan 2006). In addition to providing high-quality protein and fat and carbohydrates, soybeans are also rich in fiber, iron (Fe), calcium (Ca), zinc (Zn), and B vitamins (Lindsay and Claywell 1998).

Table 1–Major nonmeat protein sources.

Type of protein	Sources	References
β-conglycinin	Soybean	Koshiyama and Fukushima (1976), Mitsuda and others (1965), Thanh and Shibasaki (1977), Staswick and others (1984), Sun and others (2008)
Glycinin		
Vicilin	Legumes	Duranti and Gius (1997), Kang and others (2007), Plietz and others (1983), Plietz and others (1984)
Legumin		
Albumins	Oil seeds	Prakash and Narasinga Rao (1986), Marcone (1999), Minakova and others (1996)
Globulins		
Glutelins		
Gluten	Wheat, rye, and barley	Singh and MacRitchie (2001), Green and Cellier (2007)
Gliadins		
Glutenins		
Mycoprotein	*Fusarium venenatum* (Filamentous fungus)	Rodger (2001), Denny and others (2008)

Soybeans contain the highest amount of protein of any grain or legume.

Soybean proteins are composed of 2 major components, β-conglycinin and glycinin (Table 1). β-Conglycinin (Koshiyama and Fukushima 1976) has a sedimentation coefficient of 7S whereas glycinin (Mitsuda and others 1965) has a sedimentation coefficient of 11S. β-conglycinin is a trimeric protein composed of 3 subunits with a molecular mass ranging between 150 and 200 kDa (Thanh and Shibasaki 1977). In contrast, glycinin is a hexameric protein with a molecular mass ranging between 300 and 380 kDa (Staswick and others 1984; Sun and others 2008).

Legume protein. The legume family of plants accounts for 27% of primary crop production worldwide and is second in importance only to cereal grasses (Riascos and others 2010). In many regions of the world, legume seeds are a unique supply of protein in the diet. In most species, seed protein content varies from 20% to 30% of total dry weight (Riascos and others 2010). Legumes are also good sources of energy, minerals, and B vitamins (Moussa and others 1992). They occupy an important place in human nutrition, especially in the diets of low-income earners of developing countries (Oboh and others 2009). Legumes are considered the poor man's meat (Serdaroglu and others 2005).

Legume seeds accumulate large amounts of proteins during their development. Most are devoid of catalytic activity and are stored in membrane bound organelles (protein bodies) in cotyledonary parenchyma cells. These types of proteins are termed "storage proteins." However, because of their solubility in salt solutions and insolubility in water, the storage proteins are also named globulins (Derbyshire and others 1976). Although storage proteins are of importance in the human diet, their sole physiological function in the seed is to provide a source of amino acids and nitrogen for seed germination (Lambert and Yarwood 1992). In contrast, the catalytic proteins, such as albumins that are soluble in water, are represented by many different enzymes and will only be marginally discussed here.

Globulins are classified as 7S and 11S proteins on the basis of their sedimentation coefficients. The well-studied 7S and 11S proteins of peas are named vicilin and legumin, respectively, so the corresponding proteins of other seeds are often referred to as vicilin- and legumin-like globulins (Table 1) (Duranti and Gius 1997). The 7S globulin (vicilin) is a polypeptide trimer of molecular weight 40000 to 75000, thus making a native protein of molecular weight 150000 to 170000 (Plietz and others 1983). The vicilins normally do not have cysteines and, therefore, do not contain disulfide bonds (Casey and others 1986). The 11S proteins are also oligomers. The widely accepted structural model of 11S proteins is the model proposed for the *Vicia faba* legumin (Plietz and others 1984). In this model, 6 monomers are arranged in a trigonal antiprism compact structure. The monomeric unit of all legumin-like globulins consists of an acidic polypeptide chain (between 25000 and 50000 Da; Casey and others 1986) that is disulfide bonded to a basic chain (approximately 20000 Da) respectively named α and β subunits (Duranti and Gius 1997).

Oilseed protein. The proteins found in oilseeds are classified as biologically active proteins (enzymes) storage proteins, or structural proteins (Fukushima 1991; Marcone 1999). Of these proteins, the storage proteins are the most abundant and prominent in oilseeds. The levels of protein in these seeds are 13% to 17% for safflower (Prakash and Narasinga Rao 1986), 23% for cottonseed (Sunilkumar and others 2006), approximately 25% for rapeseed (Serraino and Thompson 1984), and 37% for soybean (Lampart-Szczapa 2001).

All crops contain a mixture of albumin (water-soluble), globulin (salt-soluble), and glutelin (alkali-soluble) proteins (Table 1) (Prakash and Narasinga Rao 1986; Minakova and others 1996; Marcone 1999). Most of these proteins contain the same 4 protein fractions with sedimentation coefficients of 2S, 7S, 11S, and 15S (Prakash and Narasinga Rao 1986), but the proportions of these fractions are highly variable. A similarity between the 11S proteins in oilseeds and legumes was found (Grinberg and others 1989; Lampart-Szczapa 2001) with similar molecular weights, subunits, amino acid profiles, and secondary structure (Marcone 1999). Beside these similarities, however, oilseed proteins have distinct differences in their tertiary structure (Marcone 1999).

Cereal protein. Cereals are the world's most important food crop, and cereal products are the most important foods. Cereals are utilized as seed (rice, barley, oats, and maize), flour (wheat, rye, and maize), or flakes (barley, oats, and maize). The protein contents, represented as the percentage of dry matter, of the above cereals are as follows: wheat (8% to 17.5%), maize (8.8% to 11.9%), barley (7% to 14.6%), rice (7% to 10%), oats (8.7% to 16%), and rye (7% to 14%) (Guerrieri 2004). The cereal proteins are as follows: albumins (soluble in water), globulins (soluble in salt solutions), gliadins (soluble in alcohol/water mixtures), and glutelins (soluble in dilute acid or alkali) (Singh and MacRitchie 2001; Guerrieri 2004).

Wheat gluten, an economically important co-product in the recovery of wheat starch in the wet processing of wheat flour, is an abundant plant protein source (Wang and others 2006; Xiong and others 2008). Wheat gluten is unique among cereals and other plant proteins in its ability to form a cohesive blend with viscoelastic properties once it is plasticized. The gluten protein is subdivided into 2 approximately equal groups based on their extractability (gliadin) and inextricability (glutenin) in aqueous alcohols (Table 1) (Singh and MacRitchie 2001).

Gliadins and glutenins are made up of approximately 80% of the protein contained in wheat seed. Gliadins are monomeric proteins with intramolecular disulfide bonds with low or medium molecular weights. According to electrophoresis mobility when separated on polyacrylamide gels at acid-PAGE, the wheat gliadins are divided into 4 groups as follows: α (fastest mobility), β, γ, and ω groups (lowest mobility) (Woychik and others 1961; Guerrieri 2004). Glutenins in the wheat fractions are formed by the high molecular weight (HMW) glutenins and low molecular weight (LMW) glutenins (Stevenson and Preston 1996). These proteins contain different polypeptides connected by intermolecular disulfide bonds, and the polypeptides are called subunits that are classified into 4 groups according to their molecular mobility in SDS-PAGE after reduction (A, B, C, and D regions). The A region (80000 to 120000 Da) corresponds to the HMW glutenins and the B, C, and D regions (30000 to 51000 Da) are LMW glutenins (Jackson and others 1983; Guerrieri 2004). The LMW-glutenin subunit (LMW-GS) containing B, C, or D regions represents approximately 60% of the total glutenins.

The high molecular weight glutenin subunit (HMW-GS) are minor components in quantity but are a key factor in bread making because they are major determinants of the gluten elasticity (Guerrieri 2004). The cysteine residues in the primary structure of the HMW-GS and LMW-GS allow identification of different polymer building subunits: chain extenders (subunits with 2 or more cysteine residues that can form intermolecular disulfide bonds) and chain terminators (with only one residue of cysteine available for intermolecular disulfide bonds) (Guerrieri 2004). In the first case we obtain stronger dough, in the last the opposite effect. The chain extender proteins with longer repetitive domain increased the stability and the strength of the gluten, in the durum wheat the higher presence of the LMW-GS, with a short repetitive domain reduced gluten elasticity (Guerrieri 2004). LMW-GS has the ability to form large aggregates that are related to dough strength. Although the HMW and LMW glutenins form the disulfide cross-linked gluten matrix, a small proportion (5% to10%) of α-, β-, and γ-gliadins occupy the matrix cross-link function (Mondal and others 2009). The ω-gliadins also may take part in the polymer formation. Gliadins can also function as chain terminators such as the LMW-GSs.

Vital wheat gluten is approved by FDA as GRAS (Generally Recognized as Safe) for use as a dough strengthener, formulation aid, nutrient supplement, processing aid, stabilizer, thickener, surface finishing agent, and texturizing agent. The viscoelasticity of gluten is a function of the interaction between glutenins and gliadins. In its pure form, glutenin exhibits a tough, rubbery texture when fully hydrated. Gliadin, a single-chain molecule, becomes extremely thick when hydrated and offers little resistance to extension (Foster 2006).

When heated above 85 °C, the hydrated gluten mass coagulates irreversibly without loss of its unique structural order yielding a firm, nonsticky, moist, and resilient gel (Kalin 1979). Its adhesive and film forming property binds the system's particulate matter (meat tissues and fat globules). Thermosetting properties of hydrated gluten complement film forming and adhesive properties make gluten an option for meat, poultry, and seafood applications (Foster 2006).

Mycoprotein. During the 1960s, nutritionists and politicians across the world were concerned that the predicted growth in the world's population would lead to global protein shortages in the future. Food scientists were seeking to develop a microbial protein source that would be inexpensive and palatable. Ultimately, this search has focused on a filamentous fungus that is commonly found in soil. In 1967, an organism (*Fusarium venenatum*) was identified in a field in Marlow, Buckinghamshire, U.K., which was eventually exploited to produce mycoprotein (Denny and others 2008). Mycoprotein is the generic name given to the ribonucleic acid (RNA)-reduced biomass comprising the hyphae (cells) of *F. venenatum* A3/5 (ATCC PTA-2684) in a continuous fermentation process (Table 1) (Rodger 2001).

At the beginning of research and development, more than 3000 fungal isolates from around the world were analyzed, and *F. venenatum* A3/5 (ATCC PTA-2684) was finally selected as the best organism for mycoprotein production (Wiebe 2004). In order to bring mycoprotein from the *F. venenatum* A3/5 to the market, it was necessary for scientists to invest many years in researching the safety of the organism and final products (Edelman and others 1983). It was then clear that mycoprotein could be consumed by human volunteers or laboratory animals without serious effects if the RNA content of the cells was reduced to safe levels (Solomons 1987). The ATCC PTA-2684 strain did not produce mycotoxins (O'Donnell and others 1998) because the growth conditions used for production were not suitable for mycotoxin production (Johnstone 1998). *Fusarium venenatum* A3/5 was approved for sale as food by the Ministry of Agriculture, Fisheries and Food in the United Kingdom in 1984.

Mycoprotein is commercially produced by continuous-flow fermentation of *F. venenatum* on a glucose substrate (Denny and others 2008). The CO_2 evolution rate, reflecting the biomass concentration, determines the flow rate (Rodger 2001). Cultures are maintained at 28 to 30 °C with a pH of 6.0 (Wiebe 2004). The continuous fermentation process is typically conducted for approximately 6 wk. During the production process, mycotoxins are tested at 6-h intervals to ensure that the mycoprotein is mycotoxin free (Wiebe 2004). The RNA content of the fungal biomass must be reduced in order to meet required safety standards (Edelman and others 1983). After harvesting from the fermenter, the culture broth is subjected to a short heat treatment to reduce its RNA content from 10% to less than 2% (dry weight), which is achieved by heat activation of the endogenous RNAse enzymes (Denny and others 2008). This fungal biomass is heated in a separate tank to temperatures above 68 °C (optimal 72 to 74 °C) for 30 to 45 min (Ward 1998). The heat-treated culture broth is then centrifuged, and the mycoprotein is recovered as a paste (Wiebe 2004).

A panel of experts evaluated the suitability of mycoprotein for food use in the United States. Four studies were performed to assess the tolerance of humans to mycoprotein, and the results demonstrated that mycoprotein is well tolerated by humans and has an extremely low allergenic potential (Miller and Dwyer 2001). Mycoprotein is the main ingredient in a variety of products including meat style pieces, fillets, cold-cut style slices, nuggets, burgers, sausages, ready meals, pastries, and pies (Denny and others 2008).

Advantages and disadvantages of nonmeat protein

Soy protein. The major disadvantage of soy protein is the strong off-flavors associated with the products. There are 2 types of off-flavors. One is grass and bean flavor, and the other is bitter and astringent flavor. The grass and bean flavor is developed through the action of lipoxygenases present in soybeans. The bitter and astringent flavor is caused by saponins and isoflavones (Okubo and others 1992). The off-flavors of isoflavones are increased by the hydrolysis of their aglycones through the action of β-glucosidases in soybeans (Matsuura and Obata 1993). For a long time, the removal of off-flavors has been a primary concern with the utilization of soy protein. Germination, either alone or in combination with heat treatment, overcomes some disadvantages such as objectionable flavor and odor (Suberbie and others 1981). However, the concern is currently changing to the physiologically active substances of soy protein in food systems. Saponins and isoflavones were previously considered as undesirable substances, but now they are considered as useful substances because of their anticarcinogenic activities (Fukushima 2004).

Protein Digestibility-Corrected Amino Acid Score (PDCAAS) is now widely used as a routine assay for protein quality evaluation, replacing the more traditional biological methods (for example, measurement of the protein efficiency ratio [PER] in rats). In order to make up for the shortcomings of the PER evaluations, the WHO and the FDA of the United States have adopted a new method for evaluating protein quality called the PDCAAS. This method uses an amino acid score that is a comparison between the amino acid pattern of a protein and human amino acid requirements and is a factor for digestibility to arrive at a value for the protein quality. According to the PDCAAS method, soybean protein products received scores between 0.95 and 1.00 (Golbitz and Jordan 2006). The PDCAAS values for beef protein, milk protein, and egg protein are 0.92, 1.00, and 1.00, respectively (Singh and others 2008). According to the current PDCAAS method, the quality of soybean protein is comparable to animal protein.

The validity of the PDCAAS method in assessing the protein quality of foods and diets was assessed by FAO/WHO (2001). The general consensus at these meetings was that, PDCAAS is a valuable tool for routine assessment of protein quality but, in its present form, it has several disadvantages. These include (Schaafsma 2005), 1st, the suitability of the essential amino acid composition of the currently recommended reference proteins. The current reference pattern is restricted to the indispensable amino acids and does not involve amino acids that become indispensable under specific physiological or pathological conditions, such as cystine, tyrosine, taurine, glycine, arginine, glutamine, and proline. Second, according to the PDCAAS method, values higher than 100% are truncated to 100%. This truncation procedure is valid when it comes to the evaluation of mixtures of proteins in total diets, or when a particular protein would be the only protein source in the diet. In those situations, digestible dietary essential amino acid concentrations in excess of those in the reference pattern of preschool-age children do not provide additional nutritional benefits. However, truncation of PDCAAS values of supplementary protein sources that could be used to improve the nutritional value of mixtures of proteins does not include a credit for the extra amino acids provided by the supplementary protein and does not provide any information about capacity for improvement. Third, invalidity of correction for fecal (compared with ileal) digestibility, that is, measurement of the fecal digestibility of proteins (determined in rats) may not provide an accurate correction for protein digestibility in the PDCAAS method. Ileal digestibility may be a preferred approach.

Soybean is a source of high-quality protein due to its relatively well-balanced composition of amino acids (Sun and others 2008). Soy protein products are used to complement cereal proteins (Singh and others 2008). The quality of the cereal protein is improved in soy/cereal mixtures because soy protein is a rich source of lysine, which is the first limiting essential amino acid in most cereal proteins (Dubois and Hoover 1981; Klein and others 1995).

Not only are soybean proteins nutritious, they also have a major role in food functionality. At 100 °C glycinin forms a hard, turbid, and inelastic gel, whereas β-conglycinin forms a soft, transparent, and elastic gel (Utsumi and others 1997). Glycinin is more stable thermally than β-conglycinin, but the emulsifying and emulsion-stabilizing abilities of glycinin are weaker than those of β-conglycinin. The greater hydrophobicity and structure that is more easily unfolded in β-conglycinins make their emulsifying ability stronger than that of glycinin. In contrast, larger numbers of sulfhydryl groups and their topology in glycinin make the glycinin gel harder and more turbid in comparison with the β-conglycinin gel (Utsumi and others 1997).

The use of soy in various food applications is of major importance to food industries. Because soy protein ingredients are being applied in many diverse food systems, they are increasingly regarded as versatile ingredients. It is well known that soy protein ingredients have appropriate functional properties for food applications and consumer acceptability. Important functional properties of soy protein in food systems are gelling/textural capabilities, water absorption, fat absorption, emulsification, elasticity, and color control (Singh and others 2008).

Legume protein. Legumes refer to the edible seeds of leguminous plants belonging to the family Leguminosae, which include beans and pulses (Oboh and others 2009). Legumes are distributed worldwide and are consumed regularly in some form in almost every country in the world (Oboh and others 2009). They are relatively low in sulfur-containing amino acids when compared to animal proteins. When combined with rice, the PDCAAS values for all bean products improved significantly (Kannan and others 2001).

The term pulses, as used by the FAO (1994), is reserved for crops harvested solely for the dry grain and thereby excluding crops harvested green for food (green peas, green beans, and so on) which are classified as vegetable crops. Also excluded are those crops used mainly for oil extraction (for example, soybean and groundnuts) and leguminous crops (for example, seeds of clover and alfalfa) that are used exclusively for sowing purposes. Pulses (pea, chickpea, lentil) contain high amounts of lysine, leucine, aspartic acid, glutamic acid, and arginine and provide well-balanced essential amino acid profiles when consumed with cereals and other foods rich in sulfur-containing amino acids (Boye and others 2010).

A number of allergenic food proteins have been characterized in legume crops including peanuts, soybeans, lentils, common beans, mungbeans, chickpeas, peas, and tree nuts (Singh and Bhalla 2008; Riascos and others 2010). Unfortunately, legumes including soybeans and peanuts are common sources of food allergens. Peanut allergies often cause acute and severe reactions, but allergies to soybeans are assumed not to cause severe reactions (van Boxtel and others 2008). An allergy is generally defined as an adverse immune-mediated hypersensitivity to normally harmless environmental substances called allergens. An allergen is defined by its ability to induce a specific immune response in genetically predisposed individuals through the production of elevated levels of a specific immunoglobulin E (IgE), which is in a class of antibodies that are crucial in allergies (Singh and Bhalla 2008). A protein that is stable under different conditions (such as resistant to heat denaturation or protease degradation) is more likely to go through mucosal barriers and induce an allergic reaction than one that is degraded in the digestive tract (Breiteneder and Mills 2005; Goodman and others 2008). Soybean allergies affect approximately 0.5% of infants (Anonymous 2010). Most allergies to soy that are mediated by IgE diminish with age. Occasionally, soy allergies

Table 2–Positive and negative physiological effect of antinutrients from plant origin.

Antinutrient	Positive effect	Negative effect	References
Protease inhibitors	Anticarcinogenic activities	Impairing intestinal protein digestion	Clemente and others (2004), Guillamon and others (2008)
α-Amylase inhibitors	Used as dietary supplements to control body weight in obesity therapy	Reduce the rate of digestion of starch	Mosca and others (2008), Karthic and others (2008)
Lectins (phytohemagglutinin)	Stimulation of lymphocytes (T cell)	Outbreaks of gastroenteritis, nausea, and diarrhoea	Gollob and others (1995), Harada (1999), Nasi and others (2009)
Polyphenols (especially tannins)	High antioxidant activity	Decreasing the activities of digestive enzymes and protein and amino acid availabilities	Oboh and others (2009), Troszynska and others (2006), Salunkhe and others (1982)
Phytic acid	Anticarcinogenic activities	Mineral depletion and micronutrient deficiency	Thavarajah and others (2009)

may occur in older children and adults. IgE-mediated peanut allergies tend to occur in young children, but unlike many other childhood allergies, they generally persist throughout life (Sadler 2004). A recent meta-analysis suggests that the intake of peanuts causes allergic reactions in between 0.2% and 1.5% of children (Jensen and others 2008). Currently, the sole strategy for managing food hypersensitivity involves strict avoidance of the trigger to the sensitivity (Riascos and others 2010).

Legume seeds are an important protein source in human nutrition. They contain antinutrients such as protein inhibitors, lectins, polyphenols, and phytates, which are either partially or completely eliminated by different methods of processing (Fernandez-Quintela and others 1997; Bhat and Karim 2009). Proteins such as amylase inhibitors, lectins, and trypsin inhibitors are likely to protect legume seeds against predators (Guillamon and others 2008).

It is well documented that protein inhibitors are important in determining the quality of legume seeds and their antinutritional effect in the irreversible inhibition of different digestive enzymes (Leterme and others 1992). The most characterized protein inhibitors are α-amylase inhibitors (Moreno and others 1990) and trypsin inhibitors (protease inhibitors) (Horisberger and Tacchini-Vonlanthen 1983; Domoney and others 1993). Pancreatic α-amylase hydrolyzes starch to maltose and oligosaccharides in the small intestine, whereas intestinal α-glucosidase hydrolyzes disaccharides and oligosaccharides to glucose. Inhibition of α-amylase hydrolysis reduces the rate of digestion of starch (Table 2) and results in a decrease in the postprandial blood glucose levels in diabetic patients (Karthic and others 2008). Protease inhibitors in legume seeds can have a major impact on nutritional value because they inhibit pancreatic serine proteases, thus impairing intestinal protein digestion (Table 2) (Guillamon and others 2008). However, the effects of protein inhibitors are usually found only when the seeds are consumed uncooked because heat denaturation inactivates these proteins (Vidal-Valverde and others 1994; Singh and others 2008). On the other hand, protease inhibitors can act as anticarcinogenic agents (Table 2) (Clemente and others 2004; Guillamon and others 2008). Furthermore, commercial protein concentrates of common beans enriched with α-amylase inhibitors, the so-called starch blockers, are used more frequently as dietary supplements to control body weight in obesity therapy (Table 2) (Mosca and others 2008).

Among the numerous lectins studied thus far, the lectins of the legumes are important because they represent the largest family of these proteins (Nasi and others 2009). In the seeds of the common bean (*Phaseolus vulgaris*), the protein fraction that has a sugar-binding property and hemagglutinating ability (Goldstein and Hayes 1978; Etzler 1985; Lis and Sharon 1986) is called phytohemagglutinin. Some lectins, such as the phytohemagglutinin from the common bean (*P. vulgaris*), pose a possible risk because the consumption of raw or incorrectly processed beans has been shown to cause outbreaks of gastroenteritis, nausea, and diarrhea (Table 2) (Nasi and others 2009). In contrast, lectins have specific roles as mediators of cell recognition in a variety of biological processes because of their binding specificity. Lectins play an important role in the stimulation of lymphocytes (T cells) (Table 2) (Gollob and others 1995; Harada 1999).

Polyphenols, such as tannins, crosslink with proteins by reacting with lysine or methionine amino acids making them inactive during digestion (Davis 1981) or making the proteins insoluble (Salunkhe and others 1982). Polyphenols are implicated in decreasing the activities of digestive enzymes and proteins and amino acid availabilities (Table 2) (Salunkhe and others 1982). In contrast, oxidative stress occurs when excessive free radicals react with proteins, cell walls, and DNA, which causes damage to cell structures, thereby leading to degenerative diseases (Oboh and others 2009). A practical way to fight degenerative diseases is to improve body antioxidant status. Legume seeds are a rich source of polyphenols, especially tannins, that have high antioxidant activity (Table 2) (Troszynska and others 2006; Oboh and others 2009).

Phytic acid is present in legume seeds and is responsible for the reduction of the bioavailability of essential minerals (Desphande and Cheryan 1984). Phytic acid binds important micronutrients, such as Fe and Zn, forming salts that are mainly excreted. This phenomenon can lead to mineral depletion and micronutrient deficiency in humans (Table 2) (Thavarajah and others 2009). Phytic acid also has a strong affinity to bind with other cations such as potassium (K), copper (Cu), cobalt (Co), magnesium (Mg), and Ca (in decreasing order) (Crea and others 2008). Phytic acid may affect starch digestibility through interaction with amylase and/or binding with salivary minerals, such as Ca (Yoon and others 1983). On the other hand, dietary phytic acid may have a beneficial health role, for example, as an antioxidant or anticancer agent (Table 2) (Thavarajah and others 2009).

Several strategies have been developed to remove or inactivate antinutritional factors in seeds. Some of these processes are based on chemical and physical treatments, including dehulling, soaking, cooking, and thermal treatments (Melcion and vander Poel 1993; Vidal-Valverde and others 1994; Arntfield 2004; Bhat and Karim 2009). The removal of the hulls from seeds is an effective way to reduce antinutritional factors. Hulls can be easily removed through milling or mechanical abrasion, but this is not the case for some of the smaller seeds, where the hull tends to adhere to the endosperm making separation difficult (Arntfield 2004). The process of cooking was performed by boiling the seeds in distilled water (seed: water ratio 1:6.7 w/v) (Vidal-Valverde and others 1994). The cooking liquid and seeds were separated using a strainer; the seeds were ground and freeze dried. The process of soaking was performed with the seeds in solutions, for example, distilled water, 0.1% citric acid, and 0.07% sodium bicarbonate (Vidal-Valverde and others 1994). The proportion of seed to soaking medium was 1:3 w/v. The soaking period, 9 h at room temperature, was chosen to obtain maximum seed weight and hydration. The soaking solution was drained, and the soaked seeds were ground and freeze dried. Polyphenols are significantly reduced by soaking and cooking. Phytate levels in plant foodstuffs are lowered by cooking.

Antinutrients can be eliminated or reduced significantly by thermal heating (for example, irradiation). Soaking followed by irradiation at 6 kGy, the phytohemagglutinating activity was reduced by 50% and irradiation in the range of 2 to 6 kGy revealed significant reduction in trypsin inhibitor activity in *Sesbania* spp. (Bhat and Karim 2009). The most practical, effective, and commonly used method to abolish lectin activity is aqueous heat treatment. Seeds are 1st fully soaked in water, and under these conditions, the lectin activity in fully hydrated soybeans (*G. max*), kidney beans (*P. vulgaris*), faba beans (*V. faba*), and lupin seeds (*Lupinus angustifolius*) could be eliminated by heating at 95 °C for 1 h or at 100 °C for 10 min (Grant and others 1982; Grant and van Driessche 1993).

Preparation of protein isolates or isolates involving extraction and precipitation of the legume protein has been used to obtain products with reduced levels of antinutritional factors (Swamylingappa and Srinivas 1994). Furthermore, genetic selection, germination, and fermentation also lead to a reduction or modification in the activity of the antinutritional compounds (Frias and others 1995; Kothekar and others 1996; Kozlowska and others 1996).

Legumes have been investigated regarding their potential use in developing functional foods. Gradually, interest has grown in the utilization of legume proteins for flour, isolates, and concentrates (Saio 1993; Doxastakis 2000; Serdaroglu and others 2005). To improve the nutritional quality, texture, and other functional properties of any product, the use of plant protein products as food ingredients is increased. However, these applications are limited to proteins from soybeans, whereas, comparatively, other vegetable proteins are used less.

Lupins (*Lupinus albus* L.), peas (*Pisum sativum* L.), and broad beans (*V. faba* L.) are extensively grown in different regions of the world. The protein contents for lupins, peas, and broad beans are 34.7%, 23.4%, and 32.5%, respectively, (w/w on dry matter) (Gueguen and Cerletti 1994). The high protein content makes them important sources of protein, which gives them potential to be added to various food products as novel ingredients (Papavergou and others 1999; Doxastakis 2000; Pozani and others 2002; Makri and others 2005).

Pea proteins, such as vicilin, have been reported to form heat-induced gels (Bora and others 1994). Concentration of 140 g/L was required to form a gel for broad bean protein isolate, meanwhile the isolate obtained from soybean and pea seeds needed 160 and 180 g/L, respectively (Fernandez-Quintela and others 1997). Broad bean protein isolate required the lowest concentration to form a gel, whereas pea protein isolate required the highest concentration.

The protein solubility percentage of the pea, broad bean, and soybean protein isolates revealed similar patterns (Fernandez-Quintela and others 1997). Regarding oil and water absorption capacities, the broad bean protein isolate had the highest values, whereas the soybean possessed the poorest capacity for these functional properties (Fernandez-Quintela and others 1997). Vose (1980) found that pea protein isolate exhibited similar emulsification properties to soybean protein isolate. In the case of lupin proteins, the acetylated and succinylated lupin isolates had better functional properties than the unmodified isolates (Cosme and others 1993).

Oilseed protein. Many of the oil-producing plants contain an appreciable level of protein, which has great potential for use in the human diet. Soybeans, rapeseeds, cottonseeds, sunflower seeds, and peanuts are the most for abundant protein meal and represent 68%, 12%, 7%, 4%, and 2% of world protein meal production, respectively (ASA 2009). Some oilseed proteins are deficient in sulfur-containing amino acids when compared with animal proteins (Moure and others 2006), but this limitation is easily overcome by supplementation with a cereal or mycoproteins.

Fiber, phytic acid, and phenolics are common to all oil-producing plants, and these could limit food applications. The high fiber content of most oilseed proteins limits the availability of nutrients in the meal and also creates processing problems (Prakash and Narasinga Rao 1986). A dehulling step is an effective option for decreasing the fiber content of most oilseeds because the fiber content is largely associated with the hull of the seed. In addition to reducing the level of fiber, the removal of the hulls from oil-producing plants is also an effective way to reduce other antinutritional factors such as phytic acid and phenolic compounds (Arntfield 2004). A number of treatments, such as hydrothermal treatment, soaking, fermentation, and germination, can be performed on seeds in order to remove antinutritional compounds, thus improving nutritional quality and functional properties (Moure and others 2006).

The removal of hulls is a desirable step in the protein isolation process. Protein isolation is an important step in incorporating proteins from oil-producing plants into food products. Functional properties of oilseed proteins provide valuable information regarding their effective use. The functional properties that receive the most attention when characterizing isolated oilseed proteins are fat binding capacities, emulsification properties, foaming capacities, gelation, and water absorption capacities (Arntfield 2004).

The functional properties of sunflower protein products have been reported to be comparable to those of soybean flour indicating the potential of these products for use in a variety of food products. Sunflower meal absorbed 107% water and 208% fat (Lin and others 1974), and sunflower proteins have strong emulsification properties (Sosulski 1979). Studies of the functional properties of peanut proteins indicated that they have a strong capacity toward water absorption, fat absorption, and emulsification (El-Zalaki and others 1995). Safflower proteins have fat binding and emulsification properties that are equivalent to soybean proteins (Betschart and others 1979). The fat absorption and emulsification properties of canola proteins were found to be good but not comparable to soybean proteins (Thompson and others 1982). Pawar and others (2001) demonstrated that oil absorption and emulsification properties can be influenced by the protein isolation technique, which can be improved when antinutritional factors, such as phytates and polyphenolic compounds, are reduced during isolate preparation.

Yu and others (2007) demonstrated that after heating, a 7.5% peanut protein concentrate suspension became a solution, a 10% peanut protein concentrate suspension produced a soft gel, and a 12.5% peanut protein concentrate suspension turned into a firm gel. Isolated sesame globulins were shown to produce harder gels with less syneresis than gels from soy protein (Yuno-Ohta and others 1994). Furthermore, gels from canola protein isolates were generally not as good as those from soybeans (Owen and others 1992). Transglutaminase (TG) has been proposed as a useful tool to improve the textural properties of food proteins (Pietrasik and others 2007). TG has the potential to improve gelation through the formation of covalent intramolecular and intermolecular bonds. Pinterits and Arntfield (2008) reported that enzymatic modification with TG can improve the properties of heat-induced canola protein isolate gels, thus improving its potential as a food ingredient.

Protein isolation is a very important step when incorporating proteins from oil-producing plants into food products. Pawar and others (2001) emphasized the need for good isolation procedures that reduce antinutritional compounds such as phytates and polyphenols. The influence of phytates and polyphenols on the gelation of sunflower proteins was reported by Pawar and others (2001). There is not a unique methodology or protocol for extracting protein from oilseeds or flours leading to optimal products reviewed by Moure and others [2006].

Cereal proteins. Protein supplementation of cereals is desirable because cereals have low protein contents and have an imbalanced essential amino acid composition (Singh and others 2008). The concept of cereal/legume complementation has been applied to improve protein quality. Researchers have shown that legume proteins (rich in lysine) and cereals (low in lysine) mixed in the right proportion could provide a balanced mixture of amino acids (Wondimu and Malleshi 1996).

Gluten is the protein found in wheat, rye, and barley, and is harmful to some individuals. Celiac disease (CD) is a disorder that is characterized by a permanent intolerance of gluten proteins (Green and Cellier 2007), and this disorder is not IgE mediated (Sadler 2004). The T-cell lymphocytes in the small intestine respond abnormally to gluten with an ensuing inflammatory process in which the absorptive epithelium of the small intestine becomes damaged (Sadler 2004). This forms the basis of several malabsorption problems leading to associated diseases, such as diarrhea, osteoporosis, and iron-deficiency anemia (Hamer 2005; Green and Cellier 2007). The symptoms of CD are related to intestinal damage, and the severity of the response is dependent on the number of T cells that can be triggered. CD occurs in nearly 1% of the population in many countries (Green and Cellier 2007), and approximately 0.9% to 1.2% of the Western population has the disorder (Hamer 2005). The prevalence of CD in different countries in the Middle East, North Africa, and India is similar to that in Western countries where CD was traditionally considered to be rare (Malekzadeh and others 2005). The development of CD is the result of an interaction between the environment (gluten exposure) and a genetic susceptibility (Hamer 2005). Nutritional therapy is the only accepted treatment for CD and involves the lifelong elimination of wheat, rye, and barley from the diet (Green and Cellier 2007).

Wheat gluten is a food commodity ingredient that is used mainly in the bakery industry to improve the rheological properties of flours due to its viscoelastic properties, and wheat gluten is also frequently used in processed meat products (Riaz 2004; Xiong and others 2008). The water insolubility of wheat gluten is one of the major limitations for its extensive use in food processing (Kong and others 2007) and limits its potential as a general functional protein additive in meat products. Limited hydrolysis improves gluten solubility (Batey 1985; Agyare and others 2008), and wheat gluten can be enzymatically hydrolyzed by several commercially available proteases (Kong and others 2007).

Mycoprotein. Mycoprotein's nutritional benefits arise from its chemical composition. The cell walls of the hyphae (cells) are the source of dietary fiber (chitin and glucan). The cell membranes are the source of polyunsaturated fat and the cytoplasm is the source of high-quality protein (Rodger 2001). The composition of the fiber is approximately one-third chitin and two-thirds β-1,3 glucan and 1,6 glucan (Hoseyni and others 2010). The amino acid composition of mycoprotein indicates the presence of all essential amino acids. The PDCAAS value of mycoprotein is 0.91, based on an estimate of 78% digestibility (Miller and Dwyer 2001). It may be a valuable supplement to cereal-based and legume-based diets. Mycoproteins contain no cholesterol and are low in saturated fats (Turnbull and others 1990). Mycoprotein has a favorable fatty acid profile and a fiber content that is comparable to other vegetarian protein sources. Mycoproteins also have a naturally low sodium content. Mycoprotein is a good source of Zn and selenium (Se), but the levels of Fe and vitamin B12 are low in comparison to red meat (Denny and others 2008). Because of its high fiber content, the consumption of mycoprotein significantly reduces blood cholesterol levels (Turnbull and others 1992; Denny and others 2008) and may encourage reduced energy intake (Turnbull and others 1993). Mycoprotein may be useful in the management of obesity and type 2 diabetes because it appears to show beneficial effects on glycemia and insulinemia (Denny and others 2008).

Texturized vegetable protein

Introduction. Recent improvements in human nutrition, agricultural production, and world markets have resulted in an increased interest in texturized vegetable proteins. Texturized vegetable protein is recognized as one of the hot list ingredients for its ability to contribute to 2 top food trends including the continued quest for high-quality, low-fat foods and the thriving field of functional and nutraceutical foods (Riaz 2004). The United States Dept. of Agriculture (USDA) has defined texturized vegetable protein products as "food products made from edible protein sources and characterized by having a structural integrity and identifiable structure such that each unit will withstand hydration and cooking, and other procedures used in preparing the food for consumption" (USDA 1971). The products are produced in a variety of shapes and sizes. The most popular shapes are chunks, granules, and flakes. When prepared for consumption by hydration, cooking, retorting, or other procedures, they retain their structural integrity and characteristic chewy texture (Anonymous 1972). The generic term, "texturized soy protein" (TSP), typically means defatted soy flours or concentrates that are mechanically processed by extruders to obtain meat-like chewy textures when hydrated and cooked (Singh and others 2008).

Texturized vegetable protein consumption in different regions of the world is based on religious or cultural reasons and the vegetarian diet for Hindus is an example of this. Vegetable protein foods are of interest to people following Jewish (kosher) dietary guidelines. Islam is one of the world's fastest growing religions, so the development of nonmeat protein alternatives is highly desirable to food processors as the global market for foods certified as halal is rapidly growing. Texturized vegetable proteins are accepted as halal foods (Lusas 1996). They are often regarded as a healthy choice because they are cholesterol free, low in fat, and low in calories. An additional reason for using vegetable proteins is because they have a lower price than muscle proteins and, consequently, can reduce the cost of the meat product.

Raw materials sources. The approval of a health claim for soy-based foods on October 26, 1999 by the FDA in the United States has resulted in an increased interest in texturized soy-based products. The most popular raw material source for the production of texturized vegetable proteins is the soybean. In 2008, 220.9 million metric tons of soybeans were grown worldwide (ASA 2009).

Several raw materials from whole soybeans can be used in extrusion texturization. These raw materials include defatted soy flour, soy protein concentrate, and soy protein isolate and their protein percentages are 50% to 55%, 65% to 70%, and 85% to 90%, respectively (Riaz 2004; Golbitz and Jordan 2006). The benefits of extrusion cooking are the addition of texture, denaturing of the proteins, inactivation of trypsin inhibitors, and control of bitter flavors (Bjorck and Asp 1983; Hayakawa and others 1988). Approximately 1 million metric tons of functional soy proteins are produced annually, with 55% of that used in processed muscle foods including meat, poultry, and seafood (Hoogenkamp 2007).

Wheat is a key driver of global food inflation. The Intl. Grains Council (IGC) forecasts world wheat production to reach 645 million metric tons in the 2008 to 2009 season, which is up from 604 million metric tons in 2007 to 2008 (Launois 2008). Wheat gluten is a protein that has unique properties. Wheat gluten has been used to produce texturized products in the extrusion process (Riaz 2004). Wheat gluten represents approximately 72% of wheat protein (Kong and others 2007). The acceptance of textured vegetable proteins that contain wheat protein is rapidly increasing.

Rapeseed is a crop of major economic importance. In 2008, 48.4 million metric tons of rapeseed were grown worldwide (ASA 2009). Rapeseed protein is only used in animal feed despite its high nutritional potential for human nutrition (Bos and others 2007). Introduction of new cross-links by TG can be beneficial to functional properties, thus extending applications for nonmeat proteins (Pietrasik and others 2007). Enzymatic modification with TG can improve the gelation properties of rapeseed protein (Pinterits and Arntfield 2008), and these improvements could offer new markets for rapeseed protein as a food ingredient. Rapeseed protein is suitable for the production of texturized product.

In 2008, 46.2 million metric tons of cottonseed were grown worldwide (ASA 2009). Interestingly, the 44 million metric tons of cottonseed (9.4 million metric tons of available protein) produced each year could provide the total protein requirements for half a billion people for 1 y at a rate of 50 g/d (Sunilkumar and others 2006). However, it is underutilized because of the presence of toxic gossypol within its seed glands. Options for reducing free gossypol from cottonseed glands include the following: using a 2-stage solvent extraction method that utilizes aqueous and anhydrous acetone (Gerasimidis and others 2007) and transfers the glandless mutant into commercial varieties to produce gossypol-free cottonseed (Riaz 2004) and using RNA interference (RNAi) technology to disrupt gossypol biosynthesis in cottonseed tissue by interfering with the expression of the δ-cadinene synthase gene during seed development (Sunilkumar and others 2006). Meat extenders have been extruded from some cottonseed protein products.

In addition to the above-mentioned raw materials sources, sunflower, peanut, sesame, pea, and bean proteins have also been used in the extrusion process for texturization (Riaz 2004; Strahm 2006). The most important characteristics of these raw materials are protein levels, protein quality, oil levels, fiber levels, sugar levels, sugar types, and particle size (Strahm 2006).

Uses of texturized vegetable protein. Traditionally, soy flour and soy protein concentrates have been the principal sources for raw material for the majority of the commercially textured protein ingredients. However, products comprised of wheat gluten and other vegetable proteins provide an endless array of textured vegetable protein ingredients that can be utilized as meat extenders and meat analog products (Orcutt and others 2006; Strahm 2006). Textured vegetable proteins provide an excellent source of high-quality protein and an alternative to animal meat (Figure 1).

Figure 1–Texturized vegetable proteins provide an alternative to meat.

Texturized vegetable protein can be added to meat as an extender or it can be consumed directly as a meat analog. The difference between meat extenders and meat analogs is the dependence on texturization of raw materials by extruders (Riaz 2004). If cooked alone, meat extenders are not similar to meat in appearance, texture, or mouthfeel. These types of textured products are mixed with meat for further processing to improve overall functional properties. On the other hand, meat analogs are considerably similar to meat in appearance, color, flavor, and texture when hydrated and cooked (Riaz 2004; Singh and others 2008). The additions of minor ingredients or chemicals are often used to increase the range of raw ingredients suitable for production of a specific texturized vegetable protein product (Riaz 2004). These ingredients can improve the final texture and aid in texturization. Some of these additives are food flavor, color, pH modifier, surface-active substrates, emulsifiers, wheat gluten, and surfactants. These additives can be used to assist the food scientists in controlling the functional properties, structures, mouthfeel, and/or density of the processed material (Riaz 2004). Analogs, which are produced to resemble meat in appearance, color, flavor, and texture, represent the ultimate adoption of vegetable proteins (Singh and others 2008).

Meat extender. Meat extenders are available in flaked form (>2 mm), in minced form (>2 mm), and in chunk form (15 to 20 mm), and they can absorb 2.5 to 5 times their original weight in water (Riaz 2004). If too little water is used to hydrate the product, the extended meat product will be dry. Nonmeat proteins are often used as alternative gelling agents in comminuted meat products to enhance the yield and texture by improving water-binding properties (Pietrasik and others 2007). Soy protein ingredients are widely used in meat products as extenders. Soy can extend meat products while providing an economical, high-quality protein source (Egbert and Borders 2006). In addition, soy proteins can provide functional properties to a formulation such as gelling/textural capabilities, fat emulsification, and water binding. In coarsely chopped meats, such as meat patties, sausages, chili, salisbury steaks, pizza toppings, and meat sauces, textured soy protein concentrates and soy flours are the preferred ingredients to obtain the desired texture (Jindal and Bawa 1988). Soy isolates are also used in meatballs, ground meat, bolognas, and frankfurters for improving the texture and quality (Hettiarachchy and Kalapathy 1997). Soy-extended beef is used to achieve significant cost savings (Singh and others 2008). Poultry breasts pumped with slurries of soy protein isolate and flavors are also becoming popular. Wheat gluten can be used alone or in combination with soy flour or soy concentrate to produce meat extenders. Vegetable protein ingredients, including vital wheat gluten, functional soy concentrates, and isolates are being used to bind meat cuts and trimmings to make pressed loaves and poultry rolls (Singh and others 2008). Meat extenders have been extruded from cottonseed proteins, peanut proteins, sesame proteins, sunflower proteins, pea proteins, and bean proteins (Riaz 2004; Strahm 2006). Legume flours, such as blackeye beans, chickpeas, and lentils, slightly increased the toughness of meatballs and can be successfully used in meatball formulations as extenders (Serdaroglu and others 2005).

Meat analog. Meat analogs are produced to resemble meat in appearance, color, flavor, and texture. If a firmer texture is desired for a finished product, a food scientist might need to compromise on juiciness to achieve the desired result. When functional ingredients are used to increase firmness, moisture becomes more tightly bound, thus reducing juiciness (Egbert and Borders 2006).

Meat analogs can be made in different sizes ranging from 6 to 20 mm (Riaz 2004). Meat analogs can be formed into sheets, disks, patties, strips, and other shapes. They usually absorb at least 3 times their weight in water when cooked in boiling water for at least 15 min (Riaz 2004). When hydrated, the textured vegetable proteins provide a meat-like texture that contributes to mouthfeel (Egbert and Borders 2006). TSP products and textured wheat gluten are used to enhance mouthfeel or simulate "meat texture" in meat analogs. Textured soy flour, textured soy concentrate, textured wheat gluten, and textured protein combinations such as soy and wheat are used at a level 10% to 25% for this purpose (Table 3). Meat analogs have a striated, layered structure similar to muscle meat. Analogs simulating coarse ground-meat products may contain textured proteins (such as textured soy flour and concentrates) that are available in various colors and particle sizes (Egbert and Borders 2006). Soy-based burgers and other forms of meat analogs are successful because of their healthy image (cholesterol free, low in fat, and low in calories), good taste, and low cost. There are also analogs of hamburgers, steak, chicken patties, hot dogs, sausage, sliced lunch meat, Canadian bacon, stuffed turkey, and many other meat products. Textured wheat proteins are also available in a variety of sizes, shapes, and colors that can replace meat at a cost savings while enhancing sensory properties due to their neutral taste and unique textures (Foster 2006). Typical meat analog ingredients and their purpose are shown in Table 3 as described by Egbert and Borders (2006).

All the characteristic attributes found in a meat product—texture, flavor, color, and so on, must be added to the analog by the product developer. Acceptable texture and flavor often prove to be the biggest development challenges (Egbert and Borders 2006). It is up to the food scientist and the food industry in general to advance this technology even further toward improved taste and texture.

Mycoprotein as meat analog. A filamentous fungus was chosen for the production of a meat substitute because it was believed that the mycelia would add a fibrous texture, comparable to that of meat, to the final product (Edelman and others 1983). The harvested hyphae of the fungus have similar morphology to animal muscle cells. In meats, muscle cells are held together by connective tissue. To make a similar-product texture with mycoprotein, the fungal biomass is mixed with a binding agent such as egg albumin, flavorings, and other ingredients depending on the desired final products (Rodger 2001; Denny and others 2008). After heating, the protein binder is converted to a gel that binds the hyphae together. The resulting products have similar textural properties to those found in meat products (Rodger 2001). Suitable food processing technology is used to form the required shape and size.

Prospects and challenges of nonmeat proteins

Soy protein. Meat-alternative products have traditionally been used by vegetarians. However, the products appeal to a wider range of consumers today. Several new-generation soy protein ingredients, such as TSP, combine good functionality with low beany flavor and low cost offering all the nutritional quality of soy proteins. Textured soy proteins are extremely versatile food ingredients due to their meat-like textures after hydration and amino acid composition that provides similar protein quality to that of animal proteins. These attributes will be helpful for their increasing acceptance as major ingredients in meat and as meat analogs throughout the world. On account of its extraordinary qualities, the soybean is known as "the meat that grows on crops."

Table 3–Typical meat analog ingredients and their purpose (Egbert and Borders 2006).

Ingredient	Purpose	Usage level (%)
Water	Ingredient distribution Emulsification, juiciness, cost	50 to 80
Textured vegetable proteins: textured soy flour, textured soy concentrate, textured wheat gluten, textured protein combinations such as soy and wheat	Water binding, Texture/mouthfeel Appearance; protein fortification/nutrition Source of insoluble fiber	10 to 25
Nontextured proteins: isolated soy proteins, functional soy concentrate, wheat gluten, egg whites, whey proteins	Water binding, emulsification Texture/mouthfeel Protein fortification/nutrition	4 to 20
Flavors/spices	Flavor: savory, meaty, roasted, fatty, serumy Flavor enhancement (for example, salt) Mask cereal notes	3 to 10
Fat/oil	Flavor, texture/mouthfeel Succulence, Maillard reaction/browning	0 to 15
Binding agents: wheat gluten, egg whites, gums and hydrocolloids, enzymes, starches	Texture/"bite," water binding, may contribute to fiber content, can determine production processing conditions	1 to 5
Coloring agents: caramel colors, malt extracts, beet powder, FD&C colors	Appearance/eye appeal Natural or artificial	0 to 0.5

Soybeans can supplement or replace our daily protein requirements that we usually derive from animal and marine sources.

The primary problem that limits the incorporation of a large amount of textured soy protein into alternative meat products is an undesirable flavor (Katayama and Wilson 2008). The development of soybean cultivars with low undesirable flavors will be beneficial for the production of nontraditional soy products. Off-flavor-associated components, such as saponins and isoflavones, have both favorable and unfavorable properties. Removal of undesirable components from soybeans to a satisfactory extent is possible through the creation of new soybean cultivars by genetic modification because these soybean components are controlled at a DNA level (Fukushima 2004). Alternatively, Katayama and Wilson (2008) reported that the disadvantage of the beany flavor that limited the incorporation of a large amount of textured soy protein into meat-alternative products for human consumption had been overcome by the use of vegetable-based chicken flavor. This study was successful in the utilization of the soybean components through the development of a chicken-flavored textured soy protein that was accepted by the consumers (Katayama and Wilson 2008).

As we have previously discussed, the 2 major components of soybean proteins, β-conglycinin and glycinin, are responsible for different physicochemical properties. To achieve the desirable physicochemical properties of soybean proteins, the modification of soybean cultivars by either conventional breeding or modern genetic engineering will be the continuing challenge for plant biotechnology.

Legume protein and oilseed protein. Despite their nutritional value, peanuts and soybeans are also a source of food allergens. Peanut allergies often cause acute and severe reactions, but allergies to soybeans are assumed not to cause severe reactions (van Boxtel and others 2008). β-conglycinin, also called 7S protein, is a trimeric soybean protein composed of α-, α'-, and β-subunits (Mitsuda and others 1965; Thanh and Shibasaki 1977). β-conglycinin has a low methionine content and undesirable functional properties (Fukushima 2004). Ogawa and others (1991) reported that the α- and β-subunits of β-conglycinin are important allergenic proteins in soybeans. The identification of the α- and β-subunits as important allergens increased interest in decreasing the β-conglycinin from commercial soybean varieties (Jung and Kinney 2002), and there was some progress on the removal of allergenic proteins.

The 3 proteins, Ara h 1, Ara h 2, and Ara h 3, are considered important peanut allergens that belong to the legume seed storage protein families and have been reviewed by Kang and others (2007). Ara h 1 protein has homology to vicilin proteins in other legume seeds. Ara h 2 protein belongs to a conglutin storage protein family, and Ara h 3 protein is a legumin-type storage protein that has high sequence similarity to glycinin. Glycinin, also called 11S protein and belonging to the legumin protein family, is the main storage protein in the soybean family (van Boxtel and others 2008; Sun and others 2008). van Boxtel and others (2008) reported that the legumin proteins Ara h 3 and glycinin do not maintain their IgE-binding properties during digestion with pepsin. These allergens are unable to sensitize via the gastrointestinal tract and cause systemic food allergy symptoms. These legumin allergens may not be important food allergens as was previously assumed (van Boxtel and others 2008). The Ara h 2 glycoprotein is the most potent allergen in peanuts with an approximate 50-fold greater potency than Ara h 1 (Singh and Bhalla 2008). Dodo and others (2008) reported the application of RNAi technology for silencing Ara h 2 in peanuts. The first reported attempt to use genetic engineering to generate hypoallergenic peanuts with reduced levels of Ara h 2 and a subsequent decrease in peanut allergenicity. A concern for this approach is the potential for reversion of the silenced genes (Riascos and others 2010), and the success of this approach will depend on the long-term stability of the gene suppression.

The continuing consumer interest in vegetarianism and in the choice of occasional meat-free meals as part of a varied diet are the key driving forces behind consumer demand for high quality and convenient meat-alternative products. The importance of legume protein flour, isolates, or concentrates in the food industry due to their high protein contents represents an alternative in the preparation and development of new foods. To improve the nutritional quality, texture, and other functional properties of the product or for economic reasons, the use of legume protein products in food as ingredients has increased.

The protein from oilseeds is also an alternative to animal protein for food applications. World oilseed production reached 391.5 million metric tons in 2008, and the world oilseed protein meal consumption was 224.7 million metric tons in that same time period (ASA 2009). In addition to soybeans, the other oilseeds, such as rapeseeds, cottonseeds, sunflower seeds, and peanuts, are the most abundant for protein meal. Similar to legume proteins, oilseed proteins are also deficient in sulfur-containing amino acids when compared with meat proteins, and they are found to contain antinutrients. These limitations are easily overcome, respectively, by supplementation with cereal and physicochemical treatments. Therefore, oilseed proteins may have an important role in human nutrition. In contrast, the potential negative impact of antinutritional factors is somewhat balanced by the fact that they may have a health-promoting role. Phytochemicals in foods have attracted attention mainly due to their role in preventing diseases caused by oxidative stress (Oboh and others 2009). These phytochemicals, such as polyphenols, phytic acids, and flavonoids, act as antioxidants to eliminate reactive oxygen species and scavenge-free radicals. Research has shown that the positive or negative effect of these antinutritional compounds depends on the level in the various plant proteins, dose, and time of consumption. However, a wide gap still exists in our knowledge with regard to exploring the actual gene pool and evaluating beneficial secondary metabolites, phytochemicals, and other nutritional features in noncereal grains.

The value of noncereal plant proteins in the human diet is based not only on their nutritional quality but also on functional properties. They have found increasing importance in the manufacturing of various functional food products. The successful use of noncereal plant protein preparations depends on the versatility of their functional properties, which are influenced by intrinsic factors (composition and conformation of proteins), methods of protein isolation, and environmental factors (composition of the model system or food) (Soetrisno and Holmes 1992). To obtain the advantages of these nutritious proteins, it is critical that their incorporation into food products remains appealing to the consumer. There are several limitations in the physicochemical properties of noncereal plant proteins. Therefore, challenges remain in improving the functional properties and nutritional quality of noncereal plant proteins through genetic modification.

Cereal proteins. Wheat gluten is a protein that has unique properties. In bread formulations, gluten addition can help compensate for low-protein flours. Products comprised of wheat gluten provide an endless array of textured vegetable protein ingredients that can be utilized as meat extenders and meat analog products (Orcutt and others 2006). Wheat gluten can be used in combination with soy flour or soy concentrate to produce meat extenders (Riaz 2004). Gluten can be used as an extender in ground meat patties and as a binder for sausage products. Wheat gluten can bind chunks or trimmings to create restructured items. In poultry rolls, the binding ability of gluten can reduce cooking losses during processing and preparation and improve slicing characteristics. Hydrated gluten may be extruded, texturized, or spun into fibers to produce a variety of meat analogs.

As mentioned previously, CD is induced in some individuals by the ingestion of gluten, which is derived from wheat, barley, and rye (Table 1) (Green and Cellier 2007). The gluten protein can be subdivided into 2 approximately equal groups based on their extractability (gliadin) and inextricability (glutenin) in aqueous alcohols (Singh and MacRitchie 2001). Gliadins are monomeric proteins and are divided into 4 groups as follows: α-, β-, γ-, and ω-gliadins (Guerrieri 2004). Undigested molecules of gliadin, such as a peptide from an α-gliadin fraction consisting of 33 amino acids, are resistant to degradation by gastric, pancreatic, and intestinal brush-border membrane proteases in the human intestine and, thus, remain in the intestinal lumen after gluten ingestion (Shan and others 2002). These peptides pass through the epithelial barrier of the intestine, possibly during intestinal infections or when there is an increase in intestinal permeability, and interact with antigen-presenting cells in the lamina propria (Green and Cellier 2007). Multiple T-cell-activating gluten peptides are found mainly in α-gliadins (Arentz-Hansen and others 2000; Sollid 2002; Koning 2003). Peptides derived from α-gliadins are recognized by T cells from most CD patients (Arentz-Hansen and others 2000). Wheat varieties with low extents of T-cell stimulatory epitopes may be tolerated by many CD patients (Janatuinen and others 2002; Vader and others 2003). Using RNAi technology, it is possible to silence sections of the wheat genes (α-gliadins) that may lower the content of T-cell stimulatory gluten epitopes. However, an unwanted loss of technological properties may occur because of an altered ratio of glutenin and gliadin proteins. This ratio may be compensated by the addition of monomeric proteins without T-cell stimulatory epitopes to the flour or by the introduction through genetic modification of CD-safe gliadin genes.

Mycoprotein. Mycoprotein, the main ingredient in a variety of quorn products is naturally low in fat and contains high fiber content and high-quality protein (Figure 2), enabling us to offer an alternative vegetarian source of protein to meat. The protein quality is high because mycoprotein has an excellent pattern of amino acids (Miller and Dwyer 2001). Mycoprotein can lower cholesterol, help with weight control, and also help reduce blood glucose and waist measurement, all of which could improve heart health (Aldridge 2008).

An important consideration in assessing the effect of mycoprotein on blood lipid profiles is that there appears to be a dose-dependent relationship. In 2 studies (Nakamura and others 1994; Homma and others 1995), a reduction in cholesterol concentration was seen with 24 g (dry weight) mycoprotein per day, but not with 18 g (dry weight) mycoprotein per day. These results therefore look promising in terms of opportunities for new product development toward producing foods that contain physiologically meaningful levels of mycoprotein.

Consumers choose meat-alternative products primarily for their nutritional benefits, but, at the same time, they also demand good taste and variety. Many a versatile, convenient item can be made from mycoprotein, a powerhouse ingredient with less fat, fewer calories, and more fiber than meat, and with good taste and texture (Anonymous 2009). Furthermore, a number of mycoprotein products have been approved by the Halal Food Authority—the packaging for these products now carries the halal logo (Marlow Foods 2009). Islam is said to be the world's fastest growing religion, so the development of nonmeat protein alternatives is highly desirable for foods certified as halal.

Figure 2–Mycoprotein, the main ingredient in a variety of Quorn products is a high protein, high fiber, low-fat food ingredient (picture is a courtesy of Marlow Food Ltd., United Kingdom).

Mycoprotein is the main protein ingredient in a variety of mycoprotein products available throughout Europe and the United States (Denny and others 2008). Unfortunately, many peoples of the rest of the world are not familiar with mycoprotein. There are lots of great things about mycoprotein that very few people understand. Because of this, it is not so easy to establish mycoprotein as an alternative protein source to meat in the rest of the world. In addition to development of new products, improvement of consumer knowledge using ad campaigns to make a positive choice as an alternative protein source to meat is also the challenges to food manufacturers.

Future trends

Worldwide, the food market has become more complex, and the demand for more sophisticated meat alternatives has grown. Food manufacturers face the challenge of providing nutritious, economical, and healthy foods while at the same time ensuring that the product has an appealing taste and texture. An important reason for the increased acceptance of soy protein is their low cost. Soy protein will have an opportunity to get the attention it deserves as a highly nutritious and functional food ingredient. Consumers are changing their attitudes toward "soy as an ingredient or platform for health." They should be viewed as vital components that will enable the food technologists to fabricate new foods.

Physicochemical, thermal, chemical, and enzymatic technologies applied to legume and oilseed meals, concentrates or isolates can be used to obtain products with desirable properties for food applications. Genetic engineering can enhance the quality of plant-based food products through the silencing of genes. More research will be required to place these proteins in food applications. Minor components, such as phytic acid and phenolic compounds that have been considered antinutritional, may be used for disease prevention because of their important health promotion properties.

Texturized wheat gluten is commercially available in several forms differing in size, shape, density, color, and texture. These products can be used as ingredients for extension in meat and vegetarian foods. The popularity of texturized wheat gluten is rapidly increasing. Furthermore, researchers are trying to develop wheat varieties that have a minimum amount of CD toxic proteins while maintaining technological properties (Hamer 2010).

Mycoprotein is the main ingredient in a variety of products. It required nearly 2 decades of research and development before it was introduced to the market, but since its introduction, it has been established as one of Europe's leading meat-free products (Wiebe 2004) and now is available in the U.S.A. The expansion of the mycoprotein market into the U.S.A. and an increasing number of European countries has demonstrated the general acceptability of the product. Food manufacturers are developing new mycoprotein products to provide more choices for consumers who are seeking a tasty and convenient approach to healthy eating. Including mycoprotein regularly in diets may be a good way to manage obesity, heart disease, and diabetes, and there is hope that it will be successful in the world.

In addition to the above-mentioned nonmeat proteins, edible mushrooms can be used as an alternative protein source to meat. Edible mushrooms have been part of the human diet in many cultures for years. Of the more than 10000 known species of fungi, approximately 700 are edible and more than 200 are thought to have medicinal values (Dikeman and others 2005), but only a few of the species are commercially cultivated. Edible mushrooms are a well-known valuable part of the diet because they are a good source of proteins, essential amino acids, vitamins (such as thiamin, riboflavin, and niacin), and essential minerals (such as Ca, P, Mg, Cu, Fe, and Zn). Edible mushrooms are also low in calories, fat, and sodium (Tong and others 2008; Valdez-Morales and others 2010). The primary bioactive components in mushrooms are polysaccharides and glycoproteins (Dikeman and others 2005). In addition to being a nutritious food source, mushrooms also show potential in providing medicinal functions, such as antitumor, immunomodulating, hyperlipidemic, reducing blood pressure, antiinflammatory, hypoglycemic, antiviral activities, antibiotic activities, and other health-promoting properties (Breene 1990; Wasser and Weis 1999; Talpur and others 2002; Van Nevel and others 2003; Barros and others 2007b; Barros and others 2007a; Valdez-Morales and others 2010). Many of these effects are dependent on the isolation of bioactive compounds, processing, and fruit maturity at time of harvest. In summary, mushrooms are valuable health foods with long recognized nutritional value (Valdez-Morales and others 2010). The information obtained in this study will allow people to choose mushrooms for their diet, and it should also allow food scientists to search for optimal preparation strategies for using the various products.

Furthermore, *Spirulina* has been used as food for centuries (Belay 2008). *Spirulina* is blue-green algae (*Cyanobacterium*) belonging to the family Oscillatoriaceae and form unbranched, multicellular helicoidal filaments with a length of approximately 200 to 300 μm and a breadth of 5 to 10 μm (Chronakis 2001). The edible forms that are commercially grown and sold as *Spirulina*, are in the genus *Arthrospira platensis* and *Arthrospira maxima* (Belay 2008; Chamorro-Cevallos and others 2008). *Spirulina* has a high protein content, 60 to 70 wt%, and high productivity with a photosynthetic conversion rate of 8% to 10% compared to only 3% in most terrestrial plants (Chronakis 2001). It is a rich source of proteins, vitamins, essential amino acids, minerals, and essential fatty acids (Chamorro-Cevallos and others 2008). Several toxicity tests including some sponsored by the United Nations have proven its safety (Belay 2008). Today, there are several companies producing *Spirulina*, which is sold in many food stores around the world (Chamorro-Cevallos and others 2008).

Vinnari (2008) reported a selection of strategies that can be used to help decrease meat consumption: (1) technological development in alternative protein sources and especially in the development of artificial meats is required when considering the importance of taste as a reason for meat consumption, (2) use ad campaigns to increase consumer knowledge about animal rights and vegetarianism, (3) political actions are needed in order to make the general population understand that meat consumption is a waste of energy resources, as well as an inefficient use of farm land, (4) place higher taxes on meat products.

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